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## ABOUT THE COVER

The sea urchins are collected for their edible roe, a highly prized delicacy and a vital fishery commodity in many coastal communities in its distribution range. Despite their economic value, information on their biology remains limited. In this December 2025 issue of *The Palawan Scientist*, Molina et al. reported the population structure and the lunar periodicity of spawning of sea urchins in Davao Occidental, Philippines providing insights essential for their sustainable management and conservation.

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## EDITORIAL

# Integrating One Health Across Biodiversity, Biotechnology, and Environmental Sustainability

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**Dear Readers,**

It is a great honor and privilege to write this editorial for the December 2025 issue of *The Palawan Scientist*. Reading the various articles featured in this issue has reminded me of how diverse our research endeavors are. I am impressed by the diversity of fields featured in this issue, as it calls for a **ONE HEALTH** approach, a widely recognized multidisciplinary collaboration between human, animal, and environmental health. It emphasizes how biodiversity conservation, sustainable food systems, environmental preservation, and technological development are all intertwined.

I started my veterinary training with a straightforward view of the type of work I would be doing: treating animal diseases, identifying pathogens around us, and exploring how animals can stay healthy. The global call to action for the One Health approach has become increasingly prominent over the years, and attention on that is starting to change. The call for a One Health approach has influenced not just how we conduct research but also how we understand or perceive our responsibilities. Instead of considering only the animal in front of me, I am now thinking about the people who lived with them, the environment they lived in and the factors that influenced their health. It was increasingly difficult to ignore how all of these pieces were connected. Today, our responsibilities extend beyond diagnosing infections and preventing outbreaks; they now include understanding the crucial connections between people, animals, and the environment. A significant part of our work today focuses on environmental challenges, public health, and the interactions among communities, animals, and ecosystems. It is not a sudden transformation but rather a gradual widening of perspective.

Research on marine organisms, endemic plants, natural products, pollution control technologies, genetic tools, and socio-cultural innovations collectively advances our understanding of the One Health paradigm. Reading through the wide range of articles in this issue, there is a profound understanding of the inherent interconnectedness of humans, animals, plants, and the shared ecosystems. Furthermore, there is a strong emphasis on ongoing research priorities dedicated to serving the public interest. Studies on *Tripneustes gratilla*, *Tridacna noae*, and the recently identified moray eel underscore how environmental changes and biodiversity loss continue to affect coastal communities. Our understanding of conservation efforts, crop development, and ecological shifts has significantly improved through studies of the Victoria–Anepahan Mountain Range and DNA barcoding of *Allium chinense*. Taken together, understanding conservation and habitat features is critical in ensuring adequate biodiversity protection and management.

Other articles in this issue explore innovative solutions for environmental health—a part of One Health. Studies on the mitigation of pollution, plant antioxidants, and plasma-activated water demonstrate how technology can be used to restore ecological quality amid environmental contamination and strengthen agricultural resilience. Studies on the biological properties of plants also highlight the significant role of natural resources as antioxidants, antiglycemics, antibacterials, and antifungals as alternatives to address the growing global concerns about antimicrobial resistance (AMR) and the development of new drugs. Such innovations demonstrate how biotechnology can enrich healthcare, food systems, and conservation efforts in a One Health context. On the other hand, the study on insect-eating festivals in Muslim Mindanao as a tool for promoting entomophagy demonstrated the importance of cultural knowledge and community-driven interventions to sustainably support food practices while respecting cultural traditions.

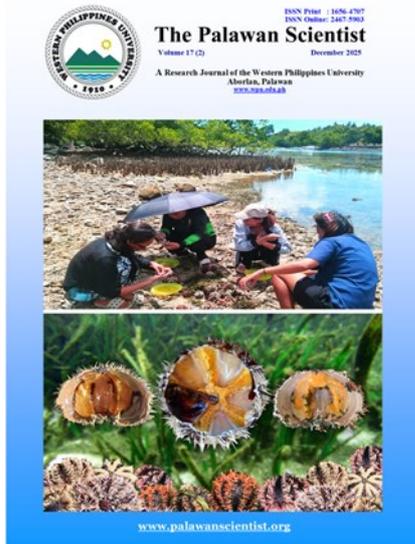
This issue also features an article on the use and role of ChatGPT in higher education across Southeast Asian countries, with a focus on Indonesia, Malaysia, and the Philippines. The article highlights both the negative and positive impacts of using AI tools. It reflects on how digital transformation affects the community, as well as the challenges posed by technological dependence, ethical practices, and research integrity. Given these opportunities and challenges, AI literacy will have a direct impact on society, and education policy guidelines will be necessary. It is also important to understand the role of digital tools in education.

Collectively, the papers in this issue encourage us not only to understand One Health as a scientific framework but also to recognize it as a reminder of our shared responsibility. There are two themes that emerge from the articles in this issue. First is an honest appreciation of the extent to which our health depends upon nature. Next is the dedication and commitment to research that serves the public good, research that does not stay within the four corners of the laboratories or conference halls but touches people, communities, policy, and everyday life. These articles remind us why the integration of human, animal, and environmental health has never been more important.

We hope this issue inspires every reader. May these pages ignite new debates, enhance collaborations, and inspire us to reflect on the gentle question: **What can we do to care for the world so it will care for us in return?**

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# Population status and lunar periodicity of spawning of *Tripneustes gratilla* (Echinodermata: Echinoidea), in Jose Abad Santos, Davao Occidental, Philippines

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## ABSTRACT

The population structure and lunar periodicity of spawning were investigated from January to February 2024 across three sites in Jose Abad Santos, Davao Occidental, Philippines. Monthly field surveys were conducted using the belt transect method to assess population structure, while laboratory analyses were performed to examine the lunar periodicity of spawning across different lunar phases. A total of 160 individuals of *Tripneustes gratilla* (Linnaeus 1758) were recorded with an estimated mean density of  $645.83 \pm 171.50$  ind ha<sup>-1</sup> and mainly dominated by adult size classes having a test diameter (TD) of 61 - 65 mm followed by pre-adults of 51 - 55 mm TD and only very few recruits 33 - 40 mm TD. The relationship between the TD and weight of *T. gratilla* displayed a high positive correlation. It exhibited a negative allometric growth with a *b* value of less than 3 in three sites. The result of the gonad index (GI) and fecundity showed that the highest GI and total number of eggs occurred during the full moon while the lowest occurred during the new moon with notes on sexual maturity at 54 mm TD. While the findings of this study provide valuable insights, they are not conclusive regarding the population structure and lunar spawning periodicity, as the study was conducted over only two months. Long-term monitoring is needed for a more accurate understanding of the status of *T. gratilla* at these sites.

**Keywords:** fecundity, gonad index, lunar phase, size structure, test diameter-weight relationship

## INTRODUCTION

*Tripneustes gratilla* (Linnaeus 1758), also known as the "collector sea urchin", is a rounded sea urchin distinguished by its unique physical characteristics. Its body is covered with colorful, short spines and tube feet (Toha et al. 2017), which it uses for defense and movement. They are highly reproductive (prolific) marine invertebrates

(Lawrence and Agatsuma 2007). In some species of sea urchin, for example, a single female can release up to 100 million eggs during one spawning period (Küçükdermenci et al. 2017). The sexual maturity of *T. gratilla* has a test diameter (TD) of 50 - 70 mm based on the observation of Junio-Meñez et al. (1998) done in the laboratory and field. The *T. gratilla* spawns year-round but the timing of spawning peaks may vary among localities (Tuason and Gomez 1979).



Spawning time are influenced by several abiotic factors (water temperature, photoperiod, moon cycle) and biotic factors (food availability and phytoplankton abundance) (Brockington et al. 2001; Perez et al. 2010; Vaitilington et al. 2005; Muthiga 2005; Lawrence and Agatsuma 2013). The peak of the spawning phase is identified by the variance in the gonad index (GI) (Vladimir et al. 2004; Gaudette et al. 2006). The development of sea urchin gonads can be assessed by observing an increase in the GI value (Lutfiyani et al. 2021). The GI values range is used to reference the maturity of the sea urchin gonads. The maximum value of GI is reached by sea urchins when it is close to the spawning time and decreases from spawning until spawning is completed (Nasrullah et al. 2018).

The gonad of sea urchin has a high concentration of bioactive compounds such as polyunsaturated fatty acids (PUFAs) especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Dincer and Cakli 2007), and it is thought to be beneficial in the treatment of numerous diseases such as cancer, cardiovascular diseases and arrhythmia (Pulz and Gross 2004). Hence, it is economically important to support small-scale fisheries and commercial trade (Andrew et al. 2002). However, *T. gratilla* is at risk of overexploitation due to uncontrolled harvesting for local food and livelihood (Junio-Meñez et al. 2008b). A scenario occurred in 1992 where overexploitation and improper management led to the depletion of wild stock sea urchins (Regalado et al. 2010; Junio-Meñez et al. 2008b). In the Philippines as of 2015, catch landings of *T. gratilla* accounted for 140 metric tonnes (MT) (Sonu 2017). The world landings of sea urchins peaked at 120,000 MT in 1995 and now decreased to about 82,000 MT in 2018. The decreasing pattern reflects the overexploitation of most fishery grounds and highlights the need for appropriate conservation policies, stock enhancement, fishery management, and aquaculture development (Rahman et al. 2022). Sea urchins are primarily exploited through gleaning; gleaners only need to walk in the seagrass areas during low tide to pick the sea urchins, place them in a basket, and carry them to shore (Furkon et al. 2019; 2020). Most of the gleaners prefer to catch sea urchins during full moon or new moon because it contains more roe during that lunar phase (Bin Nurhasan et al. 2011). The excessive gleaning of sea urchins has resulted in a low number and small test-diameter size of individuals in the wild (Nane and Paramata 2020).

Several initiatives have been launched to sustainably manage the *T. gratilla* fishery in the Philippines. This includes improvement of fisheries management (i.e. establishment of marine protected area and size quota) (Junio-Meñez et al. 2008b), and community-based sea urchin culture (Ungson 2004). In Nalvo, Sta. Maria, Ilocos Sur, a community-based sea culture, is adapted to provide income for fishermen while sustainably managing the sea urchin fishery

(Ungson 2004). The International Union on Conservation of Nature (IUCN) Red List has not evaluated the *T. gratilla* yet. Since there is no management system in place or local restrictions on the sea urchin fishery, overexploitation of *T. gratilla* in particular is now an issue (Toha et al. 2017).

Sea urchin fishing is a primary source of food and revenue for the locals, therefore socioeconomic restrictions make it difficult to implement localized fisheries regulations, such as size limits and closed seasons (Junio-Meñez et al. 2008b). Furthermore, it is not wise to introduce a total banning on sea urchin collection without a sound scientific basis, as adopting so will not always be advantageous for the seagrass and coral reef ecosystems and might be difficult to enforce (Tamti et al. 2021). For instance, outbreaks of sea urchins in the macrophyte communities may lead to overgrazing, which could be detrimental to the nearby ecosystems (Eklof et al. 2008; Wallner-Han et al. 2015). There is a need to explore alternative or complementary approaches to marine resource management. There are few published works done in the Philippines, especially in Bolinao, Pangasinan (Junio-Meñez et al. 2008b), La Union (Prado et al. 2012), Southern Guimaras (Regalado et al. 2010), Kalayaan Islands, Palawan (Balisco 2015), Northern Mindanao, and in Sta. Cruz, Southern Mindanao (Bangi et al. 2013) with regard to the investigation of the population structure of this highly valuable sea urchin species. However, localized studies are still insufficient. Factors that control the reproduction of *T. gratilla* are still poorly understood (Muthiga 2005; AbouElmaaty et al. 2023). Understanding its reproductive cycle, however, is crucial for effective fishery management and aquaculture initiatives (AbouElmaaty et al. 2023). In Jose Abad Santos, Davao Occidental, no studies have been conducted on *T. gratilla* to date. However, its collection and harvesting are common due to the area's open-access nature. If left unmanaged, this could lead to the collapse of the fishery. Thus, this study was conducted to determine the population status (i.e. population density, size structure, and test diameter-weight relationship) and lunar periodicity of spawning (i.e. gonad index and fecundity) of *T. gratilla* that would serve as a useful reference for the local government unit and other key stakeholders in the formulation of strategic measures to sustainably manage this important resource that has been long exploited.

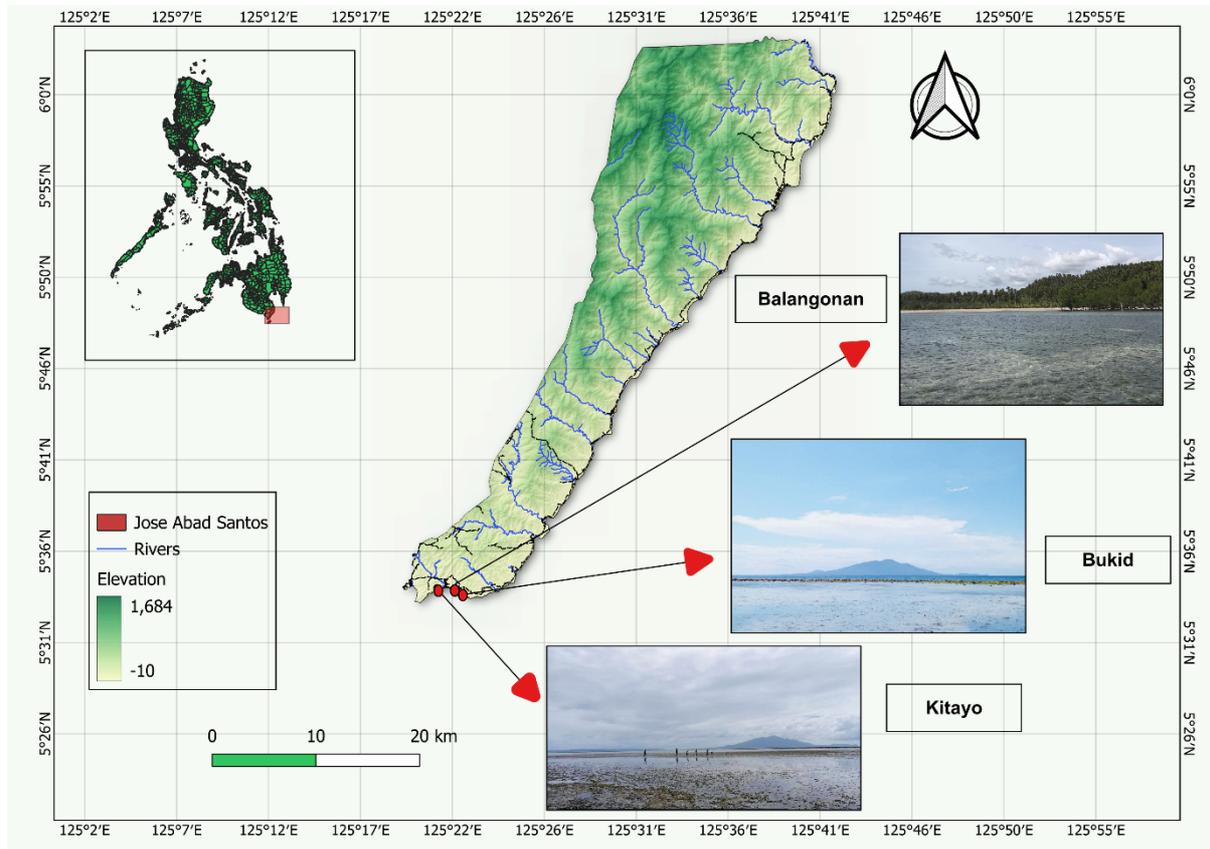
## METHODS

### Study Area

Jose Abad Santos is a coastal municipality in the province of Davao Occidental. It comprises 26 barangays, with 23 located along the coast. Among the 23 coastal barangays, the barangay Kitayo, Balangonan, and Bukid have abundant *T. gratilla*

where locals have open access to fishing that is sold in bottles in the local market, resulting in an overharvesting due to the demand in these regions. These coastal barangays encompass various ecosystems including the extensive seagrass beds, serving as a primary food and shelter to the abundant

*T. gratilla* in the area. The three sampling sites are geographically positioned at Kitayo (5°33'45.99"N and 125°20'45.90"E), Balangonan (5°33'42.63"N and 125°22'11.17"E), and Bukid (5°33'33.57"N and 125°22'26.45"E) (Figure 1).



**Figure 1.** Map of the study area in Jose Abad Santos, Davao Occidental. The red dots are the three sampling sites namely: Barangay Kitayo, Balangonan, and Bukid.

### Sampling Procedure

To determine the population density of *T. gratilla*, sampling was done from 06 to 20 January and 03 to 17 February 2024 in the three established sampling sites. Three 100 x 4 m belt transects, 50 m away from each other were laid perpendicular to the shoreline of each study site. For ease of observation, sampling was conducted in the daytime during the lowest low tide. The sampling was conducted by wading or snorkeling up to 1 m to 1.5 m depth. All *T. gratilla* found within 2 m on each side of the transect were recorded and collected for the test diameter-weight measurement. All *T. gratilla* individuals collected were brought to the shore for TD and weight measurements. The total wet weight of sea urchins was measured in grams (g) using an analytical weighing scale (Sartorius ENTRIS224-1S, Germany). The TD was measured in millimeters (mm) with the jaws of calipers positioned between spines while making sure that the measurement was from the center of an

ambulacrum to the opposite inter-ambulacrum and that the sea urchin was not tipped (Lawrence 1975). All live specimens of *T. gratilla* collected were carefully returned to the area where they were found.

Gonad index is the percentage of the total body weight of the urchin that is made up by the gonad (James and Siikavuopio 2012), and fecundity is the number of eggs in the ovaries that will mature during a particular spawning season (Cailliet et al. 1986). To determine the GI and fecundity of *T. gratilla* during each lunar phase, 10 individuals from each site with a TD of >50 mm (considered sexually mature; Junio-Meñez et al. 1998; Lawrence and Agatsuma 2013) were sampled for 2–3 days every lunar phase (full moon, last quarter, first quarter, and new moon) (Table 1). Samples were bought from the gleaner who collected the sea urchin individuals from the three sampling sites. Samples were placed in a pail and were brought to the laboratory for GI and fecundity measurements. In the laboratory, the TD and weight of

the samples were measured and then dissected by removing the Aristotle Lantern to expose its gonads. To accurately assess the GI of an individual urchin, the whole urchin was weighed (total weight), and the gonads were removed, cleaned, and then weighed (weight of gonad). Gonadal maturity stages were determined by ocular observation and through microscopic analysis. Gonadal stages were based on the work of Perez et al. (2010) (Table 2; Figure 2). For

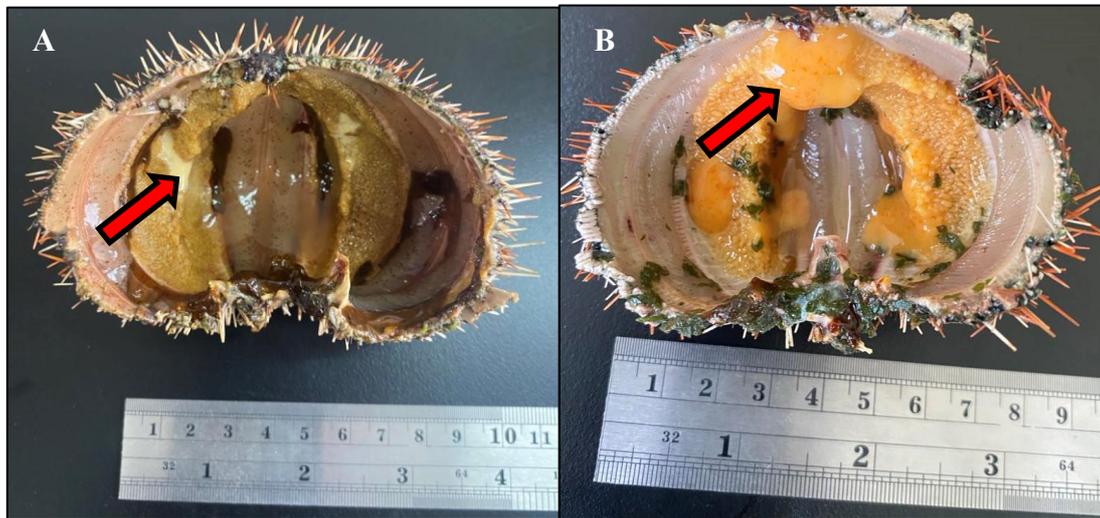
fecundity measurements, only mature ovaries were sampled. Before the actual estimation of the fecundity of *T. gratilla*, mature ovaries were fixed in Gilson’s fluid and stored in an individual container. Fecundity was determined by the gravimetric method. The total number of eggs in each ambulacrum was weighed and a sub-sample of at least 0.01 g was counted using a compound microscope.

**Table 1.** The number of samples with a test diameter >50 mm was collected for gonad index and fecundity measurements in every lunar phase from January to February in three sampling sites of Jose Abad Santos, Davao Occidental.

Sampling sites	January				February				Total
	Last quarter 4 Jan. 2024	New moon 11 Jan. 2024	First quarter 18 Jan. 2024	Full moon 26 Jan. 2024	Last quarter 3 Feb. 2024	New moon 10 Feb. 2024	First quarter 16 Feb. 2024	Full moon 24 Feb. 2024	
Kitayo	10	10	10	10	10	10	10	10	80
Balangonan	10	10	10	10	10	10	10	10	80
Bukid	10	10	10	10	10	10	10	10	80

**Table 2.** Gonadal maturity stages were based on the work of Perez et al. (2009) but were modified for better understanding.

Gonads	Male	Female
Immature	The gametes look pale-soft tissue	The gametes look pale and circular with large distinct nuclei
Mature	Extreme milky sperm appears as white fluid	Egg/ova are uniform perfectly spherical bodies with small but distinct nuclei and are yellow-gold in color
Spent	The gonads are almost empty, although small clusters of sperm may be found	The gonads appear empty, containing only a small number of relict ova



**Figure 2.** Image of mature male (A) and mature female (B) *Tripneustes gratilla*. The red arrow is the distinguishable characteristic of a male with creamy white sperm and yellow gold eggs in female.

**Data and Statistical Analyses**

Population density was estimated from the number of individuals per transect over the total area of the belt transect. The resulting values were then extrapolated to individuals per 10,000 m<sup>2</sup> or 1 hectare.

An estimated density (D) of *T. gratilla* was obtained using the formula:

$$D = \frac{\text{total number of } T.\text{gratilla per transect}}{\text{total area of belt transect} \times 10,000 \text{ m}^2} \quad (\text{Eq. 1})$$

The size structure of *T. gratilla* was analyzed using TD data, which were grouped into size classes at 5 mm intervals. The percentage of each size class was calculated by dividing the number of individuals in a specific class by the total number of individuals of all size classes, then multiplying the result by 100.

The relationship between TD length (L) and total weight (W) was determined using the equation:

$$W = aL^b \quad (\text{Eq. 2})$$

W is the total weight in grams, L is the total length in millimeters, a is the intercept and b is the slope of the regression. Parameter estimation was made through logarithmic transformation of L-W data pairs and ordinary least-squares linear regression (Pauly 1984):

$$\text{Log } W = \text{Log } a + b * \text{Log } L \quad (\text{Eq. 3})$$

when b is equal to 3, the growth is isometric, if b is greater than 3, the growth is positively allometric and if less than 3, the growth is negatively allometric (Elmasry et al. 2023; Rahman et al. 2013; Suryanti et al. 2024). The b value is interpreted as negatively allometric (b < 3); isometric (b = 3), and positively allometric (b > 3) (Rahman et al. 2013; Suryanti et al. 2024).

The gonad index was calculated using the formula of Lawrence et al. (1965):

$$GI = \frac{\text{Gonad weight (g)}}{\text{Total weight of } T. \text{ gratilla}} \times 100 \quad (\text{Eq. 4})$$

The fecundity (total number of eggs) in the ovaries was obtained from the equation of Holden and Raitt (1974):

$$F = \frac{nG}{g} \quad (\text{Eq. 5})$$

Where F is the fecundity of *T. gratilla*, n is the number of eggs in the subsample, G is the total weight of the ovaries, and g is the weight of the subsample in the same units.

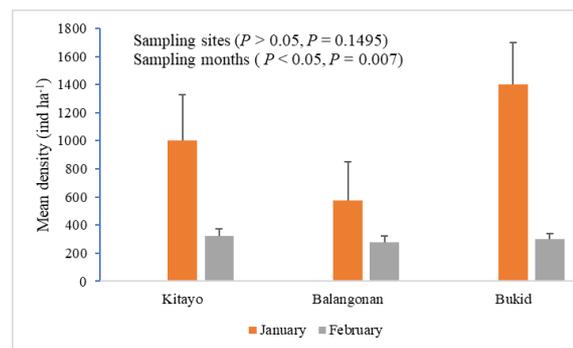
Density data were analyzed for normality using the Shapiro-Wilk test and for equal variances using Levene's test, both at a significance level of 0.05. A non-parametric approach was used since the data did not meet the assumptions of normality and equal variances. Specifically, the Kruskal-Wallis test was applied to compare *T. gratilla* density across sampling sites, while the Mann-Whitney U test was used for pairwise comparisons between sampling months. For the gonad index and fecundity data, normality was tested with the Kolmogorov-Smirnov test (P < 0.05). As these data met the assumptions of normality and equal variances, a one-way Analysis of Variance (ANOVA) was used to determine significant differences (P < 0.05) across four lunar phases. All

statistical analyses were done using Statistical Package for the Social Sciences (SPSS Software version 20).

## RESULTS

### Density

A total of 160 individuals of *T. gratilla* were recorded during the belt transect survey from January to February 2024 in the three sites in Jose Abad Santos, Davao Occidental, Philippines with an estimated mean density of  $645.83 \pm 171.50 \text{ ind ha}^{-1}$ . Mean density was significantly higher in January at  $991.67 \pm 415.56 \text{ ind ha}^{-1}$  compared to February at  $300 \pm 25 \text{ ind ha}^{-1}$ . The highest population density was recorded in January in Bukid with a mean population density of  $1400 \pm 346.41 \text{ ind ha}^{-1}$  while the lowest population density was recorded in February in Balangonan with a mean population density of  $275 \pm 43.59 \text{ ind ha}^{-1}$  (Figure 3). The density of *T. gratilla* significantly varied between January and February (P < 0.05, P = 0.007) but not across three sampling sites (P > 0.05, P = 0.1495).



**Figure 3.** Population density (ind ha<sup>-1</sup>) of *Tripneustes gratilla* from January to February 2024 in the three sampling sites of Jose Abad Santos, Davao Occidental, Philippines. Vertical bars are the standard deviation.

### Size structure

The largest and smallest individual was recorded in Balangonan with a TD of 85 mm (199.56 g) and 33 mm (33.21 g). The size structure of *T. gratilla* individuals recorded in this study was generally dominated by size classes at 5 mm intervals was 61 - 65 mm TD (23.75%) followed by 51 - 55 mm TD (23.125%). The least size classes observed were with TD ranging from 33 - 40 mm at only 0.625%. The body weight of *T. gratilla* with size classes of 31 - 35 mm ranges from 33.21 to 35.27 g while the size classes of 51 - 55 mm weigh ranges from 38.27 to 153.08 g. In addition, the body weight of individuals > 61 mm of *T. gratilla* ranges from 95.35 to 199.56 g (Figure 3). Most of the *T. gratilla* found in Bukid is comprised of size classes with TD of 51 - 55 mm (28.57%) followed by 61 - 65 mm (22.85%) (Figure 4). On the other hand, Balangonan and Kitayo were

dominated by 61 – 65 mm TD size classes. Smaller individuals with size classes of 31 – 35 mm TD were only observed in Balangonan with a relative frequency of 2.78%.

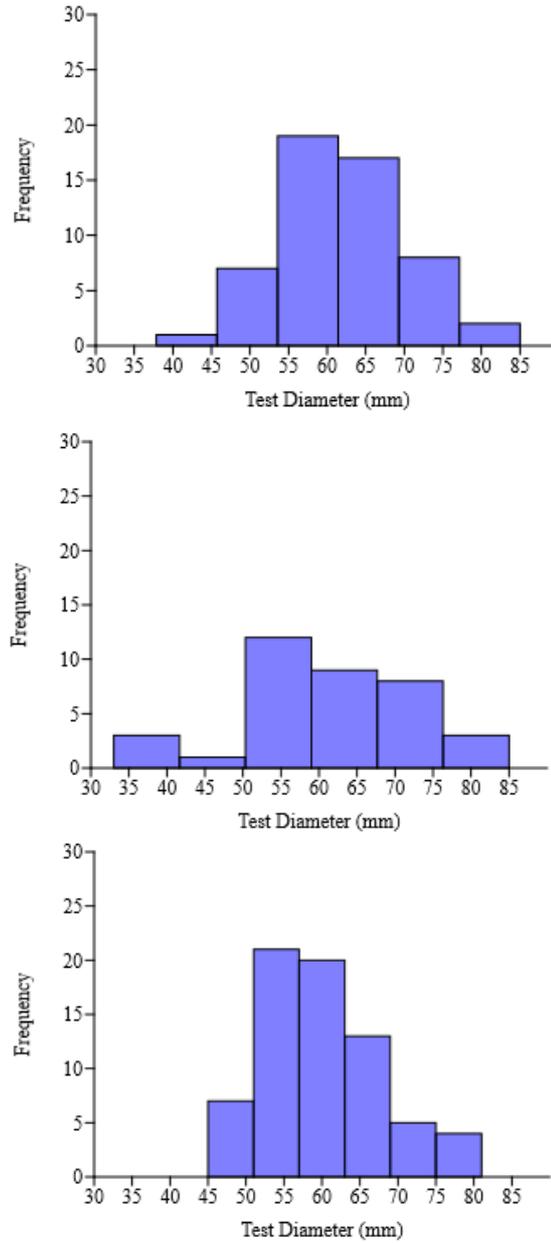
**Test Diameter-Weight Relationship**

The relationship between TD and body weight is best explained by the equation  $W = 0.0786TD^{2.41}$  in Kitayo,  $W = 0.2596TD^{2.20}$  in Balangonan, and  $W = 0.0102TD^{2.49}$  in Bukid (Figure 5). In addition, TD and body weight on the samples collected for gonad index and fecundity is represented by the equation  $W = 0.012654 TD^{2.51}$  in the last quarter,  $W = 0.00729TD^{2.56}$  in the new moon,  $W = 0.248575TD^{2.22}$  in the first quarter, and  $W = 0.451508TD^{1.95}$  in the full moon (Figure 6). The relationship between the TD and weight of *T. gratilla* displayed a high positive correlation in all three sites and across four lunar phases. Results of regression analysis on the test diameter and weight of *T. gratilla* showed negative allometric growth with a *b* value of less than 3 in all three sampling sites.

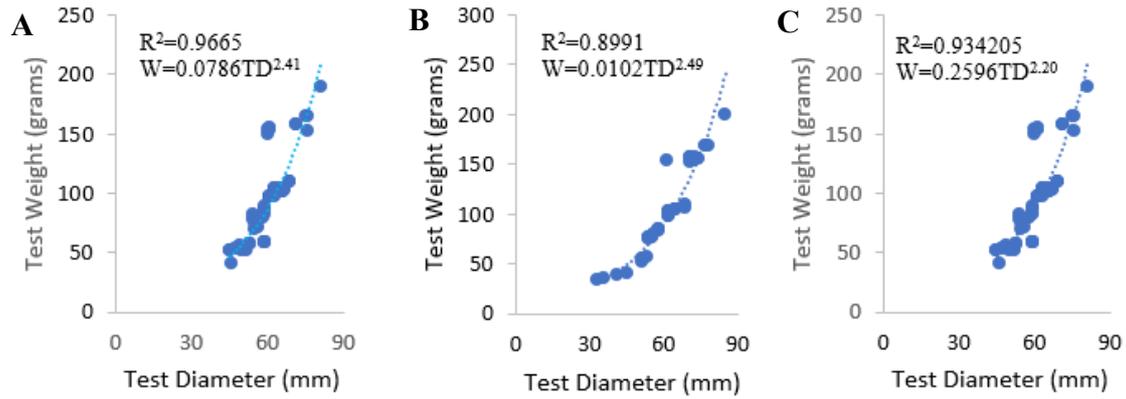
**Gonad Index and Fecundity**

The male: female sex ratio of *T. gratilla* collected was 124 males: 116 females with TD ranges from 50 – 91 mm and weight ranges from 56.16 – 315.6 g. The size of mature gonads recorded was 54 mm TD and weighed up to 53.10 g. The mean GI varied significantly in four lunar phases and three sites ( $P < 0.05$ ,  $P = 0.000430$ ). The GI was observed to be highest during the full moon in three sampling sites with a mean GI of  $6.77\% \pm 0.18$  followed by the first quarter at  $GI = 5.97\% \pm 0.16$ . On the other hand, the lowest GI value was observed during the new moon with a mean GI of  $4.98\% \pm 0.20$  (Figure 7). A total of 22 to 25 mature ovaries for each lunar phase were examined for fecundity estimates. The estimated fecundity of *T. gratilla* varied in each lunar phase ( $P < 0.05$ ,  $P = 0.00000$ ) and fecundity was relatively the highest during the full moon with a mean number of eggs of  $129,842 \pm 8319.99$  while the lowest fecundity was found during the new moon with a mean number of eggs of  $25,090 \pm 9773.96$ . The mean fecundity during the last quarter was  $93,623 \pm 5985.85$  and  $87,283 \pm 8263.54$  eggs during the first quarter (Figure 8). The highest fecundity occurs in Bukid during the

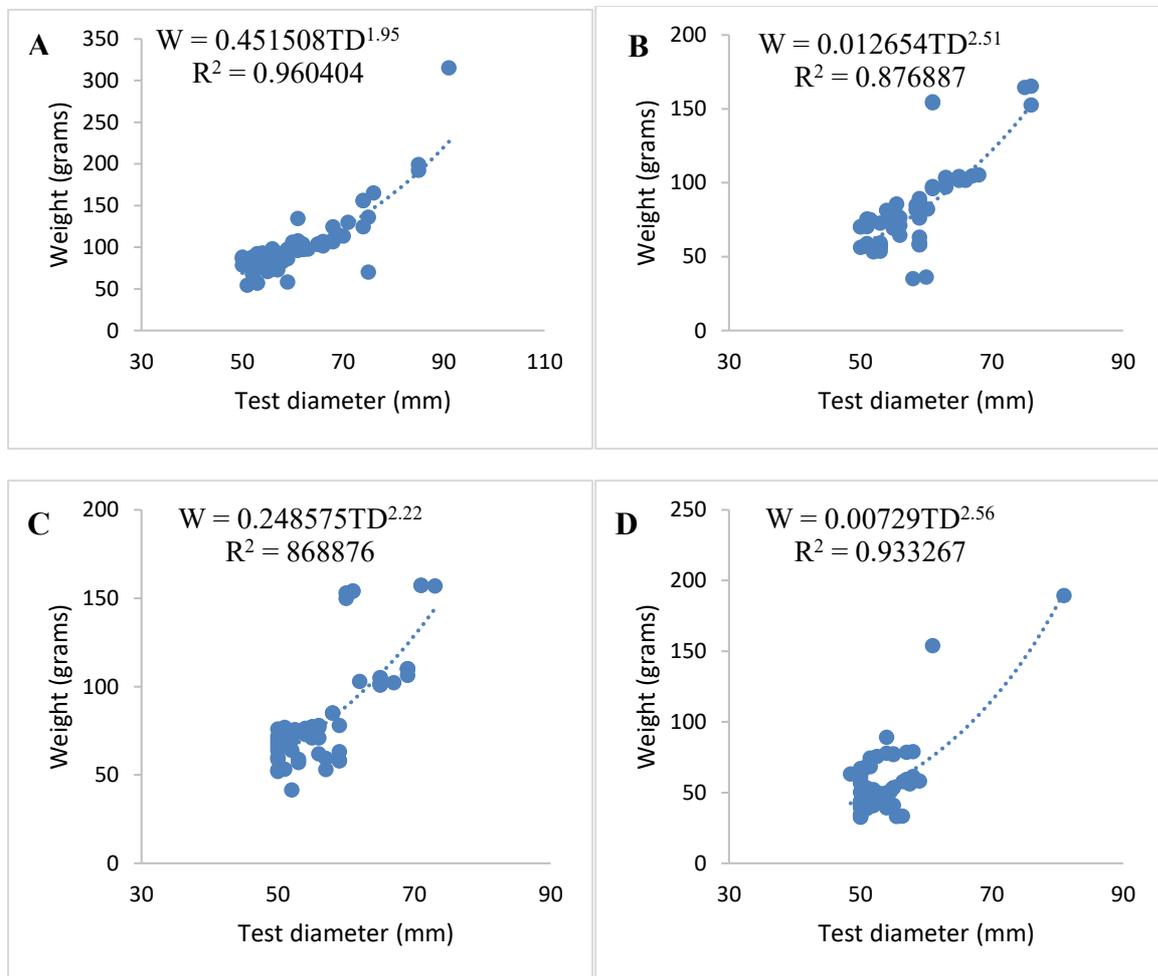
full moon while the lowest mean recorded was in Kitayo during the new moon.



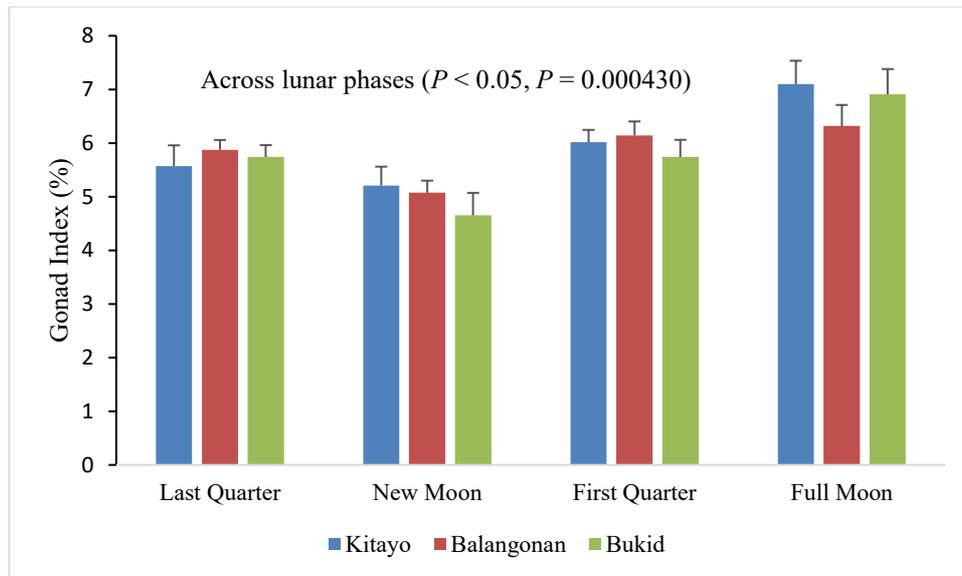
**Figure 4.** Size structure of *Tripneustes gratilla* from January to February 2024 in the three sampling sites of Jose Abad Santos, Davao Occidental Philippines: Kitayo (top), Balangonan (middle), and Bukid (bottom).



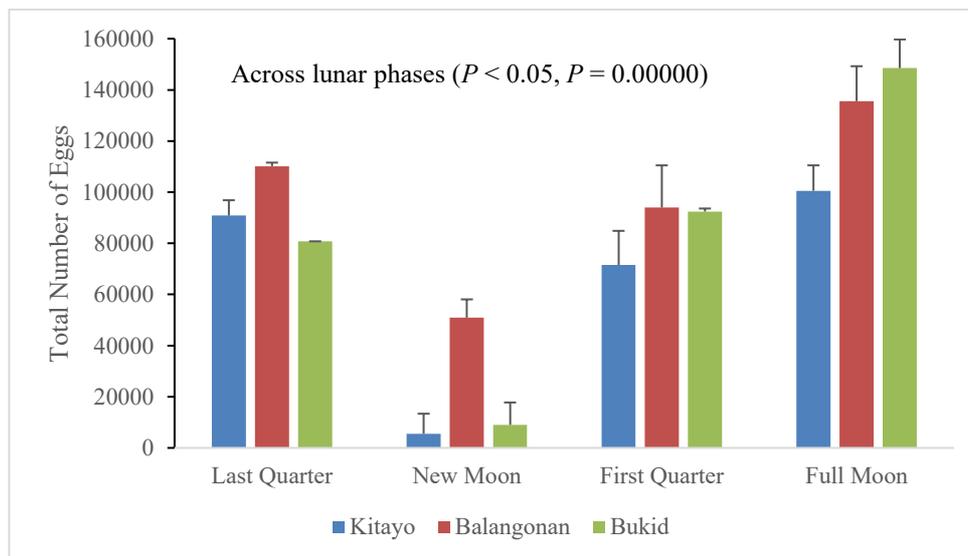
**Figure 5.** Test diameter-weight relationship of *Tripneustes gratilla* (n = 160) was collected from January to February 2024 in the three sampling sites of Jose Abad Santos, Davao Occidental, Philippines: Kitayo (A), Balangonan (B), and Bukid (C).



**Figure 6.** Test diameter-weight relationship of *Tripneustes gratilla* collected in each lunar phase from January to February 2024 in the three sampling sites of Jose Abad Santos, Davao Occidental, Philippines: Full moon (A), Last Quarter (B), New Moon (C), and First Quarter (D).



**Figure 7.** Gonad index of *Tripneustes gratilla* from four lunar phases in three sampling sites of Jose Abad Santos, Davao Occidental, Philippines. Vertical bars are the standard deviation.



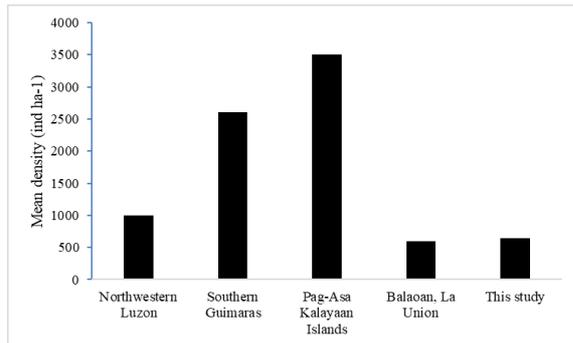
**Figure 8.** Fecundity of *Tripneustes gratilla* from four lunar phases in three sampling sites of Jose Abad Santos, Davao Occidental, Philippines. Vertical bars are the standard deviation.

## DISCUSSION

A total of 160 individuals of *T. gratilla* were recorded with an estimated mean density of  $645.83 \pm 171.50$  ind  $ha^{-1}$  in the three study sites of Jose Abad Santos, Davao Occidental, Philippines from January to February 2024. This result is comparably lower to estimates reported in the Philippines from northwestern Luzon at  $1,000$  ind  $ha^{-1}$  (Junio-Menez et al. 2008b), in southern Guimaras, Iloilo at  $2,600$  ind  $ha^{-1}$  (Regalado et al. 2010) and Pag-Asa Island, Kalayaan, Palawan at  $3,500$  ind  $ha^{-1}$  (Balisco 2015), but higher in Balaoan, La Union at  $600$  ind  $ha^{-1}$  (Prado et al. 2012) (Figure 9). The lower density observed,

compared to other localities, may be related to the level of exploitation in the area. In locations with limited accessibility and stricter enforcement, sea urchin densities tend to be higher. For example, Balisco (2015) reported high sea urchin density on Pag-asa Island, a relatively inaccessible area with only a few resident families and assigned military personnel, where the degree of exploitation is lower compared to more accessible areas. The significant difference in density between January and February could be attributed to the fishing pressure done by gleaners. Based on the actual observation, only a few gleaners captured sea urchins due to the low demand and consistently bad weather in the sites during January. It

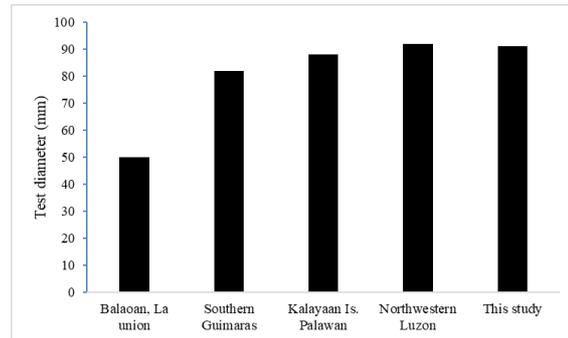
is possible that by the time the field assessments were done in February, these gleaners may have already collected most of the *T. gratilla* such that during our samplings only a few individuals were left. The current data may point out that *T. gratilla* collection was quite common and uncontrolled in the three sampling sites. The negative pressure inflicted by this activity may have resulted in fewer individuals observed. However, a year-round study should be made including the catch assessment to understand better the changes in the density of *T. gratilla* in the area.



**Figure 9.** Population density of *Tripneustes gratilla* from different localities in the Philippines.

The individual of *T. gratilla* is considered recruit (<40 mm TD), pre-adults (40 – 60 mm TD), and adults (>60 mm TD) based on the reports of Bangi et al. (2013), and Prado et al. (2012). The largest and heaviest individual recorded during the belt transect survey was found in Balangonan, with a TD of 85 mm and a weight of 199.56 grams. However, individuals collected for GI and fecundity measurements reached a TD of 91 mm and weighed up to 315.6 grams. The largest individual recorded was comparably larger than those in Balaan, La Union at 50 mm TD (Prado et al. 2012), and southern Guimaras in Iloilo at 82 TD (Regalado et al. 2010), in Pag-Asa Island, Kalayaan, Palawan at 88 mm TD (Balisco 2015) but smaller than in northwestern Luzon at 92 mm TD (Bangi et al. 2013) (Figure 10). The size structure of *T. gratilla* individuals recorded during the belt transect survey was generally dominated by adult size classes of 61 – 65 mm TD and only very few recruit individuals with a TD of 33 – 39 mm were observed (Figure 9). The low number of recruits recorded may be attributed to sampling limitations caused by the cryptic nature of juveniles hiding in rock crevices, algae, and seagrass leaves, which makes observation difficult (Balisco 2015). However, it is very alarming to note that all gleaners capture *T. gratilla* at all sizes regardless if it is below 40 mm TD in the three sampling sites. This nonselective harvesting practice may lead to the collapse of sea urchin fishery, if continuously unregulated (Ungson 2004). The prohibition of harvesting small-sized sea urchins in the area and

establishing a size catch limit (>60 mm TD) (Junio-Meñez et al. 2008b) is highly recommended for the sustainable management of this important resource.



**Figure 10.** Largest size of *Tripneustes gratilla* recorded from different localities in the Philippines.

The relationship of the TD and weight in three sampling sites exhibited a negative allometric growth ( $b < 3$ ) with a high positive correlation. Similar findings were also observed in the studies of Regalado et al. (2010) and Balisco (2015) which indicated that *T. gratilla* in their respective sampling areas displayed negative allometric growth. Negative allometry describes a condition in which a sea urchin's TD increases faster than its body weight, meaning weight does not increase proportionally with TD as it would if both grew at the same rate (Elmasry et al. 2023; Rahman et al. 2013; Suryanti et al. 2024). Factors such as diet, reproductive behavior, and the number of samples of *T. gratilla* would affect their growth (Balisco 2015). In other literature, this negative allometry reflects poor conditions for growth such as low food availability, increased fishing pressure, high predation, and pollution (Siddique and Ayub 2016; Mon et al. 2020; Elmasry et al. 2023). The seagrass in Kitayo is mostly dominated by *Syringodium isoetifolium* (Ascherson Dandy 1939) and *Cymodocea rotundata* (Ascherson and Schweinfurth 1870) while in Balangonan is dominated by *Thalassia hemprichii* (Ascherson 1871) and *S. isoetifolium*. On the other hand, seagrass beds in Bukid are dominated by *T. hemprichii* with patches of *Sargassum* species. The food preferences of sea urchins are largely influenced by the availability of specific types of food dominant in their surroundings (Kasim 2009). Seagrasses and seaweeds, commonly abundant at study sites, serve as primary food sources for *T. gratilla*; however, variations in available food items may contribute to differences in growth rates. According to Junio-Meñez et al. (2008a), *T. gratilla* exhibited higher TD growth rates when fed *Sargassum* spp. Regalado et al. (2010) further suggested that seasonality and the type of available food significantly impact growth rates and maximum attainable size in sea urchins. While this study did not investigate the availability or types of food in the study sites, further research is necessary to determine if these factors affect the reproductive biology of *T. gratilla* in these areas.

The peak of the spawning phase is determined by the variance in the gonad index (Vladimir et al. 2004). This is by far the first study on the spawning aspects such as gonad index and fecundity of *T. gratilla*, done in Davao Occidental. The highest gonad index during the full moon compared to other lunar phases indicates that the spawning period peaks during the full moon and then decreases towards the new moon. Sea urchins reach their greatest GI value near the time of spawning and then it starts to decline until spawning is over (Nasrullah et al. 2018). Other sea urchin species also exhibit spawning peaks during the full moon. For example, *Diadema savignyi* (Audouin 1809), *Echinothrix diadema* (Linnaeus 1758), *Diadema setosum* (Leske 1778), and *Strongylocentrotus intermedius* (Agassiz 1864) have been observed to spawn during the full moon (Zhadan et al. 2018). Additionally, *Lytechinus variegatus* (Lamarck 1816) in southern Brazil shows spawning associated with both the full moon and new moon phases (Aparecida et al. 2015). In contrast, other studies have documented spawning during the new moon. For instance, *T. gratilla* on the Kenyan coast reaches peak spawning around the new moon, which then declines to a minimum near the third quarter (Muthiga 2005). This pattern was similarly observed by Juinio-Meñez et al. (2008a) and Johnson and Ranelletti (2017). Another species, *Echinothrix calamaris* (Pallas 1774) also exhibits spawning during the new moon (Coppard and Campbell 2005). The result of fecundity showed that *T. gratilla* at 54 mm TD or more was already sexually mature. The total number of eggs in *T. gratilla* is relatively highest during the full moon while the lowest fecundity was found during the new moon. The variation in the observable total number of eggs may be attributed to the sizes of the sample collected. Gonad production increases when its size reaches 70 mm and has shown no observed decreasing pattern even when its size reaches 100 mm (Toha et al. 2017). According to Basch and Tegner (2007), larger sea urchins can enhance their reproductive output, which results in increased volume for the growth of gonadal tissue and an increase in fecundity. Thus, the gonads are in their greatest bulk ready for release. The lunar phase may play a role in the timing of the spawning of *T. gratilla* (Muthiga 2005; Juinio-Meñez et al. 2008a). However, the timing of spawning may not only be attributed to lunar phases but other factors should also be considered on the cues as to when the sea urchin spawns such as temperature, photoperiod, and food availability (Brockington et al. 2001; Perez et al. 2010; Vaitilington et al. 2005). It is recommended to regulate collections of *T. gratilla* in the sites, particularly during the full moon phase, as this may coincide with the peak of spawning. This will help ensure that a greater number of *T. gratilla* individuals can release gametes (eggs and sperm) and maximize the population's recruitment potential.

While the findings of this study provide valuable insights, they are not conclusive regarding the population structure and lunar periodicity of spawning. Continuous long-term monitoring of sea urchin populations is essential for a comprehensive understanding of the population dynamics of *T. gratilla* at these sites. Short-term data may not fully capture the variability in density and reproductive biology, whereas long-term monitoring can offer a more accurate picture of the status of *T. gratilla* in these sites. It is also crucial to consider other factors, such as seasonal habitat changes, food preferences and availability, and additional influences on spawning timing.

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## ETHICAL CONSIDERATIONS

A gratuitous permit no 01012-102524 from the Bureau of Fisheries and Aquatic Resources (BFAR) was secured in the collection of samples.

## DECLARATION OF COMPETING INTEREST

No potential conflict of interest was reported by the authors

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## REFERENCES

- AbouElmaaty TE, Yassien MH, Hanafy MH, Gobashy AA, Ahmed MI and Baeta M. 2023. Reproductive aspects of the sea urchin *Tripneustes gratilla* from the Red Sea, Egypt. *Egyptian Journal of Aquatic Biology and Fisheries*, 27(2): 475–494.
- Andrew NL, Agatsuma Y, Ballesteros E, Bazhin AG, Creaser EP, Barnes DKA, Botsford LW, Bradbury A, Campbell A, Dixon JD et al. 2002. Status and management of world sea urchin fisheries. *Oceanography and Marine Biology Annual Review*, 40: 343–425. <https://doi.org/10.1201/9780203180594.ch7>.

- Aparecida Y, Garcia T, Carlos and Borzone A. 2015. The reproductive cycle of the sea urchin *Lytechinus variegatus* (Echinodermata: Echinoidea) in southern Brazil. *Revista de Biologia Tropical*, 63: 243–250. <http://dx.doi.org/10.15517/rbt.v63i2.23158>
- Balisco RAT. 2015. Notes on the gracious sea urchin *Tripneustes gratilla* (Echinodermata: Echinoidea) in Pag-asa Island, Kalayaan, Palawan, Philippines. *The Palawan Scientist*, 7: 28–36. <https://doi.org/10.69721/TPS.J.2015.7.1.03>
- Bangi H, Cuaresma G, Quimpo GFT, Bollozos CJ and Roa-Quiaoit HA. 2013. Preliminary observations on the recruitment variability in *Tripneustes gratilla* (echinodermata: echinoidea) in three selected biogeographic regions in the Philippines. *Galaxea, Journal of Coral Reef Studies*, 15(Supplement): 107–114. <https://doi.org/10.3755/galaxea.15.107>
- Basch LV and Tegner MJ. 2007. Reproductive responses of purple sea urchin (*Strongylocentrotus purpuratus*) populations to environmental conditions across a coastal depth gradient. *Bulletin of Marine Science*, 81(2): 255–282.
- Bin Nurhasan R, Akmar S, Binti K and Rahim A. 2011. Sea urchin fishery practices in Sabah. *International Fisheries Symposium*, 5-9.
- Brookington S, Clarke A and Chapman ALG. 2001. Seasonality of feeding and nutritional status during the austral winter in the Antarctic sea urchin *Stereochinus neumayeri*. *Marine Biology*, 139: 127–138. <https://doi.org/10.1007/s002270100561>
- Cailliet GM, Love MS and Ebeling AW. 1986. *Fishes: A field and laboratory manual on their structure, identification and natural history*. Wadsworth Publishing Company. Belmont, California. 194pp.
- Coppard S and Campbell A. 2005. Distribution and abundance of regular sea urchins on two coral reefs in Fiji. *Micronesica*, 37(2): 249-269.
- Dincer T and Cakli S. 2007. Chemical composition and biometrical measurements of the Turkish sea urchin (*Paracentrotus lividus*, Lamarck, 1816). *Critical Reviews in Food Science and Nutrition*, 47(1): 21–26. <https://doi.org/10.1080/10408390600550265>
- Eklöf JS, de la Torre-Castro M, Gullström M, Uku J, Muthiga N, Lyimo T and Bandeira SO. 2008. Sea urchin overgrazing of seagrasses: A review of current knowledge on causes, consequences, and management. *Estuarine, Coastal and Shelf Science*, 79(4): 569-580. <https://doi.org/10.1016/j.ecss.2008.05.005>
- Elmasry E, Abdelrazek FA and El-Sayed AM. 2023. Growth pattern and population status of the sea urchin *Paracentrotus lividus* (Lamarck, 1816) on the Mediterranean coast of Egypt. *Egyptian Journal of Aquatic Research*, 49(3): 409-416. <https://doi.org/10.1016/j.ejar.2023.07.001>
- Furkon, Nessa N, Ambo-Rappe R, Cullen-Unsworth LC and Unsworth RKF. 2020. Social-ecological drivers and dynamics of seagrass gleaner fisheries. *Ambio*, 49(7): 1271–1281. <https://doi.org/10.1007/s13280-019-01267-x>
- Furkon, Nessa MN and Ambo-Rappe R. 2019. Invertebrate Gleaning: Forgotten Fisheries. In *IOP Conference Series: Earth and Environmental Science*, 253: 012029. <https://doi.org/10.1088/1755-1315/253/1/012029>
- Gaudette J, Wahle RA and Himmelman JH. 2006. Spawning events in small and large populations of the green sea urchin *Strongylocentrotus droebachiensis* as recorded using fertilization assays. *Limnology and Oceanography*, 51(3): 1485–1496. <https://doi.org/10.4319/lo.2006.51.3.1485>
- Holden MJ and Raitt DFS. 1974. *Manual of Fisheries Science Part 2. Methods of resource investigation and their application*. FAO Fisheries Technical Paper no. 115. Food and Agriculture Organization, Rome. 255pp.
- James P and Siikavuopio S. 2012. A guide to the sea urchin reproductive cycle and staging sea urchin gonad samples. Norwegian Institute of Food, Fishery and Aquaculture. 20pp.
- Johnson DW and Ranalletti ME. 2017. Natural spawning of a Hawaiian sea urchin, *Tripneustes gratilla*. *Invertebrate Biology*, 136(1): 31–36. <https://doi.org/10.1111/ivb.12158>
- Juinio-Meñez MA, Macawaris ND and Bangi HG. 1998. Community-based sea urchin (*Tripneustes gratilla*) grow-out culture as a resource management tool. *Canadian Special Publication of Fisheries and Aquatic Sciences*, 125: 393-399.
- Juinio-Meñez MA, Bangi HG and Malay MC. 2008a. Effect of type of feed, stocking density, and grow-out site on gonad index, growth and survivorship of cultured sea urchin (*Tripneustes gratilla*). *Philippine Agricultural Scientist*, 91(4): 439-449.
- Juinio-Meñez MA, Bangi HG, Malay MC and Pastor D. 2008b. Enhancing the recovery of depleted *Tripneustes gratilla* stocks through grow-out culture and restocking. *Reviews in Fisheries Science*, 16(1–3): 35–43. <https://doi.org/10.1080/10641260701678116>
- Kasim M. 2009. Grazing activity of the sea urchin *Tripneustes gratilla* in tropical seagrass beds of Buton Island. *Journal of Coastal Development*, 13(1): 19–27.
- Lawrence JM, Lawrence AL and Holland ND. 1965. Annual cycle in the size of the gut of the purple sea urchin, *Strongylocentrotus purpuratus* (Stimpson). *Nature*, 205: 1238-1239. <https://doi.org/10.1038/2051238a0>
- Lawrence JM. 1975. On the relationships between marine plants and sea urchins. *Oceanography and Marine Biology Annual Review*, 13: 213–286.
- Lawrence JM and Agatsuma Y. 2007. Chapter 24 Ecology of *Tripneustes*. In: Lawrence JM (ed). *Developments in Aquaculture and Fisheries Science*, 37: 499–520. [https://doi.org/10.1016/S0167-9309\(07\)80088-3](https://doi.org/10.1016/S0167-9309(07)80088-3)
- Lawrence JM and Agatsuma Y. 2013. Chapter 32 - *Tripneustes*. In: Lawrence JM (ed). *Developments in Aquaculture and Fisheries Science*, 38: 491-507. <https://doi.org/10.1016/B978-0-12-396491-5.00032-0>
- Lutfiyani E, Pringgenies D and Endrawati H. 2021. Studi histologi tingkat kematangan gonad jantan and betina bulu babi *Diadema setosum* di Pulau Panjang Jepara. *Majalah Ilmiah Biologi BIOSFERA: A Scientific Journal*, 38: 29-33. [Indonesian].
- Küçükdermenci A, Lök A, Acarlı S, Yiğitkurt S, Kırtık A and Kurtay E. 2017. Quantitative and qualitative observations of European sea urchin (*Paracentrotus lividus*, Lamarck 1816) gonad in Cesme Bay, Izmir, Turkey. *Ege Journal of Fisheries and Aquatic Sciences*, 34(3): 277–280. <https://doi.org/10.12714/egejfas.2017.34.3.05>
- Mon EE, Zin PP and Dwe KL. 2020. Length-weight relationship, condition factor, and sex ratio of tade mullet (*Liza tade* Forsskal, 1775) from Mawlamyine, Mon state, Myanmar. *Journal of Aquaculture and Marine Biology*, 9(4): 107-112. <https://doi.org/10.15406/jamb.2020.09.00285>
- Muthiga NA. 2005. Testing for the effects of seasonal and lunar periodicity on the reproduction of the edible sea urchin *Tripneustes gratilla* (L) in Kenyan coral reef lagoons. *Hydrobiologia*, 549(1): 57–64. <https://doi.org/10.1007/s10750-005-4757-2>
- Nane L and Paramata AR. 2020. Impact of overfishing on density and test-diameter size of the sea urchin *Tripneustes gratilla* at Wakatobi archipelago, South-Eastern Sulawesi, Indonesia. *Ilmu Kelautan: Indonesian Journal of Marine Sciences*, 25(2): 53–56. <https://doi.org/10.14710/ik.ijms.25.2.53-56>
- Nasrullah R, Sari W and Mellisa S. 2018. Gonadal maturity of sea urchins (*Tripneustes gratilla*) at Ahmad Rhangmayang beach sub-district of Mesjid Raya Aceh Besar District. *Unsyiah Maritime and Fisheries Student Scientific Journal*, 3(1): 23-32.
- Pauly D. 1984. *Fish Population Dynamics in Tropical Waters: A Manual for Use with Programmable Calculators*. International Center for Living Aquatic Resources Management, Manila, Philippines. 325pp.
- Perez AF, Boy C, Morriconi E and Calvo J. 2010. Reproductive cycle and reproductive output of the sea urchin *Loxechinus albus* (Echinodermata: Echinoidea) from Beagle Channel, Tierra del Fuego, Argentina. *Polar Biology*, 33(3): 271–280. <https://doi.org/10.1007/s00300-009-0702-6>
- Prado VV, Galvez GN and Rivera R. 2012. Size structure and density of sea urchin *Tripneustes gratilla* along Balaoan

- waters, La Union, Philippines. International Scientific Research Journal, 4(3): 197-203.
- Pulz O and Gross W. 2004. Valuable products from biotechnology of microalgae. Applied Microbiology and Biotechnology, 65(6): 635-648. <https://doi.org/10.1007/s00253-004-1647-x>
- Rahman MA, Yusoff FM, Arshad A, Amin SMN and Shamsudin MN. 2013. Population characteristics and fecundity estimates of short-spined white sea urchin, *Salmacis sphaeroides* (Linnaeus, 1758) from the coastal waters of Johor, Malaysia. Asian Journal of Animal and Veterinary Advances, 8(2): 301-308. <https://doi.org/10.3923/ajava.2013.301.308>
- Rahman MA, Ismail M, Parvez MS, Asadujjaman M, Ashik AA and Molla MHR. 2022. Echinoderm fisheries: their culture, conservation, bioactive compounds and therapeutic applications. Journal of Biological Studies, 5(3): 413-443. <https://doi.org/10.62400/jbs.v5i3.7053>
- Regalado JM, Campos WL and Santillan AS. 2010. Population biology of *Tripneustes gratilla* (Linnaeus) (Echinodermata) in seagrass beds of southern Guimaras, Philippines. Science Diliman, 22(2): 41-49.
- Siddique S and Ayub Z. 2016. Length-weight relationships and condition factor of the sea urchin *Echinometra matthaei* (Echinoidea: Echinodermata) on Buleji rocky shore of Karachi, Pakistan. Pakistan Journal of Marine Science, 25(1&2): 161-171.
- Sonu SC. 2017. Sea urchin supply, demand, and market of Japan. US Department of Commerce. National Oceanic and Atmospheric Administration National Marine Fisheries Service. 44pp. <https://www.st.nmfs.noaa.gov/Assets/commercial/market-news/00-Sea-urchin-Reports-Final-Nov-8-2018-2019.pdf>. Accessed on 28 September 2024.
- Suryanti S, Anggoro SA, In C and Widyorini N. 2024. Osmoregulation pattern, condition factor, and gonadal maturity level of sea urchins in the various ecosystems of Panjang Island, Jepara, Indonesia. Biodiversitas, 25(2): 502-509. <https://doi.org/10.13057/biodiv/d250208>
- Tamti H, Rappe RA, Andy Omar SB and Budimawan. 2021. Preliminary assessment of *Tripneustes gratilla* populations in seagrass beds of the Spermonde Archipelago, South Sulawesi, Indonesia. In IOP Conference Series: Earth and Environmental Science 763: 012008. <https://doi.org/10.1088/1755-1315/763/1/012008>
- Toha AHA, Sumitro SB, Hakim I., Widodo N, Binur R, Suhaemi S and Anggoro AW. 2017. Review: Biology of the commercially used sea urchin *Tripneustes gratilla* (Linnaeus, 1758) (Echinoidea: Echinodermata). Ocean Life, 1(1): 1-10. <https://doi.org/10.13057/oceanlife/o010101>
- Tuason AY and Gomez ED. 1979. The reproductive biology of *Tripneustes gratilla* Linnaeus (Echinoidea: Echinodermata) with some notes on *Diadema setosum* Leske. In: Proceedings of the International Symposium on Marine Biogeography and Evolution in the Southern Hemisphere, 2: 707-716.
- Ungson J. 2004. Stock enhancement of sea urchin (*Tripneustes gratilla*) in Northwestern Luzon, Philippines. A Management Strategy. IIFET 2004 Japan Proceedings. Tokyo. 10pp.
- Vařtilingon D, Rasolofonirina R and Jangoux M. 2005. Reproductive cycle of edible echinoderms from the southwestern Indian ocean I. *Tripneustes gratilla* L. (Echinoidea, Echinodermata). Western Indian Ocean Journal of Marine Science, 4: 47-60. <https://doi.org/10.4314/wiojms.v4i1.28473>
- Vladimir PG, Masuda Y and Matsuoka T. 2004. Age, growth, and reproduction of yellowbelly Threadfin Bream *Nemiterus bathybiu* in Kagoshima Bay, Southern Japan. Fisheries Science, 70(3): 497-506. <https://doi.org/10.1111/j.1444-2906.2004.00831.x>
- Wallner-Hahn S, de la Torre-Castro M, Eklöf JS, Gullström M, Muthiga NA and Uku J. 2015. Cascade effects and sea-urchin overgrazing: An analysis of drivers behind the exploitation of sea urchin predators for management improvement. Ocean and Coastal Management, 107: 16-27. <https://doi.org/10.1016/j.ocecoaman.2015.01.010>
- Zhadan PM, Vaschenko MA and Ryazanov SD. 2018. Assessing the effect of environmental factors on the spawning activity of the sea urchin *Strongylocentrotus intermedius* through video recording observations. Marine Ecology Progress Series, 588: 101-119. <https://doi.org/10.3354/meps12436>

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# Welcoming or banning ChatGPT in Higher Education: Insights from Indonesia, Malaysia, and the Philippines

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## ABSTRACT

The advent of artificial intelligence generative tools, specifically ChatGPT, has revolutionized the academic community, highlighting its positive contribution to both students and teachers. However, teachers have raised a lot of concerns, including ethical issues and academic integrity. This paper aims to explore the views and recommendations of professors on the use of ChatGPT by students in higher education. Using phenomenography, 22 professors in higher education from Malaysia (6), Indonesia (5), and the Philippines (11) willingly participated in the Key Informant Interviews (KII). Results revealed that the professors' views were divided into negative impacts (technology dependence and ethical problems) and positive contributions (user's convenience and enhancement of writing content). Moreover, they recommended (1) training on the proper use of ChatGPT and (2) protocols for using ChatGPT. The views and recommendations of the professors showed the inevitability of ChatGPT's use in higher education. Hence, the need for policies and guidance is also foreseeable and should be in place to guide and deter any ethical problems.

**Keywords:** academic integrity, artificial intelligence, education, technology

## INTRODUCTION

Artificial intelligence (AI) is now getting widespread attention from different sectors, such as business, education, military, agriculture, and other fields or disciplines. While AI development quickly evolves worldwide, especially in first-world countries, the ASEAN region cannot be ignored. Singapore is at the forefront of technological advancement in AI, followed by Indonesia and Malaysia, while the Philippines and other member states are catching up

(Zhao 2024). Moreover, as to the expected value of AI development, based on the Kearney Analysis, Indonesia is pegged at USD 366 billion, Malaysia at USD 115 billion, and the Philippines at USD 92 billion (Putra 2024). However, these developments are also met with several challenges such as the lack of clear strategies and policies (Putra 2024). In education, there has been a clamor for safety nets to protect the integrity of the academe with the proliferation of generative AIs. For instance, several concerns were raised regarding the use of ChatGPT, such as reports from



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Indonesia (Arista et al. 2024; Margono et al. 2024; Mulawarman et al. 2024; Shidarta and Martinelli 2023), Malaysia (Annamalai 2024; Arista et al. 2024; Tang and Chaw 2023), the Philippines (Espartinez 2024; Giray and Aquino 2024; Mabuan 2024; Ventayen 2023), and other ASEAN countries. Unfortunately, little attention is given to addressing or mitigating these technologies' negative impact on the academe.

The impact of generative AI tools in education has captured the attention of school administrators, scientists, and scholars worldwide. Both professors and students are also intrigued by how these tools can significantly impact the learning process, especially writing requirements like research tasks. Indeed, several reports have provided a glimpse into the positive contributions of AI tools, such as enhanced experiential learning (Elbanna and Armstrong 2024; Park and Kim 2025; Rejeb et al. 2024; Salinas-Navarro et al. 2024) and improvement of writing content (Kenwright 2024; Rejeb et al. 2024), particularly research requirements (Huang and Tan 2023; Tang et al. 2023). Hence, ChatGPT has caught global attention, including in Southeast Asian countries, which are considered latecomers in adopting AI in general (Putra 2024; Zhao 2024). This tool is seen as beneficial to society, specifically in the academe, in improving students' learning experience and performance. However, with the use of these AI tools, like ChatGPT, many are worried about their negative effect on academic integrity (Currie et al. 2023; Gammoh 2024; Gill et al. 2024) and about questions of accuracy (Hasanein and Sobaih 2023; Suárez et al. 2024).

Though scholars have reported both the benefits and challenges of ChatGPT (Rejeb et al. 2024; Wise et al. 2024; Zeb et al. 2024), educators are worried about this tool's harmful effect on education. Since academic integrity is a crucial component of education (Chavez 2023), particularly in higher education, where students are expected to do research and publication, the impact of ChatGPT cannot be taken lightly. The use of ChatGPT, for instance, has elicited much controversy as it can lead to cheating (Bin-Nashwan et al. 2023; Zhang et al. 2024) and overdependence on the tool itself (Heung and Chiu 2025; Mogavi et al. 2024). In addition, ChatGPT cannot replace the development of critical thinking skills, which is the essence of student formation (Kirwan 2023). In short, the education sector must address many ethical issues (Eppler et al. 2024; Stahl and Eke 2024) before these AI tools can generate unproductive effects in the academic community.

Specific concerns about plagiarism in AI-generated outputs are also an issue in publication. Journals are setting mechanisms to prevent unacceptable outputs in the realm of publication (Park 2023; Wientroub and Hefti 2023). For example, in a systematic review of the top 300 academic journals on

AI authorship policies, Lund and Naheem (2024) found that more than half have policies for acknowledging AI use, which can be placed in the acknowledgment or methods section. However, these policies also prohibit the inclusion of AI in the authorship list. In other words, AI or ChatGPT is already scrutinized by editors and peer reviewers. At the same time, specific policies evolve regarding how AI can be avoided or maximized.

Despite the apprehensions of some educators about the negative impact of ChatGPT and the aforementioned positive contributions, there are more positive contributions of this tool in the academe, as reported by scholars, such as increased student engagement (Cotton et al. 2023; Heung and Chiu 2025), positive user experience (Abdaljaleel et al. 2024), additional instructional content (Gill et al. 2024), and enhancement of student learning (Kim and Adlof 2024). Nevertheless, since ChatGPT is still considered new, though its utilization has already gained momentum in the academe, it is necessary to continue the discussion and propose more tailored policies and guidelines (Bouriami et al. 2025). Hence, this paper aims to explore the views of professors in higher education regarding their students using ChatGPT. The findings contribute to the debate on the benefits, drawbacks, and ethical implications of ChatGPT in the academe, particularly in higher education.

### Research Questions

1. What are the views of professors on students in higher education using ChatGPT?
2. What are their recommendations on the use or banning of ChatGPT?

## METHODS

### Research Design

Phenomenography is a qualitative method that focuses on and describes conceptions (Svensson 1981). While the debate continues as to the distinction between Phenomenology and Phenomenography, Cibangu and Hepworth (2016) argued that the latter is an offspring of the former, although Marton (2004) disagreed on this premise. Cibangu and Hepworth (2016) identified three lines of work for Phenomenography, namely, naturalistic Phenomenography, hermeneutic Phenomenography, and phenomenological Phenomenography. This study subscribed to phenomenological Phenomenography, which is more concerned with the phenomenon's essence (Cibangu and Hepworth, 2016). This paper is about the experiences and views of the professors in higher education on students' use of ChatGPT that are being investigated. Phenomenography is the most fitting approach in describing the phenomenon of students using AI tools, particularly ChatGPT, in

colleges and universities. Additionally, Phenomenography looks at the varying experiences of the key informants (Yates et al. 2012), eventually finding a common explanation of the phenomenon. Aside from looking at the individual experiences and views of the professors from Malaysia, Indonesia, and the Philippines, this paper captured the phenomenon through common themes elicited from their responses. While variations were recognized, a common description of the phenomenon is given importance to explain the views of professors on students' use of ChatGPT.

### Study Informants and Sample

There were 22 professors in higher education, with details as shown in Table 1, distributed as follows: Malaysia (6), Indonesia (5), and the Philippines (11). The term professor is operationally defined in this study as those with the rank of either assistant professor, associate professor, or professor teaching in higher education, meaning colleges or universities. Due to the nature of the inquiry, the researchers used purposive sampling. The informants were considered

qualified based on the following criteria: (a) They must be teaching in higher education, either undergraduate or graduate level, or both levels; (b) They have been teaching in higher education for at least three years; (c) They are familiar with ChatGPT and its usage; and (d) They can speak and understand the English language. First, the selection of informants from higher education was to capture the phenomenon and the proliferation of AI tools, like ChatGPT, in higher education (Ansari et al. 2024; Wang et al. 2024) and how institutions and teachers react. In addition, research requirements are more rigid in higher education, especially at the graduate or post-graduate level. Second, the respondents must have been teaching in higher education for a good number of years to be credible to share their views on the phenomenon. Third, since the study focuses only on one AI tool, ChatGPT, they must have experienced or be familiar with it to explain well and share their experience with students using it. Lastly, speaking and understanding English were necessary to gain valid and smooth communication between the interviewee and interviewer.

**Table 1.** Profile of the key informants (KI). NA – not available; TE- Teaching experience in years; F – female, M – male.

Code	Age	TE (y)	Sex	Country	Discipline	Highest Position held
KI 1	75	49	F	Philippines	Education	School Vice President
KI 2	76	54	F	Philippines	Business	School Vice President
KI 3	45	15	M	Philippines	Mathematics	Graduate School Dean
KI 4	55	33	F	Philippines	Applied Linguistics	Research Coordinator
KI 5	45	25	F	Philippines	Early Childhood	Graduate School Dean
KI 6	51	25	F	Philippines	Education	College Dean
KI 7	59	28	F	Philippines	Education	College Dean
KI 8	68	11	F	Philippines	Education	School Vice President
KI 9	55	30	M	Philippines	Education	School Vice President
KI 10	47	NA	F	Philippines	Elementary Education	Dean
KI 11	NA	NA	F	Philippines	Agricultural Plant Science	Research Director
KI 12	62	34	M	Indonesia	Education	Associate Professor
KI 13	36	13	F	Indonesia	Economics	Laboratory Head
KI 14	NA	15	F	Indonesia	Literature	Academic Coordinator
KI 15	62	40	F	Indonesia	Educational Technology	Associate Professor
KI 16	60	36	F	Indonesia	International Relations	Senior Lecturer
KI 17	61	12	F	Malaysia	Environmental Education	Department Head
KI 18	52	31	F	Malaysia	Education	Course Coordinator
KI 19	58	33	F	Malaysia	Education	Chair of Education
KI 20	52	20	F	Malaysia	Education	Department Head
KI 21	44	20	F	Malaysia	Chinese Studies	Head Digital Education
KI 22	50	30	F	Malaysia	English Studies	Course Coordinator

### Data Collection

Data collection was done by the researchers simultaneously for their assigned countries from 12 January to 16 March 2024. Data were collected through Key Informant Interviews (KII) using an interview protocol validated by three experts. Interviews were conducted via teleconference or video call or face-to-face. Teleconferencing is acceptable,

given its practicality and technicality (Weller 2017). It is also pragmatic because the key informants came from Malaysia, Indonesia, and the Philippines and were more accessible through the use of technology. Additionally, video conferencing in qualitative research allows researchers access to transnational informants while ensuring safety and saving financial resources (Khan and MacEachen 2022). There were

instances when the researchers conducted interviews through chat and followed-up with video calls at the informant's convenience. Moreover, some face-to-face interviews were conducted in the Philippines and Malaysia. Each interview lasted 30 to 45 minutes, consistent with what was specified in the content of the invitation letter, although there were instances when a few interviews did not reach 30 minutes, particularly the teleconferences. English was the language used, and informants from the three countries were chosen based on their capability to speak and understand the language. Initial data were gathered from those who were interviewed first. After the analysis, the data appeared to be revolving around the same themes; hence, the collection of data was halted in March 2024. Intended additional interviews for Malaysians and Indonesians were not pursued as the number of informants had already reached the range of 5–25, as suggested by Creswell (1998).

### Analysis and Interpretation of Data

The researchers followed Colaizzi's (1978) seven steps to analyze qualitative data. First, each transcript of the interviews was read and re-read for the researchers to get an idea of the informants' views. Second, significant statements were extracted from the transcripts. Third, the meaning was formulated for each significant statement. Fourth, formulated meanings were grouped to create categories or clusters of themes. Then, these clusters of themes were analyzed and grouped according to emerging themes, which were the final themes. Fifth, using the emerging themes, an exhaustive description of the phenomenon was written, supported by selected actual responses from the informants. Sixth, a description of the fundamental structure of the phenomenon was created. Seventh, the findings were sent to the informants for validation to ensure the researchers' analysis was consistent with the description of the phenomenon under study. They were asked to sign the validation form. In addition, the researchers sent the paper to three qualitative experts for review before finalizing it. Furthermore, the paper was sent for review by experts who authored qualitative papers published in journals indexed by Scopus.

### Trustworthiness

The validity and reliability of qualitative research are established by ensuring trustworthiness. The researchers adhered to the four criteria, as Guba (1981) suggested, which are credibility, transferability, dependability, and confirmability. Credibility is achieved when researchers subscribe to, as mentioned by Moustakas (1990, p. 32), "reflecting, sifting, exploring, judging its relevance or meaning, and ultimately elucidating the themes and essences that comprehensively, distinctly, and accurately depict the experience." This was done following the method of Colaizzi (1978) while ensuring that the themes were

consistent with the phenomenon's description, not the researchers' interpretation. In addition, the informants validated the findings before the paper's final output through a member-checking or validation technique. Regarding transferability, the researchers provided a detailed account of the methodology applied in the study, including an explanation of video teleconferencing as a technique in data collection. Regarding dependability, the researchers kept an audit trail of the data and the method of analysis for easy reference to the changes made during the preparation of the paper, from transcription to the final output. As for confirmability, similar to dependability, the audit trail and other documents were preserved for future reference. Additionally, the discussion of the themes included actual responses with proper source coding to ensure confirmation and validity of the data and interpretation of results.

## RESULTS

### Views of Professors on Students Using ChatGPT

The views of the professors on using ChatGPT by higher education students were divided into two: its negative effects and positive contributions. For the negative effects, these were (1) technology dependence and (2) ethical problems. For the positive contributions, these were (1) user's convenience and (2) enhancement of writing content.

**Technology dependence.** The professors expressed disappointment about students using ChatGPT because the latter's outputs are from the technology, not their efforts. They were worried about the effect of ChatGPT on their students. They feared the students would be too reliant on ChatGPT. In a way, it would diminish their capacity to think critically. Also, ChatGPT could make students lazy as the information and analysis of ideas are freely and conveniently provided to them. However, a good number of the informants were positive about using this tool. They considered it very helpful for the students but were also wary of its negative impact. A thirty-six-year-old professor from Indonesia, with 13 years of teaching experience in higher education, commented on the positive contribution of ChatGPT but also cautioned about its negative impact, saying:

"This tool helps the students generate, correlate, and summarize ideas. It makes them work faster. In contrast, however, their high dependency on this tool blocks their creative thinking." (Key Informant 13 or KI 13, Transcript 22 or T22, Page 2 or P2, Lines 51-53 or L51-53)

In short, with ChatGPT, higher education students would be very dependent on technology rather than their intellectual capacity if not guided properly.

The real drawback is for them to be passive, exerting less effort in their academic endeavors, and knowing where to go when they need information. Hence, too much dependence on ChatGPT might erode the very nature of academic formation.

**Ethical problems.** Aside from their fear of students' excessive dependence on technology, all informants pointed out ethical problems in using ChatGPT. There are many debatable areas about using ChatGPT within the boundaries between right and wrong. There is no originality aspect because ChatGPT has already done the analysis and paraphrasing. A fifty-two-year-old professor from Malaysia, with 31 years of teaching experience, shared this observation:

"Students cut and paste assignment questions into ChatGPT, and I noticed their information is funny. I double-check and confirm that the info is wrong. Actually, I see, and I already know it's wrong." (KI 20, T17, P1, L23-25)

The informants were saying that the use of ChatGPT's generated outputs is still an act of plagiarism and, therefore, academic dishonesty. They argued about the tendency of students to copy and paste what ChatGPT provides. It is where ethical issues arise.

**User's convenience.** Though the professors identified two downsides of using ChatGPT, they also recognized the advantages when used properly. One of these is the user's convenience of using the tool. ChatGPT makes writing easier because the tool provides the best information. It also increases the refinement and presentation of ideas. Also, the informants pointed out ChatGPT as a good source of ideas. One of the informants from the Philippines, a graduate school dean with over 25 years of teaching experience in higher education, acknowledged using ChatGPT herself and described how convenient it is to use this AI tool. She said:

"For students and teachers, AI is there to make our lives more convenient. I use ChatGPT. So, it is okay with me if students use ChatGPT in thesis or dissertation writing. Again, that is, as long as the content of the AI-generated output has been counterchecked by the students. And when they submit their output, they should have a thorough understanding of what they are submitting." (KI 7, T7, P2, L53-56)

The professors accepted the huge contribution of this particular information-generating tool in scouring the internet for the best possible ideas on the topics chosen by the students. In short, ChatGPT provides easy access and convenience for users, not only students but also professors.

### **Enhancement of writing content.**

According to the professors, the other positive aspect of using ChatGPT was the enhancement of writing content. They recognized the enormous contribution of ChatGPT in searching for the appropriate information tailored to what the students are looking for. They also agreed that ChatGPT is an easy source of ideas on specific topics that add value to the intended content as conceptualized by the students. However, they suggested for students to make their own judgments. An environmental education expert from Malaysia with 13 years of teaching experience in higher education suggested:

"I think it is fine. Students can learn the content, but they need to discriminate against the use of the content. This is enabled only if they have widely read about the topic and they have both subject and knowledge content." (KI 17, T12, P1, L37-40)

Moreover, the informants opined that using ChatGPT could enhance students' understanding of certain issues or ideas. Referring to ChatGPT for additional information could help students understand the ideas they want to develop or pursue, such as conceptualizing a thesis or dissertation. Hence, they agreed that students' output would be enhanced if the latter used ChatGPT for reference.

### **Recommendations on the Use or Banning of ChatGPT**

Based on the informants' responses, two common themes emerged, which do not include a recommendation to ban ChatGPT. These were (1) training on the proper use of ChatGPT and (2) protocols on the use of ChatGPT.

#### **Training on the proper use of ChatGPT.**

ChatGPT is a new technology that received mixed reactions from various sectors, including professors in higher education. Nevertheless, the professors recommended training students and professors on using it. The training aims to help students maximize the tool's potential without necessarily crossing the bridge of ethical standards. In other words, the purpose of the training is to ensure ChatGPT is used properly. For example, a sixty-two-year-old professor from Indonesia with 34 years of teaching experience in higher education recommended:

"They should freely introduce to the students how to use ChatGPT to help them write their academic writing assignments and theses, but still teach them the theories of academic writing. This is because ChatGPT cannot always write systematically and appropriately with the theory of thesis writing." (KI 12, T13, P 2, L 73-77)

The training on the proper use of ChatGPT could help professors be vigilant and capacitate them to prevent plagiarism. On the part of the students, they will also be guided on the proper use and avoid ethical issues.

**Protocols on the use of ChatGPT.** Since ChatGPT is available and is used by higher education students, the informants recommended developing protocols. These protocols are guided by specific institutional or governmental policies at different levels. While ChatGPT can be allowed, more explicit guidelines and ethical standards should be in place. These guidelines require additional work for the professors, such as scrutinizing the submitted outputs, particularly the thesis and dissertation. Others highlighted mechanisms for values formation and ethical standards in higher education to guide students in using the technology. Some professors even suggested an acceptable threshold or percentage if checked by AI detection software. For example, a research director from the Philippines with 33 years of teaching experience in higher education suggested:

"Part of the protocols also demands additional work from the professors, such as meticulously examining the submitted outputs, like the thesis and dissertation. This can be done by setting a 'threshold' or extent to which AI-generated texts can be allowed, e.g. 5%? 10%?" (KI 4, T6, P2, L75-77)

Because ChatGPT is still new but has already gained much popularity and usage, the recommendations from the informants are very valid. Having clear protocols and guidelines is truly beneficial for both professors and students. A lot of the problems and ethical concerns would be addressed if each higher education institution has an established policy related to ChatGPT and other AI-generative tools.

## DISCUSSION

The study was conducted using phenomenography, which generates the views of professors on the use of ChatGPT by higher education students. The findings revealed the two sides of reality in using ChatGPT—its positive and negative contributions—with two themes generated, respectively. For the recommendations, two themes focused primarily on capacity building and the creation of clear guidelines.

One of the informants' worries is the students' tendency to be too reliant on ChatGPT. Understandably, professors predicted the effect of ChatGPT and other AI-generated tools based on their experience with the emergence of the internet, which provides an easy and convenient way to get

information. How much more can ChatGPT help or even write for the students without much hassle and thinking? This observation by the informants is not something new, as scholars pointed out that over-reliance on this kind of technology (Gao et al. 2024; Kiryakova and Angelova 2023) could affect the development of critical thinking (Farrokhnia et al. 2023; Fuchs 2023). Indeed, it is a serious matter in the academic world. One of the 21st-century skills is critical thinking. However, there is also a 21st-century tool that can affect this particular skill. Nevertheless, several reports argued the positive contribution of ChatGPT and other AI tools to trigger and entice critical thinking skills (Essel et al. 2024; Kiryakova and Angelova 2023; Xu et al. 2024).

ChatGPT elicited so much interest right after it was launched in 2022, but later on, educators were worried about its ethical implications (Casal and Kessler 2023; Ray 2023; Stahl and Eke 2024). The professors shared similar sentiments. Their concern is that if students just copy and paste what ChatGPT provides, the tool no longer elicits critical thinking (Bai et al. 2023; Yilmaz and Yilmaz 2023) and does not help students draw out their essential understanding of information. Surprisingly, ChatGPT can generate outputs that cannot be caught by plagiarism-checker software (Khalil and Er 2023). What more do faculty members need to do in evaluating the submitted outputs of students, knowing there are AI tools with the power to make it appear truly the work of a human being? However, there are measures to address the ethical issues of using ChatGPT and other AI-generative tools. Fortunately, some plagiarism detector software has also evolved to add features for flagging AI-generated outputs (Cingillioglu 2023; Gao et al. 2024). The challenge is crafting and implementing school policies regarding accepting or rejecting AI-generated outputs.

On the positive side, the professors pointed out that the user's convenience is one advantage or contribution of ChatGPT for the students. The tool provides students with much-needed assistance in obtaining and writing information more logically. However, more than what the tool can provide, users found its ease of use (Albayati 2024; Ma et al. 2024), which is an added advantage for those accessing the internet and the tool itself. No wonder why ChatGPT, developed by OpenAI, recorded over 100 million users in just two months after its launch (Huh et al. 2023). Other developers are expected to come up with similar, if not better, services or features (Matherly 2023) as advertising revenues have shown an exponential trend (Huh et al. 2023). Google launched its most capable model, Gemini. As developers continue to push the boundaries of creativity and innovation, users will feast on the merits of using AI-generative tools, particularly in the academe.

As expected, the informants recognized how ChatGPT can greatly help students' outputs. Based on

several studies, ChatGPT improves students' writing content and skills (Khalifa and Albadawy 2024; Khalifa and Ibrahim 2024; Rejeb et al. 2024). It allows them to explore supporting literature and ideas for their thesis statements that need further deepening—something that takes time and a lot of digging in the absence of ChatGPT and other related tools. However, scholars cautioned users to check the generated data meticulously because of the reported inaccuracy (Al-Mughairi and Bhaskar 2024; Elbanna and Armstrong 2024). Scholars also warned of reporting erroneous scientific findings due to overreliance on AI tools, which can surely affect the integrity of scientific inquiry and could even lead to possible harm to society. This is also true in the academic community. When researchers cite a fraudulent scientific output, the wrong information is perpetuated with possible serious consequences. Nevertheless, as emphasized by the informants, ChatGPT is there to help students improve their writing content. Still, it is the role of the professors to check and educate the former on the proper use of it.

As to the informants' recommendation, it is advisable that professors and students in higher education should undergo proper training on using ChatGPT and other tools. The best way to do this is to integrate it into the faculty development plan and even into the curriculum for the students. Several studies have reported that professors are integrating ChatGPT into the teaching-learning process to automate tasks and create a conducive learning environment (Elbanna and Armstrong 2024), and students find many benefits in using ChatGPT (Lo 2023; Ngo 2023). Hence, teaching students how to use this tool maximizes its benefits and contributions. However, professors should be knowledgeable enough to ensure they can offer proper guidance to the students.

Because of the ethical issues surrounding the use of AI tools like ChatGPT, the professors acknowledged the inevitability of crafting and implementing protocols for using ChatGPT. For instance, ChatGPT-generated texts cannot be entirely copied (Kim et al. 2023). Professors are worried about the impact of this tool on the development of students' writing competency. However, ChatGPT can be used for editing and refinement (Kim et al. 2023). In addition, journals are also putting measures in place to ensure they do not publish articles with texts generated solely by AI (Park 2023; Wientroub and Hefti 2023). Thus, with the popularity and extensive utilization of ChatGPT and other AI generative tools, there is a need for clearer policies, protocols, or guidelines, particularly in higher education, underlining the ethical considerations and maximizing its potential for both students and professors. Professors in higher education must ensure these tools are helpful and not a barrier to societal values (Butson and Spronken-Smith 2024).

The findings show how professors view ChatGPT in higher education, particularly regarding

students using it in their academic endeavors. However, the study was limited to the perspective of the professors, not from the students themselves, who are the actual users, although in many instances, professors also use it for varied purposes. The students' experiences would be of great interest for a more in-depth understanding of how these tools can be used and what specific policies and guidelines can be crafted. The paper only focused on three countries in the ASEAN region, which cannot speak in general for all member states. Also, the paper is limited in the number of expected informants to balance the views per country, who are also distributed across different disciplines in higher education. A more focused discipline or even department, like graduate school, would be more appropriate for a specific context.

The findings present both the negative and positive impacts of ChatGPT in higher education and the recommendations from the professors. On the positive side, ChatGPT gains the patronage of students and professors because it is user-friendly and accessible. It also contributes significantly to improving writing content, which is the very nature of developing this tool. However, ChatGPT can also lead students to over-reliance on this technology, affecting their capacity to develop critical thinking skills. Additionally, many ethical concerns have to be addressed. Consequently, there is a need for training among users, especially professors, as well as the crafting and implementing of policies and guidelines to preserve academic integrity and maximize the benefits of using this tool. Based on the findings, professors in higher education did not want to ban or reject ChatGPT but instead suggested ways to maximize its potential while protecting academic integrity. In short, ChatGPT, besides its popularity, has much to offer for professors and students. However, appropriate human intervention has to be in place, such as control facilitated by clear policies and guidelines.

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## ETHICAL CONSIDERATIONS

The researchers made sure all ethical concerns were addressed before, during, and after the conduct of the inquiry.

**Free and informed consent.** First and foremost, the researchers secured permission from the individual informants. All of them agreed to participate in the key informant interviews using teleconferencing

or face-to-face interviews. They were given an informed consent form before the interview, which they signed as evidence of their free choice and willingness to participate.

**Confidentiality.** The researchers ensured that confidentiality was observed. None of the data, except for the final paper for publication, was divulged by any means of communication. To ensure that their names and identities were protected, the researchers used codes to represent themselves when their actual responses were included in the presentation of results.

**Recruitment.** Using purposive sampling, each researcher assigned to a particular country was responsible for recruiting possible informants who qualified based on the criteria. The researchers made initial contact with informants. Those who showed a positive response were given formal letters of invitation.

**Data storage.** Each researcher was responsible for storing the data collected from the informants from a specific country. Each one was tasked with keeping the data secure and discarding it once the project was done. Similarly, the same process was followed for the collated data for analysis.

## DECLARATION OF COMPETING INTEREST

The authors declare that this study was conducted in the absence of any relationships that could be considered a conflict of interest.

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## REFERENCES

- Abdaljaleel M, Barakat M, Alsanafi M, Salim NA, Abazid H, Malaeb D, Mohammed AH, Hassan BAR, Wayyes AM, Farhan SS et al. 2024. A multinational study on the factors influencing university students' attitudes and usage of ChatGPT. *Scientific Reports*, 14: 1983. <https://doi.org/10.1038/s41598-024-52549-8>
- Albayati H. 2024. Investigating undergraduate students' perceptions and awareness of using ChatGPT as a regular assistance tool: A user acceptance perspective study. *Computers and Education: Artificial Intelligence*, 6: 100203. <https://doi.org/10.1016/j.caeai.2024.100203>
- Al-Mughairi H and Bhaskar P. 2024. Exploring the factors affecting the adoption AI techniques in higher education: insights from teachers' perspectives on ChatGPT. *Journal of Research in Innovative Teaching & Learning*. <https://doi.org/10.1108/JRIT-09-2023-0129>
- Annamalai N. 2024. Factors affecting English language high school teachers switching intention to ChatGPT: a Push-Pull-Mooring theory perspective. *Interactive Learning Environments*, 1-18. <https://doi.org/10.1080/10494820.2024.2371928>
- Ansari AN, Ahmad S and Bhutta SM. 2024. Mapping the global evidence around the use of ChatGPT in higher education: A systematic scoping review. *Education and Information Technologies*, 29(9): 11281-11321. <https://doi.org/10.1007/s10639-023-12223-4>
- Arista A, Shuib L and Ismail MA. 2023. An Overview chatGPT in Higher Education in Indonesia and Malaysia. In 2023 International Conference on Informatics, Multimedia, Cyber and Informations System (ICIMCIS), Jakarta Selatan, Indonesia, pp. 273-277. <https://doi.org/10.1109/ICIMCIS60089.2023.10349053>
- Bai L, Liu X and Su J. 2023. ChatGPT: The cognitive effects on learning and memory. *Brain-X*, 1(3): e30. <https://doi.org/10.1002/brx2.30>
- Bin-Nashwan SA, Sadallah M and Bouteraa M. 2023. Use of ChatGPT in academia: Academic integrity hangs in the balance. *Technology in Society*, 75: 102370. <https://doi.org/10.1016/j.techsoc.2023.102370>
- Bouriami A, Takhdat K, Barkatou S, Chiki H, Boussaa S and El Adib AR. 2025. Insights into nurse educators' use of ChatGPT in active teaching methods: A cross-sectional pilot study. *Educación Médica*, 26(2): 101006. <https://doi.org/10.1016/j.edumed.2024.101006>
- Butson R and Spronken-Smith R. 2024. AI and its implications for research in higher education: a critical dialogue. *Higher Education Research & Development*, 43(3): 563-577. <https://doi.org/10.1080/07294360.2023.2280200>
- Casal JE and Kessler M. 2023. Can linguists distinguish between ChatGPT/AI and human writing? A study of research ethics and academic publishing. *Research Methods in Applied Linguistics*, 2(3): 100068. <https://doi.org/10.1016/j.rmal.2023.100068>
- Chavez JV. 2023. Assessing online academic integrity and humanized teaching in Zamboanga Peninsula Polytechnic State University. *Journal of Multidisciplinary in Social Sciences*, 19(1): 9-17.
- Cibangu S and Hepworth M. 2016. The uses of phenomenology and Phenomenography: A critical review. *Library & Information Science Research*, 38(2): 148-160. <https://doi.org/10.1016/j.lisr.2016.05.001>
- Cingillioglu I. 2023. Detecting AI-generated essays: the ChatGPT challenge. *The International Journal of Information and Learning Technology*, 40(3): 259-268. <https://doi.org/10.1108/IJILT-03-2023-0043>
- Colaizzi PF. 1978. Psychological research as the phenomenologist views it. In: Valle R and King M (eds). *Existential phenomenological alternatives in psychology*. New York: Oxford University Press, pp. 48-71.
- Cotton DR, Cotton PA and Shipway JR. 2023. Chatting and cheating: Ensuring academic integrity in the era of ChatGPT. *Innovations in Education and Teaching International*, 61(2): 228-239. <https://doi.org/10.1080/14703297.2023.2190148>
- Creswell J. 1998. *Qualitative Inquiry and Research Design: Choosing Among Five Traditions* (2nd ed). Sage Publication, Inc, California, United States of America. 395pp.
- Currie GM. 2023. Academic integrity and artificial intelligence: is ChatGPT hype, hero or heresy?. *Seminars in Nuclear Medicine*, 53(5): 719-730. <https://doi.org/10.1053/j.semnuclmed.2023.04.008>
- Elbanna S and Armstrong L. 2024. Exploring the integration of ChatGPT in education: adapting for the future. *Management & Sustainability: An Arab Review*, 3(1): 16-29. <https://doi.org/10.1108/MSAR-03-2023-0016>
- Eppler M, Ganjavi C, Ramacciotti LS, Piazza P, Rodler S, Checcucci E, Rivas JG, Kowalewski KF, Belenchon IR, Puliatti S, et al. 2024. Awareness and use of ChatGPT and large language models: a prospective cross-sectional global survey in

- urology. *European Urology*, 85(2): 146-153. <https://doi.org/10.1016/j.eururo.2023.10.014>
- Espartinez AS. 2024. Exploring student and teacher perceptions of ChatGPT use in higher education: A Q-Methodology study. *Computers and Education: Artificial Intelligence*, 7: 100264. <https://doi.org/10.1016/j.caeai.2024.100264>
- Essel HB, Vlachopoulos D, Essuman AB and Amankwa JO. 2024. ChatGPT effects on cognitive skills of undergraduate students: Receiving instant responses from AI-based conversational large language models (LLMs). *Computers and Education: Artificial Intelligence*, 6: 100198. <https://doi.org/10.1016/j.caeai.2023.100198>
- Farrokhnia M, Banihashem SK, Noroozi O and Wals A. 2023. A SWOT analysis of ChatGPT: Implications for educational practice and research. *Innovations in Education and Teaching International*, 61(3): 460-474. <https://doi.org/10.1080/14703297.2023.2195846>
- Fuchs K. 2023. Exploring the opportunities and challenges of NLP models in higher education: is Chat GPT a blessing or a curse?. *Frontiers in Education*, 8: 1166682. <https://doi.org/10.3389/educ.2023.1166682>
- Gammoh LA. 2024. ChatGPT in academia: exploring university students' risks, misuses, and challenges in Jordan. *Journal of Further and Higher Education*, 48(6): 608-624. <https://doi.org/10.1080/0309877X.2024.2378298>
- Gao Y, Wang Q and Wang X. 2024. Exploring EFL university teachers' beliefs in integrating ChatGPT and other large language models in language education: a study in China. *Asia Pacific Journal of Education*, 44(1): 29-44. <https://doi.org/10.1080/02188791.2024.2305173>
- Gill SS, Xu M, Patros P, Wu H, Kaur R, Kaur K, Fuller S, Singh M, Arora P, Parlikad A, et al. 2024. Transformative effects of ChatGPT on modern education: Emerging Era of AI Chatbots. *Internet of Things and Cyber-Physical Systems*, 4: 19-23. <https://doi.org/10.1016/j.ioteps.2023.06.002>
- Giray L and Aquino R. 2024. Use and impact of ChatGPT on undergraduate engineering students: A case from the Philippines. *Internet Reference Services Quarterly*, 28(4): 453-462. <https://doi.org/10.1080/10875301.2024.2384028>
- Guba E. 1981. Criteria for assessing the trustworthiness of naturalistic inquiries. *Educational Communication and Technology Journal*, 29: 75-91. <https://doi.org/10.1007/BF02766777>
- Hasanein AM and Sobaih AEE. 2023. Drivers and Consequences of ChatGPT Use in Higher Education: Key Stakeholder Perspectives. *European Journal of Investigation in Health, Psychology and Education*, 13(11): 2599-2614. <https://doi.org/10.3390/ejihpe13110181>
- Heung YME and Chiu TK. 2025. How ChatGPT impacts student engagement from a systematic review and meta-analysis study. *Computers and Education: Artificial Intelligence*, 8: 100361. <https://doi.org/10.1016/j.caeai.2025.100361>
- Huh J, Nelson MR and Russell CA. 2023. ChatGPT, AI advertising, and advertising research and education. *Journal of Advertising*, 52(4): 477-482. <https://doi.org/10.1080/00913367.2023.2227013>
- Huang J and Tan M. 2023. The role of ChatGPT in scientific communication: writing better scientific review articles. *American Journal of Cancer Research*, 13(4), 1148-11545.
- Kenwright B. 2024. Is it the end of undergraduate dissertations?: Exploring the advantages and challenges of generative ai models in education. In: Hai-Jew S (ed). *Generative AI in teaching and learning*. IGI Global, pp. 46-65. <https://doi.org/10.4018/979-8-3693-0074-9.ch003>
- Khalifa M and Albadawy M. 2024. Using Artificial Intelligence in Academic Writing and Research: An Essential Productivity Tool. *Computer Methods and Programs in Biomedicine Update*, 8: 100145. <https://doi.org/10.1016/j.cmpbup.2024.100145>
- Khalifa AA and Ibrahim MA. 2024. Artificial intelligence (AI) and ChatGPT involvement in scientific and medical writing, a new concern for researchers. A scoping review. *Arab Gulf Journal of Scientific Research*, 42(4): 1770-1787. <https://doi.org/10.1108/AGJSR-09-2023-0423>
- Khalil M and Er E. 2023 June. Will ChatGPT G et You Caught? Rethinking of Plagiarism Detection. In: Zaphiris P and Ioannou A (eds). *Leaning and Collaboration Techniques. HCII 2023. Lecture Notes in Computer Science*, Springer Nature, Switzerland, pp. 475-487. [https://doi.org/10.1007/978-3-031-34411-4\\_32](https://doi.org/10.1007/978-3-031-34411-4_32)
- Khan TH and MacEachen E. 2022. An alternative method of interviewing: Critical reflections on videoconference interviews for qualitative data collection. *International Journal of Qualitative Methods*, 21: 1-12. <https://doi.org/10.1177/16094069221090063>
- Kim M and Adlof L. 2024. Adapting to the Future: ChatGPT as a Means for Supporting Constructivist Learning Environments. *TechTrends*, 68(1): 37-46. <https://doi.org/10.1007/s11528-023-00899-x>
- Kim JK, Chua M, Rickard M and Lorenzo A. 2023. ChatGPT and large language model (LLM) chatbots: The current state of acceptability and a proposal for guidelines on utilization in academic medicine. *Journal of Pediatric Urology*, 19(5): 598-604. <https://doi.org/10.1016/j.jpuro.2023.05.018>
- Kirwan A. 2023. ChatGPT and university teaching, learning and assessment: some initial reflections on teaching academic integrity in the age of Large Language Models. *Irish Educational Studies*, 43(4): 1389-1406. <https://doi.org/10.1080/03323315.2023.2284901>
- Kiryakova G and Angelova N. 2023. ChatGPT—A challenging tool for the university professors in their teaching practice. *Education Sciences*, 13(10): 1056. <https://doi.org/10.3390/educsci13101056>
- Lo CK. 2023. What is the impact of ChatGPT on education? A rapid review of the literature. *Education Sciences*, 13(4): 410. <https://doi.org/10.3390/educsci13040410>
- Lund BD and Naheem KT. 2024. Can ChatGPT be an author? A study of artificial intelligence authorship policies in top academic journals. *Learned Publishing*, 37(1): 13-21. <https://doi.org/10.1002/leap.1582>
- Ma J, Wang P, Li B, Wang T, Pang XS and Wang D. 2024. Exploring User Adoption of ChatGPT: A Technology Acceptance Model Perspective. *International Journal of Human-Computer Interaction*, 41(2): 1431-1445. <https://doi.org/10.1080/10447318.2024.2314358>
- Mabuan RA. 2024. ChatGPT and ELT: Exploring Teachers' Voices. *International Journal of Technology in Education*, 7(1): 128-153. <https://doi.org/10.46328/ijte.523>
- Margono H, Saud M and Falahat M. 2024. Virtual Tutor, Digital Natives and AI: Analyzing the impact of ChatGPT on academia in Indonesia. *Social Sciences & Humanities Open*, 10: 101069. <https://doi.org/10.1016/j.ssaho.2024.101069>
- Marton F. 2004. Phenomenography – A research approach to investigating different understandings of reality. *Journal of thought*, 21(3): 28-49.
- Matherly C. 2023. Reconsidering internationalization and global cooperation: An interview with Dr. Cheryl Matherly. *Journal of Higher Education Policy and Leadership Studies*, 4(2): 137-142. <https://dx.doi.org/10.61186/johepal.4.2.137>
- Mogavi RH, Deng C, Kim JJ, Zhou P, Kwon YD, Metwally AHS, Tlili A, Bassanelli S, Bucchiarone, Gujar S, et al. 2024. ChatGPT in education: A blessing or a curse? A qualitative study exploring early adopters' utilization and perceptions. *Computers in Human Behavior: Artificial Humans*, 2(1): 100027. <https://doi.org/10.1016/j.chbah.2023.100027>
- Moustakas CE. 1990. *Heuristic research: Design, methodology, and applications*. SAGE Publications, Inc. <https://doi.org/10.4135/9781412995641>
- Mulawarman M, Mahfud A and Nugraini ED. 2024. The Impact of ChatGPT on Character Transformation in Educational Settings in Indonesia: A Scoping Review. *International Conference on Innovation & Entrepreneurship in Computing, Engineering & Science Education (InvENT 2024)*. Atlantis Press, pp. 319-329. [https://doi.org/10.2991/978-94-6463-589-8\\_29](https://doi.org/10.2991/978-94-6463-589-8_29)
- Ngo TTA. 2023. The perception by university students of the use of ChatGPT in education. *International Journal of Emerging*

- Technologies in Learning, 18(17): 4-19. <https://doi.org/10.3991/ijet.v18i17.39019>
- Park A and Kim T. 2025. Code suggestions and explanations in programming learning: Use of ChatGPT and performance. The International Journal of Management Education, 23(2): 101119. <https://doi.org/10.1016/j.ijme.2024.101119>
- Park SH. 2023. Use of generative artificial intelligence, including large language models such as ChatGPT, in scientific publications: policies of KJR and prominent authorities. Korean Journal of Radiology, 24(8): 715-718. <https://doi.org/10.3348%2Fkjr.2023.0643>
- Putra BA. 2024. Governing AI in Southeast Asia: ASEAN's way forward. Frontiers in Artificial Intelligence, 7: 1411838. <https://doi.org/10.3389/frai.2024.1411838>
- Ray PP. 2023. ChatGPT: A comprehensive review on background, applications, key challenges, bias, ethics, limitations and future scope. Internet of Things and Cyber-Physical Systems, 3: 121-154. <https://doi.org/10.1016/j.iotcps.2023.04.003>
- Rejeb A, Rejeb K, Appolloni A, Treiblmaier H and Iranmanesh M. 2024. Exploring the impact of ChatGPT on education: A web mining and machine learning approach. The International Journal of Management Education, 22(1): 100932. <https://doi.org/10.1016/j.ijme.2024.100932>
- Salinas-Navarro DE, Vilalta-Perdomo E, Michel-Villarreal R and Montesinos L. 2024. Using Generative Artificial Intelligence Tools to Explain and Enhance Experiential Learning for Authentic Assessment. Education Sciences, 14(1): 83. <https://doi.org/10.3390/educsci14010083>
- Shidarta S and Martinelli I. 2023. Should Indonesia Block ChatGPT?. EDP Sciences, 426: 02046. <https://doi.org/10.1051/e3sconf/202342602046>
- Stahl BC and Eke D. 2024. The ethics of ChatGPT—Exploring the ethical issues of an emerging technology. International Journal of Information Management, 74: 102700. <https://doi.org/10.1016/j.ijinfomgt.2023.102700>
- Suárez A, Díaz-Flores García V, Algar J, Gómez Sánchez M, Llorente de Pedro M and Freire Y. 2024. Unveiling the ChatGPT phenomenon: Evaluating the consistency and accuracy of endodontic question answers. International endodontic journal, 57(1): 108-113. <https://doi.org/10.1111/iej.13985>
- Svensson L. 1997. Theoretical Foundations of Phenomenography. Higher Education Research & Development, 16(2): 159-171. <http://dx.doi.org/10.1080/0729436970160204>
- Tang CM and Chaw LY. 2023. What Have People Discussed about ChatGPT in Malaysian Education? A Qualitative Content Analysis of News Articles. European Conference on e-Learning, 22(1): 314-321. <https://doi.org/10.34190/ecel.22.1.1557>
- Tang A, Li KK, Kwok KO, Cao L, Luong S and Tam W. 2023. The importance of transparency: Declaring the use of generative artificial intelligence (AI) in academic writing. Journal of Nursing Scholarship, 56(2): 314-318. <https://doi.org/10.1111/jnu.12938>
- Ventayen RJM. 2023. OpenAI ChatGPT-Generated Results: Similarity Index of Artificial Intelligence-Based Contents. In: Ranganathan G, EL Alloui Y and Piramuthu S (eds). Soft Computing for Security Applications. Advances in Intelligent Systems and Computing, 1449: 215-226. [https://doi.org/10.1007/978-981-99-3608-3\\_15](https://doi.org/10.1007/978-981-99-3608-3_15)
- Wang H, Dang A, Wu Z and Mac S. 2024. Generative AI in Higher Education: Seeing ChatGPT Through Universities' Policies, Resources, and Guidelines. Computers and Education: Artificial Intelligence, 7: 100326. <https://doi.org/10.1016/j.caeai.2024.100326>
- Weller S. 2017. Using internet video calls in qualitative (longitudinal) interviews: Some implications for rapport. International Journal of Social Research Methodology, 20(6): 613-625. <https://doi.org/10.1080/13645579.2016.1269505>
- Wibowo TO. 2024. The Social Construction of Technology for the Use of CHATGPT in Indonesia. ETTISAL: Journal of Communication, 9(1): 73-89. <https://doi.org/10.21111/ejoc.v9i1.11479>
- Wientroub S and Hefti F. 2023. Introducing Journal of Children's Orthopaedics' ChatGPT and generative AI policy. Journal of Children's Orthopaedics, 17(4): 297-298. <https://doi.org/10.1177/18632521231191687>
- Wise B, Emerson L, Van Luyn A, Dyson B, Bjork C and Thomas SE. 2024. A scholarly dialogue: writing scholarship, authorship, academic integrity and the challenges of AI. Higher Education Research & Development, 43(3): 578-590. <https://doi.org/10.1080/07294360.2023.2280195>
- Xu X, Wang X, Zhang Y and Zheng R. 2024. Applying ChatGPT to tackle the side effects of personal learning environments from learner and learning perspective: An interview of experts in higher education. PLOS One, 19(1): e0295646. <https://doi.org/10.1371/journal.pone.0295646>
- Yates C, Partridge H and Bruce C. 2012. Exploring information experiences through Phenomenography. Library and Information Research, 36(112): 96-119. <https://doi.org/10.29173/lirg496>
- Yilmaz R and Yilmaz FGK. 2023. Augmented intelligence in programming learning: Examining student views on the use of ChatGPT for programming learning. Computers in Human Behavior: Artificial Humans, 1(2): 100005. <https://doi.org/10.1016/j.chbah.2023.100005>
- Zhang L, Amos C and Pentina I. 2024. Interplay of rationality and morality in using ChatGPT for academic misconduct. Behaviour & Information Technology, 44(3): 491-507. <https://doi.org/10.1080/0144929X.2024.2325023>
- Zhao X. 2024. Technological hedging and differentiated responses of Southeast Asian countries to US-China technological competition: a case study on artificial intelligence (AI). The Pacific Review, 1-32. <https://doi.org/10.1080/09512748.2024.2408010>
- Zeb A, Ullah R and Karim R. 2024. Exploring the role of ChatGPT in higher education: opportunities, challenges and ethical considerations. The International Journal of Information and Learning Technology, 41(1): 99-111. <https://doi.org/10.1108/IJILT-04-2023-0046>

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# Evaluation of antioxidant and hypoglycemic activities and metabolite profiles of the fractionated extract of the *Crescentia cujete* Linn. fruit

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## ABSTRACT

Diabetes mellitus (DM) is a prevalent disease that is the sixth leading cause of death in the Philippines. The fruit juice of *Crescentia cujete* Linn., or “miracle fruit”, is utilized by locals from Agusan del Sur, Zamboanga del Sur, and Zamboanga Sibugay in the Philippines as an anti-diabetic and antioxidant treatment alternative or additive due to the high costs of their current prescriptions. Phytochemical screening of the fruit extract indicated the presence of alkaloids and reducing sugars with trace amounts of saponins, volatile oil, tannins, and polyphenols. Hypoglycemic and antioxidant assays were utilized to determine the potential activity. Alpha-glucosidase inhibition at 10 ppm of the fruit exhibited low enzyme inhibition activity. Methanol/H<sub>2</sub>O solvent fraction showed notable antioxidant activity in the 2,2-Diphenyl-1-PicrylHydrazyl (DPPH) assay, averaging 55.69% inhibition. For more comprehensive bioactivity comparison of *C. cujete*, the half-maximal inhibitory concentration (IC<sub>50</sub>) of  $\alpha$ -glucosidase, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) should be identified and various concentration screening protocols for the assays should be conducted to help identify the effective enzyme inhibitory effects of the sample. Mass Spectral Library included 1-Stearoyl-2-hydroxy-sn-glycero-3-phosphocholine, 15(S)-15-Methyl Prostaglandin E1, and 5,6-Dihydroxy-8Z,11Z,14Z-eicosatrienoic acid.

**Keywords:** 2,2-diphenyl-1-picrylhydrazyl,  $\alpha$ -glucosidase, GNPS, miracle fruit, phytochemical screening

## INTRODUCTION

*Crescentia cujete* Linn. is locally known as “calabas” or “kalabash” (Lim 2012), earning the name “miracle fruit” from its reputation of being a ‘miracle cure’ (Rellin et al. 2018). Traditional herbal medicine remains popular in the country, and native traditional healers from Agusan del Sur (Arquion et al. 2015),

Zamboanga del Sur (Morilla and Demayo 2019), and Zamboanga Sibugay (De Guzman et al. 2020), Philippines are utilizing the fruit, leaf, and bark decoction of *C. cujete* to treat various ailments like diabetes due to high costs of the current medications available in the market (Glaudson et al. 2016; Gonzales et al. 2022). The effect was attributed to the phytochemicals present in the fruit, such as cyanhydric



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acid, iridoids, pectins, and citric acid (Tupas et al. 2018). *Crescentia cujete* possesses significant free radical scavenging properties compared with stem bark and a clear correlation exists between the antioxidant activity and phenolic content (Das et al. 2014). Local studies presented by the Department of Science and Technology - Philippine Council for Health Research and Development (DOST-PCHRD) helped establish the status and demand of the sought-after miracle fruit for its anti-cancer, anti-tumor, and anti-diabetic properties. The widespread popularity of use and demand as an alternative herbal cure and cultivation has increased throughout the Philippines in recent years, and people are taking the opportunity to propagate the tree throughout the country (Arquion et al. 2015; Morilla and Demayo 2019; De Guzman et al. 2020; Glaudson et al. 2016; Gonzales et al. 2022). Despite its popularity as a traditional medicine, there is still a limited amount of research done pertaining to its medicinal use as an anti-diabetic and antioxidant (Glaudson et al. 2016; Gonzales et al. 2022). The study aims to fill the gap of knowledge and recognize the hypoglycemic and antioxidant potentials of the calabash fruit by conducting constituent description and metabolite profiling. The study used molecular networking to putatively identify active compounds present in the fractionated extracts of *C. cujete*.

## METHODS

### Sample Preparation and Extraction

Fruits of *C. cujete* Linn. were identified and authenticated by the University of Santo Tomas - Research Center for the Natural Sciences & Applied Sciences (RCNAS) Herbarium. The whole fruit was scraped, the seeds removed and the juice was filtered out. The scraped flesh fruit was dried in the hot air oven at 50°C temperature then it was ground into a coarsely powdered fruit sample. The powdered sample was soaked in 70% methanol for 4 hours, transferred to the percolator and the remaining menstruum was added and was macerated for 24 hours. The eluted extract was collected and kept in a 2-8°C cold storage for further fractionation. The percolation process (Mukherjee 2019) was repeated thrice to collect enough crude extract of approximately 3,950 mL.

### Phytochemical Screening

Qualitative phytochemical screening of the sample was performed to identify the chemical constituents present in the extract: alkaloids, saponins, steroids, tannins, flavonoids, reducing sugars, and volatile oils. Each test was done in triplicates (Billacura and Laciapag 2017).

**Test for alkaloids (Dragendorff's Test).** Two milliliters of 2M HCl were added to the 1 mL crude extract and placed in boiling water for 5 min. A 0.30 g of NaCl was added to the sample after it cooled

down and filtered. It was washed with 2 mL of HCl and then a sufficient amount of HCl was added to make it 5 mL, then 2-3 drops of Dragendorff's reagent were added to the sample. The presence of alkaloids can be identified if there is formation of precipitate or turbidity.

**Test for saponins (Foam Test).** One milliliter of crude extract was vigorously shaken with 5 ml distilled water. The persistent appearance of honeycomb froth above the surface indicates the presence of saponins.

**Test for steroids (Liebermann Burchard's Test).** Two milliliters of chloroform were added to the 1 mL crude extract. A few drops of concentrated sulfuric acid were added to form layers. The appearance of reddish-brown color indicates the presence of steroids.

**Test for tannins (Ferric Chloride Test).** Fifteen milliliters of hot distilled water were added to 1 mL crude extract. It was cooled down and decanted into another test tube. Three drops of 10% NaCl solution were added and then filtered. It was divided into three separate test tubes with their corresponding labels: (1) test tube A, (2) test tube B, and (3) test tube C. Test tube A was the negative control. Three drops of gelatin salt reagent were added to test tube B. Three drops of 1% FeCl<sub>3</sub> were added to test tube C. The appearance of a dark-blue color indicates the presence of hydrolyzable tannins, while the brownish-green color indicates the presence of condensed tannins.

**Test for flavonoids (Alkaline reagent Test).** Half milliliter of crude extract was treated with 1 mL 0.1 N NaOH solution and observed for yellow color that indicates the presence of flavonoids.

**Test for reducing sugars (Fehling's Test).** One milliliter of crude extract was dissolved in 3 mL of distilled water. A few drops of Fehling's A and B were added to the solution, and then mixed. It was then placed in a boiling water bath for 1-5 min and cooled down. The appearance of a red-brown precipitate indicates the presence of a reducing compound.

**Test for volatile oils.** One milliliter of crude extract was dissolved in 90% ethanol. A few drops of 1% FeCl<sub>3</sub> were added. The appearance of green color indicates the presence of volatile oils.

### Fractionation

The crude extract was partitioned using a modified Kupchan liquid-liquid partitioning method (Abdel-Mageed et al. 2020) with five different solvents: water, sec-butanol, methanol:water, dichloromethane (DCM), and hexane (Figure 1).

### $\alpha$ -Glucosidase Inhibition Assay

An optimized  $\alpha$ -glucosidase assay was performed using the fractionated extracts (Naing et al. 2019). Working stock solutions of the substrate and  $\alpha$ -glucosidase enzyme were prepared for plating.



$$\% \text{ Inhibitory Activity} = \frac{\text{Slope}_{\text{uninhibited}} - \text{Slope}_{\text{inhibited}}}{\text{Slope}_{\text{uninhibited}}} \times 100 \quad (\text{Eq. 1})$$

$$\text{DPPH scavenging effect (\%)} = \frac{\text{Absorbance of DPPH} - \text{Absorbance of sample}}{\text{Absorbance of DPPH}} \times 100 \quad (\text{Eq. 2})$$

### Ultra-High Performance Liquid Chromatography-Tandem Mass Spectrometry (UPLC-MS/MS) Analysis

Untargeted LC-MS/MS metabolite profiling of the fractionated extracts was performed using a Waters Acquity UPLC® H-Class System with a Xevo® G2-XS Quadrupole Time-of-Flight (QToF) High-Resolution Mass Spectrometer.

### Untargeted Metabolite Profiling

Molecular networking of compounds was done by the analysis of the tandem MS spectra through the Global Natural Products Social (GNPS) Molecular Networking Platform, utilizing the fragmentation pattern of the compounds in fractions of water, methanol/water, sec-butanol, and dichloromethane extracts. Molecular networking was done by checking fragmentations of compounds using MZmine (ver. 3.2.8) and then the molecular formula of compounds was predicted based on their monoisotopic mass using the ChemSpider, COCONUT, and PubChem databases.

### Molecular Networking

From the MS/MS spectra generated from the Data-Dependent Acquisition (DDA) mode, the detected metabolites were putatively identified through GNPS molecular network using the online workflow (<https://ccms-ucsd.github.io/GNPSDocumentation/>) on the GNPS website (<http://gnps.ucsd.edu>). Edges were filtered to have a cosine score above 0.7 and more than six matched peaks. The result network was visualized by using Cytoscape (version 3.10.3).

## RESULTS

### Phytochemical Screening

The crude extract exhibited the presence of secondary metabolites including alkaloids and reducing sugars (Table 1). Traces of flavonoids, saponins, volatile oils, tannins, and polyphenols were also present.

### $\alpha$ -Glucosidase Inhibition Assay

All fractions of the extract exhibited 7.44% (hexane), 0.85% (DCM), 1.94% (MeOH/H<sub>2</sub>O), 1.83% (sec-butanol), 0.70% (water), and 1.26% (crude) inhibition activity against  $\alpha$ -glucosidase (Table 2).

**Table 1.** Phytochemical constituents in the crude methanolic extract of oven-dried *Crescentia cujete* fruit. Legend: ++ (present), + (trace), - (absent).

Phytochemical Screening	Result	Indication
Alkaloids	Presence of turbidity	(++)
Flavonoids	No yellow coloration	(-)
Reducing Sugar	Precipitation was observed	(++)
Saponins	Persistent froth was observed	(+)
Steroids	No reddish-brown coloration	(-)
Tannins and Polyphenols	Brownish-green coloration detected	(+)
Volatile oil	Green coloration detected	(+)

**Table 2.** Percentage Inhibition of  $\alpha$ -Glucosidase Activity of Fractionated *Crescentia cujete* fruit extracts.

Sample No	Sample Code	% Inhibition $\pm$ SD
1	Hexane	7.44 $\pm$ 0.56
2	DCM	0.85 $\pm$ 0.48
3	MeOH/H <sub>2</sub> O	1.94 $\pm$ 0.38
4	Sec-Butanol	1.83 $\pm$ 0.37
5	H <sub>2</sub> O	0.70 $\pm$ 0.51
6	Crude	1.26 $\pm$ 1.32
Acarbose		90.79 $\pm$ 0.55

### 2, 2 - diphenyl -1- picrylhydrazyl (DPPH) Antioxidant Assay

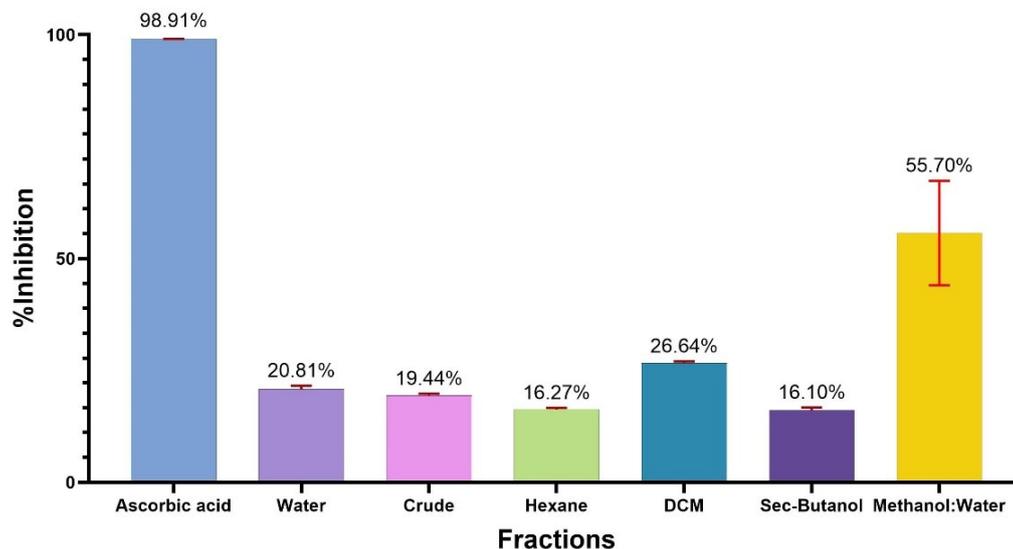
All fractions of the extract exhibited 55.69% (MeOH/H<sub>2</sub>O), 20.81% (water), 19.44% (crude), 16.27% (hexane), 26.64% (DCM), and 16.10% (sec-butanol) inhibition activity against DPPH (Figure 2).

### Dereplication of Compounds

From the MS/MS spectra generated from the DDA mode, the detected metabolites were putatively identified through GNPS molecular networking and visualized using Cytoscape (Figure 3). Three compounds were returned with spectral similarity in the NIST14 database (Table 3). In both DCM and sec-

butanol fractions, 1-Stearoyl-2-hydroxy-sn-glycero-3-phosphocholine is present with a cosine score of 0.97 (Figure 4). Only in the DCM fraction, 15(S)-15-Methyl Prostaglandin E1 is present but with low similarity to the NIST 14 spectral match with a cosine

score of 0.74 (Figure 5). In DCM and MeOH:H<sub>2</sub>O fractions, 5,6-Dihydroxy-8Z,11Z,14Z-eicosatrienoic acid is present and with the lowest similarity with only a cosine score of 0.51 (Figure 6).



**Figure 2.** Percentage Inhibition of DPPH (free radical) treated with Fractionated *Crescentia cujete* fruit extracts + DMSO measured after 15 minutes at 540 nm absorbance.

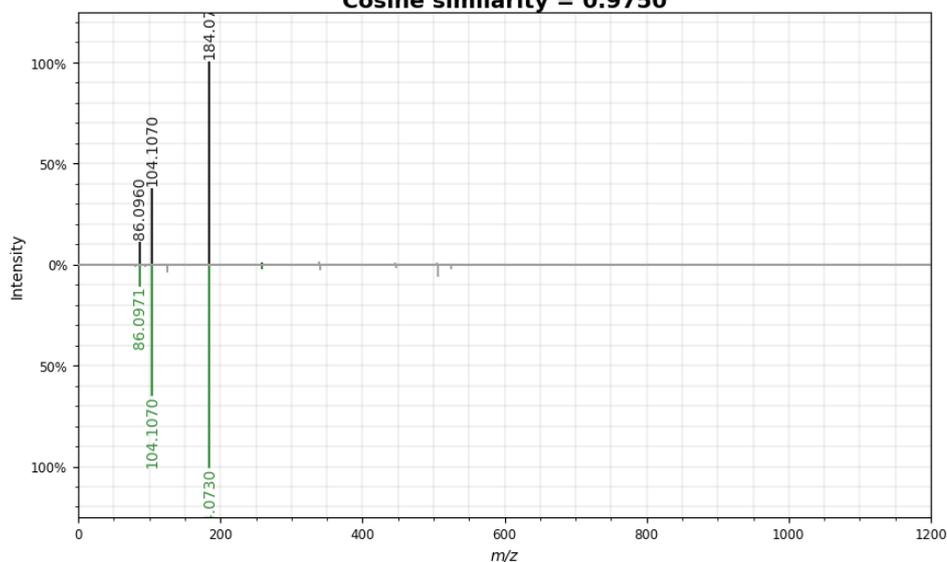
**Top: mzspec:GNPS:TASK-05816234f26642f497c96a5821a06ed2-spectra/specs\_ms.mgf:scan:114**

Precursor *m/z*: 522.4180 Charge: 0

**Bottom: mzspec:GNPS:GNPS-LIBRARY:accession:CCMSLIB00003136720**

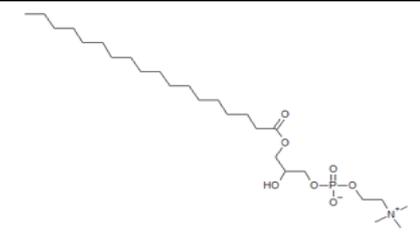
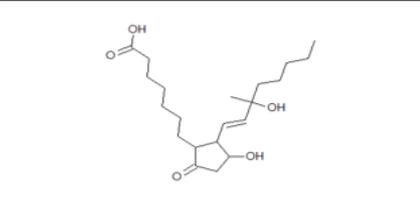
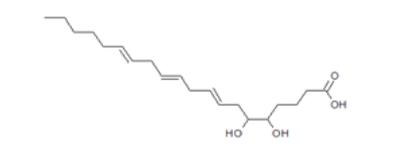
Precursor *m/z*: 524.3710 Charge: 1

**Cosine similarity = 0.9750**



**Figure 3.** Mirror match comparison of MS/MS profiles of the sample at the top (precursor *m/z* at 522.4180) and the reference 1-Stearoyl-2-hydroxy-sn-glycero-3-phosphocholine at the bottom (precursor *m/z* at 524.3710). The cosine score between the sample and the GNPS reference spectrum is 0.97.

**Table 3.** Identified compounds from molecular networking of fractionated *Crescentia cujete* fruit extracts.

Compound Name	Cosine Similarity	Structure
1-Stearoyl-2-hydroxy-sn-glycero-3-phosphocholine	0.9750	
15(S)-15-Methyl Prostaglandin E1	0.7162	
5,6-Dihydroxy-8Z,11Z,14Z-eicosatrienoic acid	0.5112	

**Top: mzspec:GNPS:TASK-05816234f26642f497c96a5821a06ed2-spectra/specs\_ms.mgf:scan:64**

Precursor *m/z*: 352.7720 Charge: 0

**Bottom: mzspec:GNPS:GNPS-LIBRARY:accession:CCMSLIB00003138511**

Precursor *m/z*: 351.2520 Charge: 1

**Cosine similarity = 0.7162**



**Figure 4.** Mirror match comparison of MS/MS profiles of the sample at the top (precursor *m/z* at 352.7720) and the reference 15(S)-15-Methylprostaglandin E1 at the bottom (precursor *m/z* at 351.2520). The cosine score between the sample and the GNPS reference spectrum is 0.71.

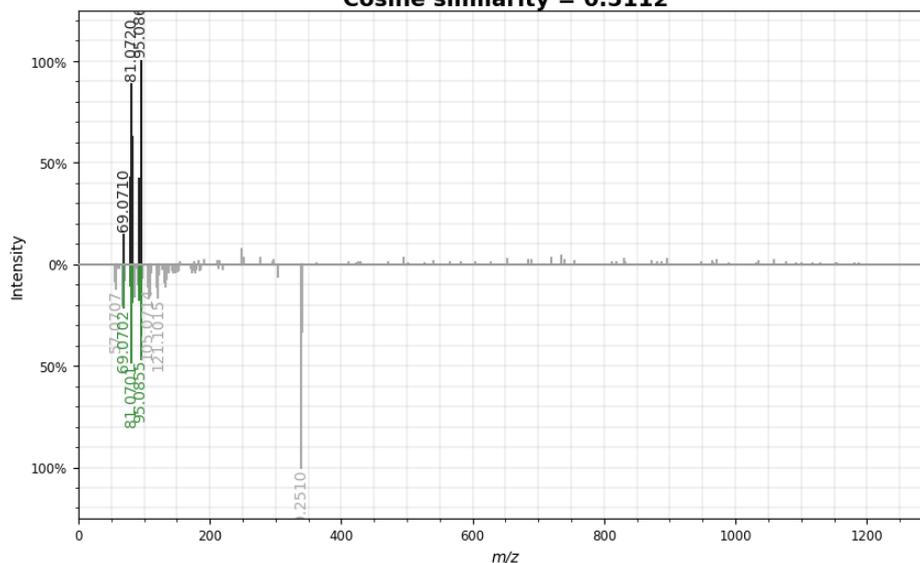
Top: mzspec:GNPS:TASK-15663149eee94e939801d6a596615e84-spectra/specs\_ms.mgf:scan:99

Precursor m/z: 338.5960 Charge: 0

Bottom: mzspec:GNPS:GNPS-LIBRARY:accession:CCMSLIB00003135090

Precursor m/z: 339.2510 Charge: 1

Cosine similarity = 0.5112



**Figure 5.** Mirror match comparison of MS/MS profiles of the sample at the top (precursor m/z at 338.5960) and the reference 5,6-Dihydroxy-8Z,11Z,14Z-eicosatrienoic acid at the bottom (precursor m/z at 339.2510). The cosine score between the sample and the GNPS reference spectrum is 0.51.

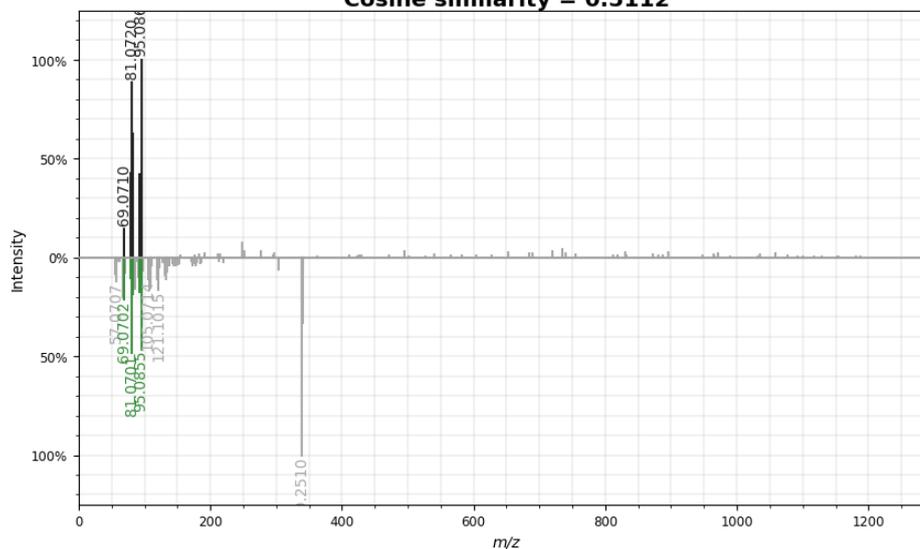
Top: mzspec:GNPS:TASK-15663149eee94e939801d6a596615e84-spectra/specs\_ms.mgf:scan:99

Precursor m/z: 338.5960 Charge: 0

Bottom: mzspec:GNPS:GNPS-LIBRARY:accession:CCMSLIB00003135090

Precursor m/z: 339.2510 Charge: 1

Cosine similarity = 0.5112



**Figure 6.** Mirror match comparison of MS/MS profiles of the sample at the top (precursor m/z at 352.7720) and the reference 5,6-Dihydroxy-8Z,11Z,14Z-eicosatrienoic acid at the bottom (precursor m/z at 351.2520). The cosine score between the sample and the GNPS reference spectrum is 0.71

### Untargeted Metabolite Profiling

A molecular network was created using the online workflow (<https://ccms-ucsd.github.io/GNPSDocumentation/>) on the GNPS website (<http://gnps.ucsd.edu>). The data was filtered by removing all MS/MS fragment ions within +/- 17 Da of the precursor m/z. MS/MS spectra were window filtered by choosing only the top 6 fragment ions in the +/- 50 Da window throughout the spectrum. The precursor ion mass tolerance was set to 2.0 Da and a MS/MS fragment ion tolerance of 0.5 Da. A network was then created where edges were filtered to have a cosine score above 0.7 and more than 6 matched peaks. Further, edges between two nodes were kept in the network if and only if each of the nodes appeared in each other's respective top 10 most similar nodes. Finally, the maximum size of a molecular family was set to 100, and the lowest scoring edges were removed from molecular families until the molecular family size was below this threshold. The spectra in the network were then searched against GNPS' spectral libraries. The library spectra were filtered in the same manner as the input data. All matches kept between network spectra and library spectra were required to have a score above 0.7 and at least 6 matched peaks. The GNPS online workflow result can be found in this URL:

<https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=195e8cd40a344097a8c021f8c191f40f>

Table 3 shows the three compounds that returned with spectral similarity in the NIST14 database.

1-Stearoyl-2-hydroxy-sn-glycero-3-phosphocholine, 15(S)-15-Methyl Prostaglandin, and 5,6-Dihydroxy-8Z,11Z,14Z-eicosatrienoic acid.

1-Stearoyl-2-hydroxy-sn-glycero-3-phosphocholine (18:0 Lyso PC) is a saturated lysophosphatidylcholine, has emerged as a potent anti-diabetic candidate due to its role as a significant dipeptidyl peptidase-4 (DPP-IV) inhibitor.

15(S)-15-Methyl Prostaglandin E1 (15-methyl PGE1) is a synthetic analog of Prostaglandin E1, modified with a methyl group in the S-configuration at carbon-15. This alteration enhances its metabolic stability and prolongs its half-life by preventing rapid inactivation.

5,6-Dihydroxy-8Z,11Z,14Z-eicosatrienoic acid (5,6-DHET) is a bioactive lipid derived from eicosatrienoic acid (20:3) and belongs to a specialized class of lipid mediators. Despite its biological significance, research specifically focusing on 5,6-DHET is limited. One study highlights reduced plasma levels of 5,6-DHET in diabetic nephropathy (DN) patients compared to those with T2DM without nephropathy. This finding suggests a protective role for 5,6-DHET in preventing DN, with potential applications as a biomarker for early detection and progression of DN in T2DM patients (Peng et al. 2021). The therapeutic potential of interventions

aimed at restoring or mimicking 5,6-DHET activity to mitigate DN warrants further exploration.

## DISCUSSION

### Phytochemical Screening

A definitive relationship between the secondary metabolites and the fruit's biological activity is difficult to establish due to a variety of phytochemicals with similar chemical structures (Saxena et al. 2013). The presence of tannins, polyphenols, and reducing sugars showed that the fruit has a potentially good source of antioxidant and hypoglycemic properties (Billacura and Laciapag 2017). The presence of alkaloids and saponins showed that *C. cujete* has potential antibacterial effect (Billacura and Laciapag 2017). Saponins may also exhibit an anti-inflammatory effect (Saxena et al. 2013).

### $\alpha$ -Glucosidase Inhibition Assay

The inhibiting activity against  $\alpha$ -glucosidase reduces glucose absorption by slowing down digestion of carbohydrates thereby controlling blood sugar levels (Feng et al. 2022). None of the six samples analyzed exhibited significant inhibitory activity against  $\alpha$ -glucosidase (Table 2). A higher concentration screening may elicit higher inhibitory activity.

### Antioxidant Assay

Antioxidants inhibit cellular damage through their free radical scavenging property (Lobo et al. 2010). The MeOH:H<sub>2</sub>O fraction exhibited potential antioxidant activity (Figure 2). The polarity of the solvent, along with compounds such as flavonols, alkaloids, polyphenols, and saponins may have contributed to the observed action (Tai et al. 2011).

### Dereplication of Compounds and Untargeted Metabolite Profiling

The GNPS spectral library is composed of MS/MS spectra coming from community deposits and aggregations from different sets of collections of libraries. Cosine scores have values ranging from 0 to 1, with 1 pertaining to absolute similarity (Wang et al. 2016). While natural products and other pertinent databases with an extensive collection of data have expensive access. As a result, the scope of the search was primarily limited to the available library collections and publicly accessible databases. A more exhaustive metabolite profiling and dereplication of compounds would be possible if given the opportunity of resources. Further solvent fractionation of the crude extract using MeOH/H<sub>2</sub>O improved the antioxidant activity by an average of 55.69%. However, no significant  $\alpha$ -glucosidase inhibitory activity can be

inferred from a single 10 ppm concentration screening of the same fractionated extracts.

To provide a more comprehensive comparison of the bioactivity of *C. cujete*, the half-maximal inhibitory concentration (IC<sub>50</sub>) of the  $\alpha$ -glucosidase, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) should be identified and various concentration screening protocols for the assays should be conducted to help identify the effective enzyme inhibitory effects of the sample. Further, isolation of the bioactive fractions could distinctly determine and identify the secondary constituents present in the sample. Nuclear magnetic resonance (NMR) spectroscopy could be performed for better identification, analysis, and structure elucidation of organic compounds.

The identification of 18:0 Lyso PC in *C. cujete* fruit highlights its potential as a natural product for Type 2 diabetes mellitus (T2DM) treatment by targeting DPP-IV, a key enzyme in T2DM management (Ambhore et al. 2023; Shaikh et al. 2021). By enhancing pancreatic insulin secretion and suppressing glucagon production, 18:0 Lyso PC improves glycemic control, with additional protective effects against obesity phenotypes in high-fat diet models (Han et al. 2021; Gilbert and Pratley 2020). Further, investigation into its bioactivity, such as determining the IC<sub>50</sub> values of the fruit extract, could validate its therapeutic efficacy and pave the way for developing cost-effective, plant-derived anti-diabetic agents.

The detection of 15-methyl PGE1 in *C. cujete* extracts may partially explain its reputed “miracle cure” properties, including potential vasoprotective and cytoprotective effects. It activates prostaglandin receptors, eliciting biological effects such as vasodilation, reduced clot formation, and cytoprotection (Kushwaha et al. 2023). Clinically, it is used for labor induction due to its uterotonic properties (Bakker et al. 2017) and in gastroenterology for its role in mucosal protection against peptic ulcers. However, the presence of this compound raises concerns about potential risks for pregnant women, as uterotonic effects could pose a threat. With a moderate cosine score of 0.71 from spectral matching, its identification remains tentative, necessitating advanced techniques such as nuclear magnetic resonance (NMR) for structural validation and bioactivity confirmation.

The fruit of *C. cujete* presents a potential natural source of 5,6-DHET. However, the spectral data in this study indicate low cosine scores in the compound's spectral match, meaning its presence in the fruit is not yet reliably confirmed. Further research employing more precise analytical methods is essential to validate *C. cujete* as a viable source of 5,6-DHET for therapeutic applications.

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## ETHICAL CONSIDERATIONS

This research adheres to the highest level of ethical standards. All protocols are approved by the College Research Ethics Committee in accordance with its existing rules and guidelines.

## DECLARATION OF COMPETING INTEREST

The authors declare that there are no competing interests to any authors.

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## REFERENCES

- Abdel-Mageed WM, Lehri B, Jarmusch SA, Miranda K, Al-Wahaibi LH, Stewart HA, Jamieson AJ, Jaspars M and Karlyshev AV. 2020. Whole genome sequencing of four bacterial strains from South Shetland Trench revealing biosynthetic and environmental adaptation gene clusters. *Marine Genomics*, 54: 100782. <https://doi.org/10.1016/j.margen.2020.100782>
- Ambhore JP, Laddha PR, Nandedkar A, Ajmire PV, Chumbhale D, Navghare AB, Kuchake V, Chaudhari P and Adhao VS. 2023. Medicinal chemistry of non-peptidomimetic dipeptidyl peptidase IV (DPP IV) inhibitors for treatment of Type-2 diabetes mellitus: Insights on recent development. *Journal of Molecular Structure*, 1284: 135249. <https://doi.org/10.1016/j.molstruc.2023.135249>
- Arquion RD, Galanida CC, Villamor B and Aguilar HT. 2015. Ethnobotanical study of indigenous plants used by local people of Agusan del Sur, Philippines. *Asia Pacific Higher Education Research Journal*, 2(2). <https://doi.org/10.56278/apherj.v2i2.102>
- Bakker R, Pierce S and Myers D. 2017. The role of prostaglandins E1 and E2, dinoprostone, and misoprostol in cervical ripening and the induction of labor: a mechanistic approach. *Archives of Gynecology and Obstetrics*, 296(2): 167–179. <https://doi.org/10.1007/s00404-017-4418-5>

- Billacura MP and Laciapag GCR. 2017. Phytochemical Screening, Cytotoxicity, Antioxidant, and Anthelmintic Property of the Various Extracts from *Crescentia cujete* Linn. Fruit. Science International-Lahore, 29(2): 31-35.
- Clarke G, Ting KN, Wiart C and Fry J. 2013. High Correlation of 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging, Ferric Reducing Activity Potential and Total Phenolics Content Indicates Redundancy in Use of All Three Assays to Screen for Antioxidant Activity of Extracts of Plants from the Malaysian Rainforest. *Antioxidants*, 2(1): 1–10. <https://doi.org/10.3390/antiox2010001>
- Das N, Islam ME, Jahan N, Islam MS, Khan A, Islam MR and Parvin MS. 2014. Antioxidant activities of ethanol extracts and fractions of *Crescentia cujete* leaves and stem bark and the involvement of phenolic compounds. *BMC Complementary and Alternative Medicine*, 14: 45. <https://doi.org/10.1186/1472-6882-14-45>
- De Guzman AAD, Jamanulla CEVA, Sabturani AM and Madjos GG. 2020. Ethnobotany and physiological review on folkloric medicinal plants of the Visayans in Ipil and Siay, Zamboanga Sibugay, Philippines. *International Journal of Herbal Medicine*, 8(3): 08-16.
- Feng Y, Nan H, Zhou H, Xi P and Li B. 2022. Mechanism of inhibition of  $\alpha$ -glucosidase activity by bavachalcone. *Food Science and Technology*, 42: e123421 <https://doi.org/10.1590/fst.123421>
- Gilbert MP and Pratley RE. 2020. GLP-1 Analogs and DPP-4 Inhibitors in Type 2 Diabetes Therapy: Review of Head-to-Head Clinical Trials. *Frontier in Endocrinology*, 11: 178. <https://doi.org/10.3389/fendo.2020.00178>
- Glaudson FGP, de Oliveira AP, Maria CSS, De Gonçalves ROJ, Miranda IMP, Pereira X, Delange DM and Roberto JAS. 2016. Phytochemical screening and antioxidant activity of methanolic fraction from the leaves of *Crescentia cujete* L. (Bignoniaceae). *Journal of Chemical and Pharmaceutical Research*, 8(2): 231-236.
- Gonzales AL, Sevilla UTA, Tsai PW and Huang SKH. 2022. Antioxidant and anti-inflammatory activities of bioactive compounds from *Crescentia cujete* L. leaves and fruit—A review. *International Journal of Advanced and Applied Sciences*, 9(11): 64-70. <https://doi.org/10.21833/jjaas.2022.11.007>
- Han AR, Park HR, Kim GJ, Kim BR, Kim YR, Park HH, Park J, Jin CH, Kim JM, Kwon SJ et al. 2021. 18:0 Lyso PC Derived by Bioactivity-Based Molecular Networking from Lentil Mutant Lines and Its Effects on High-Fat Diet-Induced Obese Mice. *Molecules*, 26(24): 7547. <https://doi.org/10.3390/molecules26247547>
- Kushwaha V, Agrawal P, Vekaria H, Das A, Shoraisham BK and Pathak B. 2023. Prostaglandins: An overview. *European Journal of Pharmaceutical and Medical Research*, 11(1): 130-139.
- Lim TK. 2012. *Crescentia cujete*. In: Lim TK (eds). *Edible Medicinal and Non-Medicinal Plants*. Springer Netherlands, pp. 480–485pp. [https://doi.org/10.1007/978-90-481-8661-7\\_64](https://doi.org/10.1007/978-90-481-8661-7_64)
- Lobo V, Patil A, Phatak A and Chandra N. 2010. Free radicals, Antioxidants and Functional foods: Impact on Human Health. *Pharmacognosy Reviews*, 4(8): 118–126. <https://doi.org/10.4103/0973-7847.70902>
- Morilla LJG and Demayo CG. 2019. Medicinal plants used by traditional practitioners in two selected villages of Ramon Magsaysay, Zamboanga Del Sur. *Pharmacophore*, 10(1): 84-89.
- Mukherjee PK. 2019. Chapter 6 - Extraction and Other Downstream Procedures for Evaluation of Herbal Drugs. *Quality Control and Evaluation of Herbal Drugs*. 195-236pp. <https://doi.org/10.1016/B978-0-12-813374-3.00006-5>
- Naing MD, Yerro JZ, Fernandez PL and Amor E. 2019.  $\alpha$ -Glucosidase Inhibition Assay. In: Guevarra A and Alvero R (eds). *Tuklas Lunas® Protocols for Drug Discovery and Development Volume IIB: Primary Bioactivity Assays*. Taguig: Philippine Council for Health Research and Development.
- Peng L, Sun B, Liu Y, Huang J, Chen G, Zhang X, Chen C, Wang DW and Wang G. 2021. Increased lipoxygenase and decreased cytochrome P450s metabolites correlated with the incidence of diabetic nephropathy: Potential role of eicosanoids from metabolomics in type 2 diabetic patients. *Clinical and Experimental Pharmacology and Physiology*, 48(5): 679-685. <https://doi.org/10.1111/1440-1681.13471>
- Rellin KFB, Dasmariñas DD and Junio HA. 2018. Untargeted Metabolite Profiling of Philippine-grown *Crescentia cujete* and its Commercial Fruit Juice using GC-MS and UPLC-HRMS. *Philippine Journal of Science*, 147(4): 647-658.
- Saxena M, Saxena J, Nema R, Singh D and Gupta. 2013. Phytochemistry of Medicinal Plants. *Journal of Pharmacognosy and Phytochemistry*, 1(6): 168-182.
- Shaikh S, Lee E-J, Ahmad K, Ahmad S-S, Lim J-H and Choi I. 2021. A Comprehensive Review and Perspective on Natural Sources as Dipeptidyl Peptidase-4 Inhibitors for Management of Diabetes. *Pharmaceuticals*, 14(6): 591. <https://doi.org/10.3390/ph140605>
- Shekhar TC and Anju G. 2014. Antioxidant Activity by DPPH Radical Scavenging Method of *Ageratum conyzoides* Linn. Leaves. *American Journal of Ethnomedicine*, 1(4): 244-249.
- StataCorp. 2023. *Stata Statistical Software: Release 13*. College Station, TX: StataCorp LLC.
- Tai Z, Cai L, Dai L, Dong L, Wang M, Yang Y, Cao Q and Ding Z. 2011. Antioxidant activity and chemical constituents of edible flower of *Sophora viciifolia*. *Food Chemistry*, 126(4): 1648–1654. <https://doi.org/10.1016/j.foodchem.2010.12.048>
- Tupas GD, Rañeses GMM, Guarino DV, Martinez C, Marundan JA, Mascardo K, Melanio A, Meling RK, Mendez M, Monteverde KA and Montojo MD. 2018. Effect of ED90 dose of Calabash (*Crescentia cujete*) Decoction on the Blood Sugar Levels of Pre-Diabetic Individuals. *DMSFI Research Journal*, 3: 21-26.
- Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M and Telser J. 2007. Free radicals and antioxidants in normal physiological functions and human disease. *The International Journal of Biochemistry & Cell Biology*, 39(1): 44–84. <https://doi.org/10.1016/j.biocel.2006.07.001>
- Wang M, Carver JJ, Phelan VV, Sanchez LM, Garg N, Peng Y, Nguyen DD, Watrous J, Kapono CA, Luzzatto-Knaan T et al. 2016. Sharing and community curation of mass spectrometry data with Global Natural Products Social Molecular Networking. *Nature Biotechnology*, 34: 828-837. <https://doi.org/10.1038/nbt.3597>

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# Efficient degradation of aniline yellow dye using photo-Fenton advanced oxidation process: Optimization via central composite design

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## ABSTRACT

Dyes are the coloring agents considered pollutants when combined in water bodies. This study used the photo-Fenton process, one of the advanced oxidation processes, to degrade aniline yellow dye (AYD). It is a primary dye that blends with any color, creating other shades of dye. Operating variables, namely initial concentration, contact time, and pH were studied in the degradation of AYD pollutants. A central composite design was applied to acquire the optimum conditions of these independent variables, resulting in the AYD degradation and eliminating up to 94.00% at pH 5 and an initial AYD concentration of 35 ppm. The removal efficiency of FeCl<sub>3</sub> resulted in an AYD removal of 2.03 mg per gram of FeCl<sub>3</sub>. The pseudo-first kinetic model best explained the mechanism of degradation and removal of AYD in aqueous solution. The results of the study showed that the photo-Fenton process using UV light from fluorescent lamp and Fenton's reagents (H<sub>2</sub>O<sub>2</sub> and FeCl<sub>3</sub>) effectively degraded AYD in water.

**Keywords:** advanced oxidation process (AOP), dye removal, photocatalysis, pollutant removal, wastewater treatment

## INTRODUCTION

The textile industry is essential for global economic growth but poses significant environmental challenges due to its extensive use of chemicals and water consumption (Mabayo and Orale 2024). It significantly contributes to worldwide water pollution by releasing many chemicals and colors into water bodies. Textile dyeing alone is estimated to account for 17-20% of industrial water contamination, posing an urgent environmental concern. Dyes are a

significant source of pollution from textile processes. Azo dyes, in particular, are among the industry's most widely utilized.

Aniline yellow dye (AYD) belongs to the azo dye family. It is widely used in microscopy for vital staining and the manufacture of yellow colors and inks. However, the indiscriminate release of AYD into water bodies poses significant environmental and health hazards. The AYD has been recognized as exceedingly hazardous, with potentially carcinogenic



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effects and adverse effects on aquatic ecosystems (Pagalan Jr et al. 2020).

Efforts to mitigate the environmental impact of dye contamination have led to exploring various treatment methods (Nerona et al. 2024; Shaltout et al. 2024; Su et al. 2024). Conventional wastewater treatment methods such as adsorption, thermal incineration, and membrane filtration struggle to remove AYD, necessitating the practical exploration of alternative approaches (Chaturvedi 2022). Among these, advanced oxidation processes (AOPs) have emerged as promising solutions for the degradation of organic pollutants. Advanced oxidation processes (AOPs) generate highly reactive hydroxyl ( $\bullet\text{OH}$ ) radicals to break down organic compounds. Among these processes, the Fenton-based advanced oxidation process has gained attention. In the Fenton process, ferrous ions ( $\text{Fe}^{2+}$ ) react with hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) to produce  $\bullet\text{OH}$  radicals. These radicals exhibit strong oxidative power and can effectively degrade organic pollutants (Giwa et al. 2021; Pandis et al. 2022). Compared to other AOPs such as ozonation and photocatalysis, the photo-Fenton process offers distinct advantages and limitations. For instance, ozonation can achieve similar removal efficiencies, but the photo-Fenton process demonstrates faster reaction times, reduced operational complexity, and higher efficacy under certain conditions. On the other hand, photocatalysis can degrade a wider range of organic pollutants but often requires more expensive catalysts and longer reaction times.

However, the Fenton process alone may not be sufficient for complete AYD degradation. To enhance its efficiency, researchers have combined it with ultraviolet (UV) light irradiation, resulting in the photo-Fenton advanced oxidation process (Ameta et al. 2018). This technique treats the solution under Fenton conditions while simultaneously exposed to UV light. The UV light accelerates the degradation rate of organic pollutants, making the process more effective. The photo-Fenton process offers several advantages, including increased reaction rates, improved degradation efficiency, and reduced energy consumption (Kumari and Kumar 2023; O'Dowd and Pillai 2020; Shalini and Setty 2022; Sriprom et al. 2018).

This study investigated the degradation of AYD contaminants using the photo-Fenton AOP including the impact of initial AYD concentration,

exploring optimal contact times, and evaluating the influence of pH on the process. These factors were selected because they play a crucial role in optimizing the degradation efficiency of the photo-Fenton process. Initial AYD concentration affects the availability of pollutants for degradation, while contact time is critical for determining how long the radicals have to act on the contaminants. The pH, which influences the stability of the reactive species in the Fenton process, is also a key parameter in controlling the efficiency of the degradation process. By systematically varying these parameters, the researchers aim to optimize the photo-Fenton process for efficient AYD removal from aqueous solutions.

## METHODS

### Experimental Design

Central composite design (CCD) was the tool responsible for giving the number of runs and the different operating variables. Three factors are being optimized. The range and levels are shown in Table 1. The values that were put in the range and levels were based on the preliminary experiment done and on the literature. A preliminary experiment was done to make sure that the percentage error was not that high and to have a result near the desired expectation.

After entering the coded level values, the CCD would give the experimental runs showing the combination of different values of independent variables, as shown in Table 2.

### AYD Solution Preparation

The 1000 mg of AYD (CAS No. 60-09-3, purity >98%) was mixed with 1 L of distilled water to produce a 1000 ppm solution subject to dilution to make the desired concentration for every run carried out from central composite design (CCD), as shown in Table 2. The ratio and proportion were performed to get the desired initial concentration values of AYD (Genesiran et al. 2015).

### Photo-Fenton Process

The primary step in the photo-Fenton process is to take the pH generated by the design expert on different runs. Hydrochloric acid (HCl, 1M) was used to obtain the desired pH and get the acidic solution.

**Table 1.** Experimental range and levels of independent variables.

Factors	Coded Level				
	-2	-1	0	1	2
Initial concentration (ppm)	10	20	30	40	50
Contact Time (min)	20	30	40	50	60
pH	2	3	4	5	6

**Table 2.** Efficiency of the degradation of the aniline yellow dye (AYD) through photo-Fenton process.

Run	Initial concentration (ppm)	Time (min)	pH	% AYD Removal
1	35	50	3	84.63
2	35	50	5	93.64
3	25	50	3	91.60
4	35	30	3	87.15
5	25	50	5	93.12
6	30	40	4	87.53
7	30	40	6	91.74
8	25	30	3	84.54
9	30	60	4	93.42
10	30	40	4	87.53
11	40	40	4	90.97
12	30	20	4	86.27
13	30	40	4	88.80
14	35	30	5	95.45
15	30	40	4	87.95
16	20	40	4	88.87
17	30	40	2	87.11
18	30	40	4	90.90
19	25	30	5	84.03
20	30	40	4	88.38

After getting the desired pH of the solution to be treated, the reagents H<sub>2</sub>O<sub>2</sub> (30%, Merck) and FeCl<sub>3</sub> (reagent grade, 97%) were added to the solution. The prepared solution was placed in the prototype photo reactor, having four fluorescent lamps positioned equally to each other. The duration of the solution inside the reactor differed depending on the time given by the CCD.

Afterward, the solution was removed from the reactor, and the absorbance was determined using a Hach DR/850 colorimeter.

**Experimental Setup**

The prototype photoreactor (Figure 1) is 2 feet tall, has a 16 cm hole diameter, and has 32 cm entire diameter. The four fluorescent tubes with 18 watts each are placed at an equal distance of 22.63 cm along the circumference of the cylinder facing each other, as adopted in the study of Genesiran et al. (2015).

The fluorescent lamps served as the light source of the reaction where the photo-Fenton treatment takes place. The magnetic bar and stirrer were used to disperse the solution during irradiation.

**Dye Removal Analysis**

In getting the percentage removal, the absorbance of each sample was tested using the DR/850 colorimeter. After obtaining the absorbance, a calibration formula was used to get the value of the dye residual. The calibration curve formula in getting dye residual is shown in Equation 1 where x and y in the absorbance is the residual (ppm).

When the value of the dye residual has been identified, percentage removal was determined using

Equation 2 where C<sub>o</sub> is the amount of initial concentration and C<sub>e</sub> is the value of the dye residual.

**Effects of the Photo-Fenton Process**

The effects of the photo-Fenton process were determined after all experimental ran. The data were analyzed using variance analysis (ANOVA) of the response surface methodology (RSM).

**Optimization of the Removal of AYD**

Three factors were optimized: the initial concentration, contact time, and pH. The recommended combinations of these independent variables were used to identify the percent removal of the AYD. The lowest percentage error obtained was considered the best conditioning for optimum degradation of AYD. The formula for getting the least percentage error is shown in Equation 3.

**Kinetic Model**

In determining the appropriate kinetic model best fitted to the degradation of AYD via AOP, initial concentration values, pH, and time at optimum conditions were used. The time was divided by 10, and 10 experimental runs were conducted at optimum pH and concentration with variable time at 3-minute increments for each run.

The equations of the pseudo-first and second order are shown in Equations 4 and 5, respectively, where C<sub>o</sub> is the initial concentration of AYD (mg/L), C<sub>t</sub> is the final concentration (mg/L), t in contact time (min), k<sub>1</sub> is the pseudo-first-order rate constant (min<sup>-1</sup>), and k<sub>2</sub> is the rate constant of pseudo-second-order (mg/L min).

$$y = 126.23x - 1.5617 \quad (\text{Eq. 1})$$

$$\text{Percent AYD removal} = \frac{C_0 - C_e}{C_0} \times 100 \quad (\text{Eq. 2})$$

$$\% \text{ error} = \frac{\text{Predicted Value} - \text{Actual Value}}{\text{Actual Value}} \times 100 \quad (\text{Eq. 3})$$

$$C_t = C_0 e^{-k_1 t} \quad (\text{Eq. 4})$$

$$C_t = \frac{C_0}{1 + k_2 C_0 t} \quad (\text{Eq. 5})$$

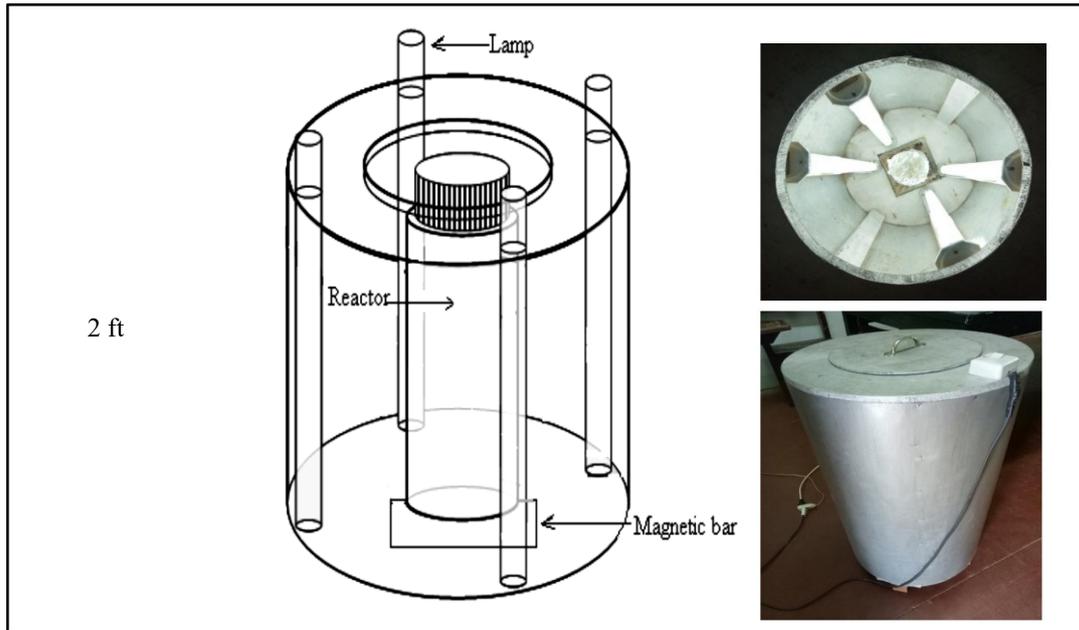


Figure 1. The prototype reactor set-up. [Redrawn from Genesiran et al. (2015)].

## RESULTS

### AYD Percent Removal via Photo-Fenton Process

The experimentation of AYD degradation through the photo-Fenton process was conducted based on the CCD experimental combination (Table 2).

Response surface methodology (RSM) modeled the values through Design Expert 7.0 software, which obtained the statistical outcomes. The data showed that the percent removal of AYD using the photo-Fenton process was about 84.03% to 95.45%.

### Model Fitting on AYD Degradation via Photo-Fenton Process

The reduced two-factor interval was found and best fitted to predict the removal of AYD through the photo-Fenton, as suggested by the CCD. The reduced model is shown in Equation 6, where  $Y$  represents the percent removal of AYD while  $A$ ,  $B$ ,

and  $C$  are the values of initial concentration (ppm), contact time (min), and pH, respectively.

The positive coefficients indicate an effect of increasing dye removal, while the negative coefficients indicate otherwise.

$$Y = 59.007 + 0.559A + 1.659B - 10.092C - 0.050AB + 0.395AC \quad (\text{Eq. 6})$$

The model's acceptability was verified through statistical analysis using ANOVA in Table 3. The result of the ANOVA implied a highly significant reduced model with a  $P < 0.0001$ , indicating that there is a 99.99% chance that the model is accurate and reliable.

Moreover, the initial concentration, contact time, and pH, including AC and AB, were considered significant with a p-value less than 5%. Overall, the reduced two-factor interval model was precise, as supported by the lack of fit p-value of 0.5649, which

is not significant. This implied that the model generated could predict the percent removal of AYD given the values of the chosen variables.

### Impact of Operating Parameters on AYD Degradation

The investigation explored the influence of operating parameters on AYD removal via photo-Fenton AOP. The initial concentration of AYD (20-40 ppm), contact time (20-60 min), and pH (2-6) were varied, and their effects were statistically significant ( $P < 0.05$ ) based on the reduced two-factor interval model.

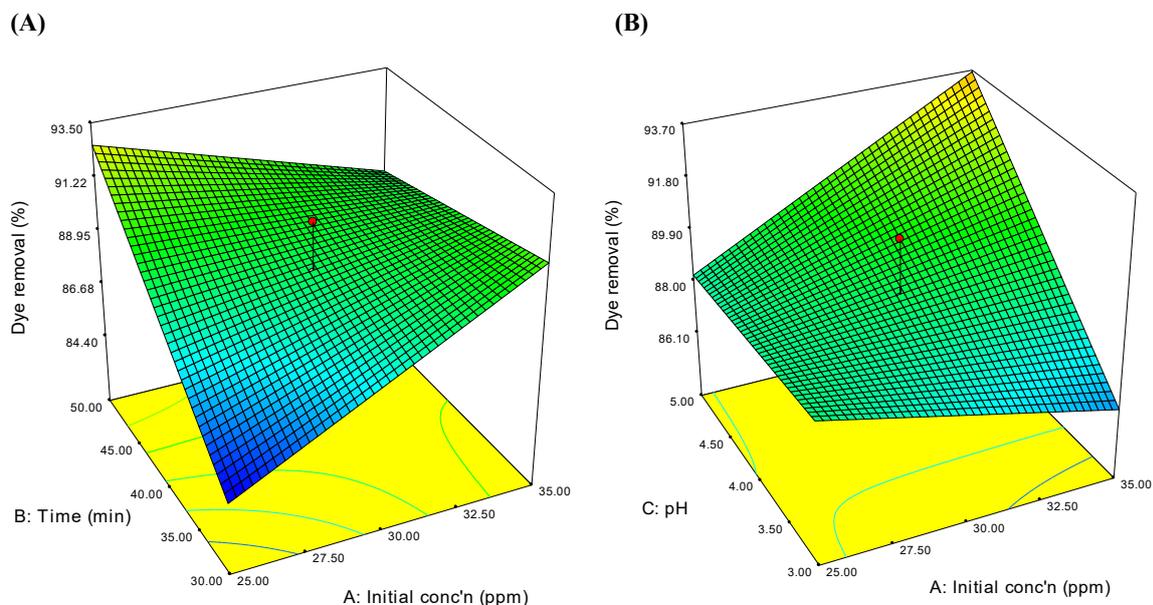
In Figure 2A, the highest removal efficiency (92.7%) was achieved when the lower initial concentration (25 ppm) was combined with increased contact time (30-50 min). This aligns with the concept

of having more hydroxyl radicals available to attack fewer dye molecules at lower concentrations and allowing for more degradation with extended reaction time.

Figure 2B analysis suggests a maximum removal of 93.7% at the highest concentration (35 ppm) and acidic pH (5). However, actual experiments achieved a higher maximum removal (95.45%) at a different concentration under the optimized contact time determined by the model. This emphasizes the importance of contact time for optimal removal. The increasing removal with increasing pH up to 5 supports the idea of favorable mildly acidic conditions for hydroxyl radical generation. Conversely, a decrease in removal at higher pH (beyond 5) suggests scavenging of hydroxyl radicals by hydroxide ions ( $\text{OH}^-$ ) at excessively high pH levels.

**Table 3.** ANOVA for the response surface reduced two-factor interval model. <sup>a</sup> = significant; <sup>b</sup> = not significant.

Source	Sum of Squares	Df	Mean Square	F Value	p-value
Model	179.99	6	30.00	19.66	< 0.0001 <sup>a</sup>
A – Initial Concentration	7.92	1	7.92	5.19	0.0403 <sup>a</sup>
B – Time	41.06	1	41.06	26.91	0.0002 <sup>a</sup>
C – pH	49.29	1	49.29	32.31	< 0.0001 <sup>a</sup>
AB	49.90	1	49.90	32.71	< 0.0001 <sup>a</sup>
AC	31.19	1	31.19	20.44	0.0006 <sup>a</sup>
Residual	19.84	13	1.53		
Lack of Fit	11.81	8	1.48	0.92	0.5649 <sup>b</sup>
Pure Error	8.03	5	1.61		
Cor Total	199.82	19			
$R^2 = 0.9007$					



**Figure 2.** 3D surface plots showing the interactive effects of (A) initial concentration of aniline yellow dye (AYD, ppm) and time (min), (B) initial concentration of AYD (ppm) and pH.

**Optimized Condition for AYD Removal**

Central composite design (CCD) was used as the experimental technique to search for the optimum values of the parameters. The optimum values were generated from the CCD, which had an initial concentration of 35 ppm, 30 min of contact time, and 5 pH, and a predicted AYD degradation of 94.26%.

The optimum values were validated through actual experimentation runs and reached a closed outcome between the predicted value (94.26%) and actual value of (94.00%), having 0.26% percent of error. This indicates that the model equation can calculate the degradation of AYD, given the operating parameters.

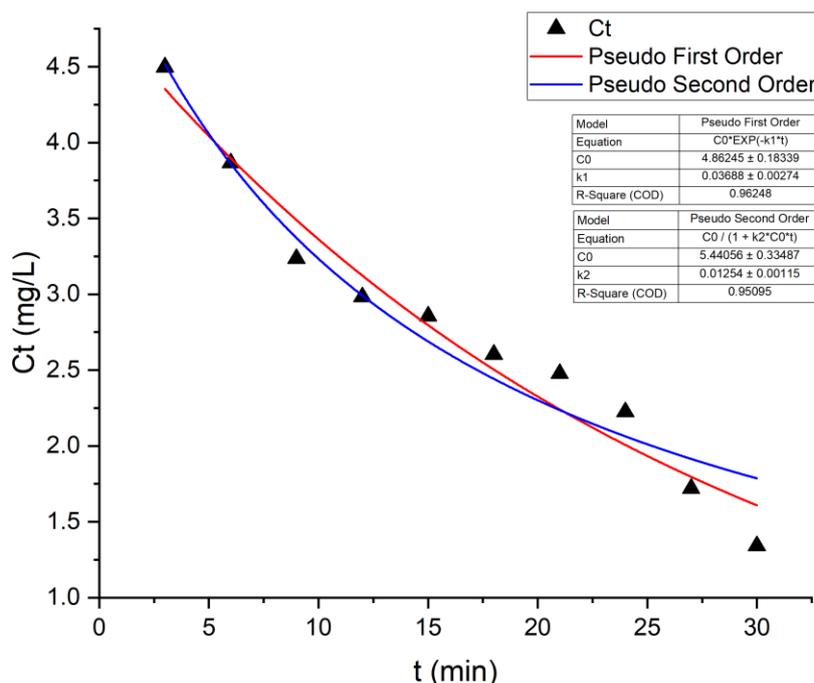
**Kinetic Modeling of AYD Degradation**

The most appropriate kinetic model was determined through kinetic experimental runs under an optimum level of 35 ppm of initial concentration

and a pH of 5 while varying the optimum contact time of 30 min by division of 10 (3, 6, 9, 12, 15, 18, 21, 24, 27, and 30). The results of the kinetic experimental runs are shown in Figure 3.

In the pseudo-first-order kinetic plot, the points were more closely positioned along the trend line than in the pseudo-second-order kinetic model plot. Therefore, the study on the degradation and removal of AYD through the photo-Fenton process exhibited that the first model had the greatest R<sup>2</sup> of 0.9625, which best defined the experimental data, compared to the second order, with R<sup>2</sup> of 0.9510.

The kinetics of AYD removal can be best described through the pseudo-first-order, which has a higher coefficient of determination than the second-order. This means that the removal of AYD is highly dependent on the amount of present •OH radicals.



**Figure 3.** Kinetic model plots for Pseudo-First Order and Pseudo-Second Order.

**DISCUSSION**

**Removal of AYD via Photo-Fenton Process**

The results presented in Table 2 demonstrate the effectiveness of the photo-Fenton AOP for degrading AYD in aqueous solution. The achieved

percentage removal of AYD ranged from 84.03% to 95.45%, indicating a significant treatment efficiency.

The data suggest that the degradation efficiency is influenced by several factors as designed in the CCD experiment (Table 2). Lower initial concentrations (Runs 3, 5, 8) generally resulted in higher degradation percentages. This can be attributed

to the greater availability of  $\bullet\text{OH}$  radicals for attacking fewer dye molecules (He et al. 2002; Macías-Sánchez et al. 2011). Increased reaction time (Runs 2, 5, 7) typically led to improved degradation. This allows for a longer exposure of AYD molecules to the oxidizing radicals. Furthermore, The optimal pH for the photo-Fenton process is typically acidic (2-3). However, the data do not show a definitive trend within the tested range (pH 3-5). Further investigation might be needed to determine the optimal pH for degrading AYD.

### Model Fitting

The statistical analysis confirmed the effectiveness of the reduced two-factor interval model in predicting the removal of AYD through the photo-Fenton AOP. The developed model (Equation 6) incorporates the solution's initial concentration (A), contact time (B), and pH (C), along with their interaction terms (AB and AC), to predict the percentage removal of AYD (Y).

The positive coefficients associated with A (initial concentration), B (contact time), and AC (interaction of initial concentration and pH) in Equation 7 indicate that increasing these factors generally leads to higher dye removal. Conversely, the negative coefficient with C (pH) suggests that an increase in pH decreases AYD removal. This aligns with the understanding that acidic pH conditions favor the photo-Fenton process (Nawaz et al. 2020). The negative coefficient of AB (interaction of initial concentration and contact time) implies a complex relationship between these factors, where the effect on removal might depend on the specific values of A and B.

The ANOVA table (Table 3) further strengthens the model's validity. The highly significant model ( $P < 0.0001$ ) indicates a 99.99% chance that the model is reliable. Moreover, the significance ( $P < 0.05$ ) of individual factors (A, B, C, AB, and AC) confirms their influence on AYD removal. The non-significant lack of fit p-value (0.5649) implies that the model adequately fits the data and can be used for prediction within the studied range.

Indeed, the reduced two-factor interval model effectively captures the relationship between the process variables (initial concentration, contact time, and pH) and the photo-Fenton degradation of AYD. The statistical analysis validates the model's accuracy and reliability for predicting the percentage removal of AYD within the investigated range.

### Interactive Effects of the Operating Variables

The study investigated the interactive effects of operating parameters on AYD removal using the photo-Fenton AOP. The chosen variables, viz. initial concentration (A), contact time (B), and pH (C) were found to significantly influence the degradation

process ( $P < 0.05$ ) according to the developed reduced two-factor interval model.

Figure 2A highlights the interplay between initial AYD concentration and contact time in achieving efficient degradation. It reveals that the combination of a lower initial concentration (25 ppm) with extended contact time (30-50 min) leads to a significant increase in AYD removal, reaching up to 92.7%. This observation aligns with established principles. At lower dye concentrations,  $\bullet\text{OH}$  radicals, the primary oxidants in photo-Fenton AOP, are more likely to encounter and degrade AYD molecules. Additionally, extending the reaction time allows for continuous generation of  $\bullet\text{OH}$ , facilitating the breakdown of a larger fraction of the dye molecules present. This explains the observed increase in removal with increasing contact time, particularly at lower initial concentrations.

Figure 2B showcases the interactive effect of initial AYD concentration and pH on degradation. While the figure suggests a maximum removal of 93.7% at the highest concentration (35 ppm) and acidic pH (5), it is crucial to consider the findings from the actual experimental runs. These runs achieved a maximum removal of 95.45% at a different concentration (possibly lower) under the optimized contact time determined by the CCD analysis. This aligns with the study by Lucas and Peres (2006), which emphasized the importance of optimal contact time for maximizing AYD removal.

The trend observed in Figure 2B further supports the findings of Kakodia et al. (2013). The increasing removal with increasing pH up to a certain point (around pH 5) suggests that mildly acidic conditions favor the generating of  $\bullet\text{OH}$  radicals in the photo-Fenton process. However, excessively high pH levels (beyond the optimum) can lead to the scavenging of  $\bullet\text{OH}$  radicals by  $\text{OH}^-$  hindering their ability to degrade AYD molecules (Kumari and Kumar 2023), which explains the decrease in removal observed when the pH goes beyond 5.

Categorically, the analysis of interaction terms (AB and AC) in Figure 2 provides valuable insights into the combined effects of operating parameters on AYD removal in the photo-Fenton AOP. Lower initial dye concentration and extended contact time generally promote efficient degradation. Maintaining a mildly acidic pH (around 5) is crucial for optimal generation and activity of hydroxyl radicals. These findings contribute to a comprehensive understanding of the process optimization for effective AYD removal.

### Optimization Studies

Using photo-Fenton AOP, the study employed a CCD to identify the optimal operating parameters for maximizing AYD removal. The model predicted an optimal removal of 94.26% at an initial

concentration of 35 ppm, contact time of 30 min, and pH of 5.

Validation experiments were conducted under these predicted optimal conditions, achieving an actual removal efficiency of 94.00%, as shown in Table 4. This close agreement between the predicted and actual values (with a mere 0.26% error) demonstrates the model’s strong predictive capability. This implies that the developed model equation (Equation 6) can be reliably used to estimate the degradation of AYD within the investigated range of operating parameters.

Moreover, Table 5 shows the efficiency of this study as compared to some related published studies. The results of this study are higher than the degradation of reactive yellow dye (91%) under optimized conditions by Mohammed et al. (2022), which required more complex parameters and a longer irradiation time. Similarly, the 92.24% removal efficiency for acid red 73 reported by Vaez et al. (2012) and the 89% removal for acid yellow 17 by Khan et al. (2018) are slightly lower than this study’s performance. Both studies relied on acidic conditions and additional reactants (e.g. Fe<sup>2+</sup> and H<sub>2</sub>O<sub>2</sub>), suggesting the method

used in this study may offer a simpler and more efficient alternative for dye removal.

The optimization using CCD highlights its effectiveness in identifying favorable conditions for photo-Fenton AOP. This approach can also be valuable for optimizing the degradation of other organic pollutants. The achieved removal efficiency (around 94%) signifies the potential of photo-Fenton AOP for treating wastewater containing AYD. However, further research is needed to evaluate the applicability of this method at larger scales and with more complex wastewater matrices. Moreover, the study underscores the importance of considering initial concentration, contact time, and pH to optimize AYD removal. Maintaining a mildly acidic pH (around 5) appears crucial for maximizing the generation and activity of hydroxyl radicals.

Future studies could explore additional factors that might influence the process, such as the type and concentration of the iron catalyst used in the photo-Fenton reaction. Additionally, investigating the degradation products of AYD could provide valuable insights into the reaction mechanism and potential environmental implications.

**Table 4.** Optimization and validation of the removal of aniline yellow dye (AYD) using photo-Fenton advanced oxidation processes (AOP).

Experiment	Variables			Response
	AYD Initial concentration (ppm)	Contact time (min)	pH	AYD removal (%)
CCD (Theoretical)	35	30	5	94.26
Validation (Actual)	35	30	5	94.00

**Table 5.** Comparison of the degradation efficiency in this study and other published studies.

Dye Removed	Optimal Conditions	Percent Removal	Reference
Aniline yellow dye	pH: 5 Initial AYD Concentration: 35 ppm Contact time: 30 min	94.00%	This Study
Reactive yellow dye	pH:6.95294 TiO <sub>2</sub> concentration: 25.5441 mg/L H <sub>2</sub> O <sub>2</sub> concentration: 383.676 mg/L RY initial dye conc.: 20.9412 mg/L irradiation time:89.6176 min	91.00%	(Mohammed et al. 2022)
Acid red 73	pH: 3 Initial dye concentration: 25 mg/L H <sub>2</sub> O <sub>2</sub> concentration: 0.5 mg/L Anion concentration: 0.69 mg/L	92.24%	(Vaez et al. 2012)
Acid yellow 17	AY 17: 0.06 mM Fe <sup>2+</sup> : 0.06 mM H <sub>2</sub> O <sub>2</sub> :0.9 mM pH: 3.0 reaction time: 60 min	89.00%	(Khan et al. 2018)

### Kinetic Modelling

Kinetic experiments were conducted under the optimized conditions (35 ppm initial concentration, pH 5) with varying contact times (3-30 min). The results (Figure 3) were used to evaluate the fit of two kinetic models: pseudo-first-order and pseudo-second-order.

The pseudo-first-order model exhibited a superior fit to the experimental data compared to the pseudo-second-order model. This is evident from the higher  $R^2$  value (0.9625) for the pseudo-first-order model compared to the pseudo-second-order model's  $R^2$  value (0.9510), as indicated in Table 6. This suggests that the removal rate of AYD is primarily dependent on the concentration of  $\bullet\text{OH}$  radicals present in the solution.

The dominance of the pseudo-first-order model implies that the degradation rate is directly proportional to the concentration of AYD molecules available for reaction with  $\bullet\text{OH}$  radicals. This finding highlights the crucial role of  $\bullet\text{OH}$  radicals in the photo-Fenton process. Optimizing process conditions to generate a sufficient concentration of these radicals is essential for efficient AYD removal.

**Table 6.** Kinetic parameters for aniline yellow dye (AYD) degradation through the photo-Fenton process.

Pseudo-first order		Pseudo-second order	
Variables	Value	Variables	Value
$K_1, \text{min}^{-1}$	0.0369	$K_2, \text{mg/L/min}$	0.0125
$C_0, \text{mg/L}$	4.8625	$C_0, \text{mg/L}$	5.4406
$R^2$	0.9625	$R^2$	0.9510

### Synthesis of Findings and Implications

This study demonstrated the effectiveness of the photo-Fenton process for aniline yellow dye degradation in aqueous solutions. The process was optimized under varying initial concentration, pH, and contact time conditions, showing a promising removal efficiency of up to 94%. These results are significant in the context of wastewater treatment, particularly for industries where azo dyes such as aniline yellow are prevalent contaminants. Future research should focus on addressing several key areas to enhance the applicability of the photo-Fenton process. First, investigate the formation and toxicity of degradation byproducts which is crucial for understanding the environmental impact of the process. Second, explore methods to enhance the sustainability and cost-effectiveness of the process, like utilizing solar light instead of artificial UV radiation, could significantly reduce energy consumption. Lastly, assess the scalability of the photo-Fenton process for large-scale wastewater treatment facilities is an essential step toward its commercial implementation. These directions will help refine the process and ensure its long-term viability in industrial applications.

### FUNDING

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### ETHICAL CONSIDERATIONS

This study did not involve humans or animals.

### DECLARATION OF COMPETING INTEREST

The authors declare that there is no competing interests to any authors.

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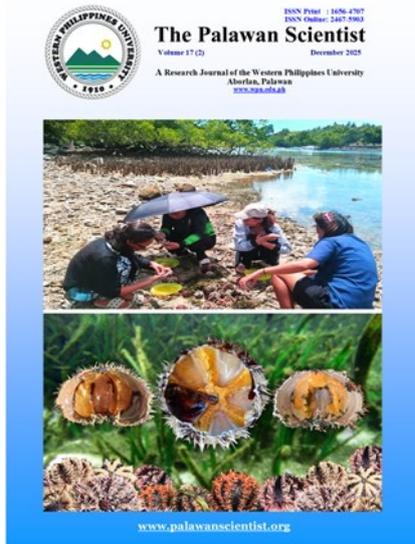
### REFERENCES

- Ameta R, Chohadia AK, Jain A and Punjabi PB. 2018. Fenton and Photo-Fenton Processes. In: Ameta SC and Ameta R (eds). *Advanced Oxidation Processes for Wastewater Treatment: Emerging Green Chemical Technology*. Academic Press, United Kingdom, pp. 49-87. <https://doi.org/10.1016/C2016-0-00384-4>
- Chaturvedi NK. 2022. Comparison of available treatment techniques for hazardous aniline-based organic contaminants. *Applied Water Science*, 12: 173. <https://doi.org/10.1007/s13201-022-01695-3>
- Genesiran DSC, Jarena LJM, Miyake RD and Pacquiaio MR. 2015. Degradation of methylene blue dye (MBD) using photo-Fenton advanced oxidation process. BS in Environmental Engineering. Misamis Oriental State College of Agriculture and Technology. 85pp.
- Giwa A, Yusuf A, Balogun HA, Sambudi NS, Bilad MR, Adeyemi I, Chakraborty S and Curcio S. 2021. Recent advances in advanced oxidation processes for removal of contaminants from water: A comprehensive review. *Process Safety and Environmental Protection*, 146: 220–256. <https://doi.org/10.1016/j.psep.2020.08.015>
- He J, Tao X, Ma W and Zhao J. 2002. Heterogeneous photo-Fenton degradation of an azo dye in aqueous  $\text{H}_2\text{O}_2$ /iron oxide dispersions at neutral pHs. *Chemistry Letters*, 31(1): 86–87. <https://doi.org/10.1246/cl.2002.86>
- Kakodia AK, Gurjar L, Ameta SC and Sharma BK. 2013. Photodegradation of Acid Violet 90 in aqueous solution by Photo-Fenton reagent. *International Journal of Chemical Sciences*, 11(2): 855–864.
- Khan J, Sayed M, Ali F and Khan HM. 2018. Removal of Acid Yellow 17 Dye by Fenton Oxidation Process. *Zeitschrift Fur Physikalische Chemie*, 232(4), 507–525. <https://doi.org/10.1515/zpch-2017-1072>
- Kumari P and Kumar A. 2023. Advanced Oxidation Process: A remediation technique for organic and non-biodegradable

- pollutant. Results in Surfaces and Interfaces, 11: 100122. <https://doi.org/10.1016/j.rsurfi.2023.100122>
- Lucas MS and Peres JA. 2006. Decolorization of the azo dye Reactive Black 5 by Fenton and photo-Fenton oxidation. Dyes and Pigments, 71(3): 236–244. <https://doi.org/10.1016/j.dyepig.2005.07.007>
- Mabayo VI and Orale R. 2024. Agricultural-based Biomass as an Efficient Adsorbent in the Removal of Dyes in Dye-contaminated Wastewater: A Mini Review. Current Applied Science and Technology, 24(1): e0257011. <https://doi.org/10.55003/cast.2023.257011>
- Macías-Sánchez J, Hinojosa-Reyes L, Guzmán-Mar JL, Peralta-Hernández JM and Hernández-Ramírez A. 2011. Performance of the photo-Fenton process in the degradation of a model azo dye mixture. Photochemical and Photobiological Sciences, 10(3): 332–337. <https://doi.org/10.1039/c0pp00158a>
- Mohammed NA, Alwared AI, Abdulhasan MJ and Salman MS. 2022. Optimization and kinetic evaluation of reactive yellow dye degradation by solar photocatalytic process. Desalination and Water Treatment, 277: 234–243. <https://doi.org/10.5004/dwt.2022.29034>
- Nawaz M, Shahzad A, Tahir K, Kim J, Moztahida M, Jang J, Alam MB, Lee SH, Jung HY and Lee DS. 2020. Photo-Fenton reaction for the degradation of sulfamethoxazole using a multi-walled carbon nanotube-NiFe<sub>2</sub>O<sub>4</sub> composite. Chemical Engineering Journal, 382: 123053. <https://doi.org/10.1016/j.cej.2019.123053>
- Nerona JD, Abella NCM, Jundam GA-GC and Gumaling RP. 2024. Response surface and artificial neural network modeling for optimization of process parameter in Cu(II) adsorption uptake of sodium-activated Philippine natural zeolite. Journal of Water Process Engineering, 58: 104851. <https://doi.org/10.1016/j.jwpe.2024.104851>
- O'Dowd K and Pillai SC. 2020. Photo-Fenton disinfection at near neutral pH: Process, parameter optimization and recent advances. Journal of Environmental Chemical Engineering, 8(5): 104063. <https://doi.org/10.1016/j.jece.2020.104063>
- Pagalan Jr E, Sebron M, Gomez S, Salva SJ, Ampusta R, Macarayo AJ, Joyno C, Ido A and Arazo R. 2020. Activated carbon from spent coffee grounds as an adsorbent for treatment of water contaminated by aniline yellow dye. Industrial Crops and Products, 145: 111953. <https://doi.org/10.1016/j.indcrop.2019.111953>
- Pandis PK, Kalogirou C, Kanellou E, Vaitsis C, Savvidou MG, Sourkouni G, Zorpas AA and Argirusis C. 2022. Key Points of Advanced Oxidation Processes (AOPs) for Wastewater, Organic Pollutants and Pharmaceutical Waste Treatment: A Mini Review. ChemEngineering, 6(1): 8. <https://doi.org/10.3390/chemengineering6010008>
- Shalini and Setty YP. 2022. Fenton/Photo-Fenton processes for wastewater treatment and disinfection. In: Mungray A, Mungray A, Sonawane S and Sonawane S (eds). Novel Approaches towards Wastewater Treatment and Resource Recovery Technologies. Elsevier, Amsterdam, Netherlands, pp. 241–252.
- Shaltout WA, El-Nagggar GA, Esmail G and Hassan AF. 2024. Synthesis and characterization of ferric@nanocellulose/nanohydroxyapatite bio-composite based on sea scallop shells and cotton stalks: adsorption of Safranin-O dye. Biomass Conversion and Biorefinery, 14(4): 4759–4776. <https://doi.org/10.1007/s13399-022-02753-1>
- Sriprom P, Champa V, Kitchaiya P and Assawasaengrat P. 2018. Optimizing Decolorization Efficiency of Methylene Blue by Photo-Fenton Process over Fe-Diatomite using Central Composite Design. Computer Aided Chemical Engineering, 43: 409–414. <https://doi.org/10.1016/B978-0-444-64235-6.50074-7>
- Su X, Wang X, Ge Z, Bao Z, Lin L, Chen Y, Dai W, Sun Y, Yuan H, Yang W et al. 2024. Koh-activated biochar and chitosan composites for efficient adsorption of industrial dye pollutants. Chemical Engineering Journal, 486: 150387. <https://doi.org/10.1016/j.cej.2024.150387>
- Vaez M, Moghaddam AZ and Alijani S. 2012. Optimization and modeling of photocatalytic degradation of azo dye using a response surface methodology (RSM) based on the central composite design with immobilized Titania nanoparticles. Industrial and Engineering Chemistry Research, 51(11): 4199–4207. <https://doi.org/10.1021/ie202809w>

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# Distribution, habitat characteristics, and conservation status of Noah's clam *Tridacna noae* (Röding, 1798) in the reefs of Palawan, Philippines

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## ABSTRACT

This study aimed to provide an updated distribution range of *Tridacna noae* (Röding, 1798) in Palawan with notes on its habitat characteristics and conservation status. Data on the distribution and habitat characteristics were obtained from reef assessments from 2010-2023 and 10 Key Informants (KIs) and plotted using QGIS v3.28 software to illustrate its distribution in a map. A total of 12 reef sites within eight municipalities in Palawan showed the presence of *T. noae*. Majority of these reefs were found in open-access coastal areas facing the West Philippine Sea. The clams were partially buried or settled on the top of dead or live coral in a depth of 1-5 meters from reef flat to reef slope surrounded by macroalgae. *Tridacna noae* remained unlisted in international, national, and local lists of protected species although it was confirmed in 2014 as a distinct species of giant clam, hence; the inclusion of *T. noae* in the lists of protected species in the Philippines is needed. Knowing the distribution, habitat characteristics, and conservation status of a certain threatened species such as the giant clam is an important step in managing and conserving wild population.

**Keywords:** QGIS, remote sensing, teardrops giant clams, threatened species

## INTRODUCTION

*Tridacna noae* (Röding, 1798) also known as Noah's clam or Teardrop clam is a species of giant clam previously considered as a variant of *Tridacna maxima* (Röding, 1798) because of similarity in morphological features (Rosewater 1965). The validation of *T. noae* as a distinct species from *T. maxima* was through morphological and genetic characteristics (Su et al. 2014). According to Su et al. (2014), the mantle of *T. noae* can be recognized by the presence of its unique ocellate spots or "teardrops" with sparse hyaline organs, while *T. maxima* has only

continuous hyaline organs on the mantle edge. In addition, both species of giant clams are said to have similar maximum shell lengths (i.e. 40 cm), although the largest observed *T. noae* so far was 32 cm (Neo 2023). Both species can be found in shallow reef areas and lagoons with water depths between 1-15 m (Borsa et al. 2015; Militz et al. 2015; Neo 2023) and typically found partially buried either on dead or live coral substrate surrounded by various macroalgae (Su et al. 2014; Neo et al. 2017).

The *T. noae* is widely distributed in the Indo-West Pacific region, extending from the north of the Ryukyu Archipelago in Japan, east of Kiribati,



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southeast of Cook Island, Western Australia, southwest of Christmas Island, west of Vietnam, south of Taiwan, and in the central Philippines (see Borsa et al. 2014; Neo et al. 2017). Over the years, reports of distribution range in some localities within the Indo-West Pacific are still expanding. For example, in 2019 and 2021, the presence of *T. noae* was reported in the west of Palawan, in the Philippines (see Ecube et al. 2019; Mecha 2024). While in 2022 and 2023, a range expansion of *T. noae* was reported in American Samoa (Marra-Biggs et al. 2022) and Cook Island (Morejohn et al. 2023).

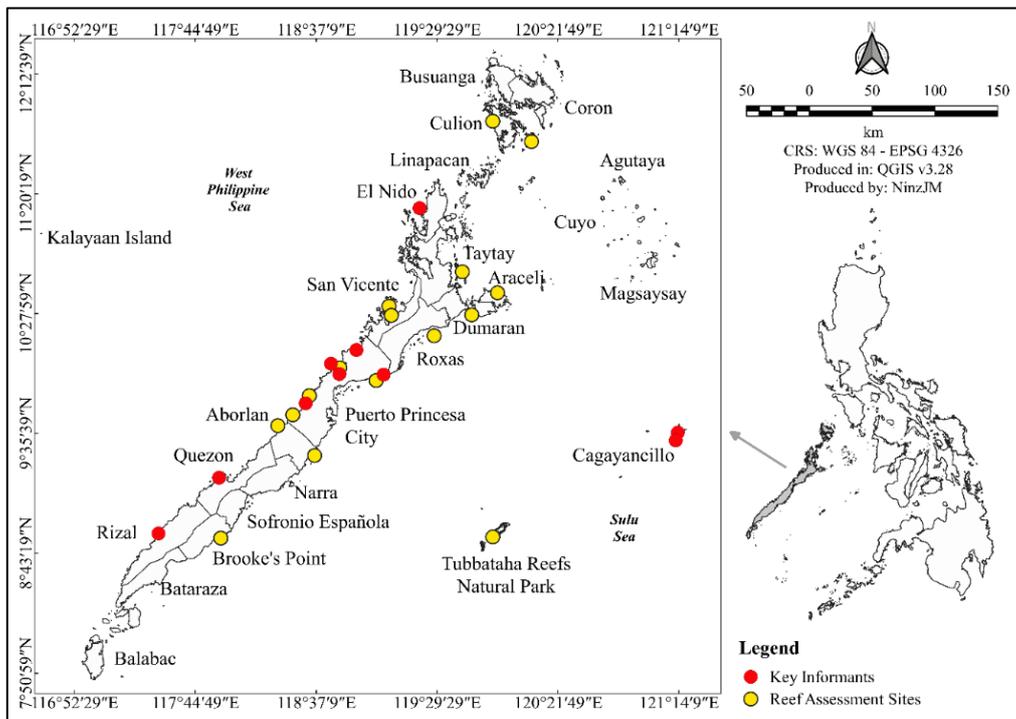
All distinct species of giant clams under Tridacninae are listed under the Appendix II of CITES in which the global trade for live, meat, and shell must be regulated due to the declining wild population (CITES 2024; Dolorosa et al. 2024; Lee et al. 2024; Vogel and Hoeksema 2024). These are *Hippopus hippopus* (Linnaeus, 1758); *H. porcellanus* Rosewater, 1982; *T. gigas* (Linnaeus, 1758); *T. derasa* (Röding, 1798); *T. squamosa* Lamarck, 1819; *T. maxima* (Röding, 1798); *T. crocea* Lamarck, 1819; *T. noae* (Röding, 1798); *T. mbalavuana* Ladd, 1934; *T. rosewateri* Sirenko and Scarlato, 1991; *T. squamosina* Sturany, 1899; *T. elongatissima* Bianconi, 1856 (Fauvelot et al. 2020). In the Philippines, the first eight of the 12 species of giant clams mentioned above can also be found on its reefs (Dolorosa et al. 2015; Ecube et al. 2019; Neo et al. 2017), however, only the first seven are formally included in the list of protected species which prohibit the collection of living specimens and byproduct (DA 2001; PCSD 2017).

In Palawan, the presence of *T. noae* was only reported by Ecube et al. (2019) and Mecha (2024), however, the study area was limited to two municipalities. Hence, this study aimed to provide an updated distribution range of *T. noae* in the reefs of Palawan with notes of its habitat characteristics and conservation status which could be used for conservation management of its wild population and hatchery propagation like the case of *T. gigas* (see MFI 2020; Mecha et al. 2020).

## METHODS

### Study Sites

The study covers the entire province of Palawan in the Philippines (Figure 1), designated as a biosphere reserve by the UNESCO - Man and Biosphere Programme (MAB) in 1990 due to its relatively intact ecosystems and its potential to showcase the co-existence of sustainable development and protection of the environment (Sandalo and Baltazar 1997). It has 1,768 islands and a 2,000 km irregular coastline (UNESCO 2021) that lies between the West Philippine Sea (WPS) and the Sulu Sea known as the apex of marine biodiversity - the Sulu-Sulawesi Marine Ecoregion (SSME) (ADB 2014) and home to eight species of giant clams in the Philippines (Dolorosa et al. 2015; Ecube et al. 2019).



**Figure 1.** Map of Palawan showing the reef assessment sites (n=16) and location of KIs (n=10) during the virtual interview.

### Distribution and Habitat Characteristics

The distribution was based from the compiled photos of individual *T. noae* opportunistically found from various personal reef survey projects in 16 reef sites from 2013-2023 in Palawan. These reef surveys were done using SCUBA gears at reef slope to reef flat with deep ranging from 2 to 15 m. Another source of information was the 10 Key Informants (KIs) employed in different NGOs from Palawan who also accidentally encountered *T. noae* from various reef surveys from 2010-2022. These KIs also responded to the posted information about *T. noae* on social media network between 2021-2023. Only individuals/divers who personally took photos of *T. noae* on the reef were included as KI. Each KI was also asked about the estimated sizes of *T. noae* and the characteristics of the habitat such as the type of substrate, the surrounding environs, and the depth of the reefs. All photos of *T. noae* presented by each KIs were also solicited. The location of reef survey sites and responses of KIs about the distribution of *T. noae* was geographically presented using the QGIS v3.28 software.

### Conservation Status

*Tridacna noae* is not included in regulations, highlighting the need for further assessment. However, the conservation status of the species was evaluated based on the International Union for Conservation of Nature (IUCN 2024) Red List, CITES (2024), Fisheries Administrative Order (FAO) 208 (DA 2001), and Palawan Council for Sustainable Development (PCSD) Resolution 15-521 (PCSD 2017). A thorough review and field survey are needed to determine whether the species is at risk, evaluate existing legal protections, and identify the necessary data for its possible inclusion in conservation policies.

## RESULTS

### Distribution Range of *Tridacna noae*

A total of 12 reef sites within eight municipalities in Palawan had documentation of *T. noae* (Figure 2). Ten of these reefs are on the west coast of Palawan, while only two reefs in the east coast. Additionally, most of the reefs are open access, except for Puerto Princesa Underground River (PPUR) which is a Marine Protected Area (MPA), and Port Barton, San Vicente which is located in a private resort (Figure 2).

### Habitat Characteristics

The majority of *T. noae* were found in the outer reef flat to reef slopes surrounded by macroalgae such as *Padina* spp., *Sargassum* spp., *Halimeda* spp. at a depth of 1-5 m (Table 1; Figures 3 and 4). Most of the clams were partially buried or on top of dead or

live massive corals, while some individuals were found within the base of branching corals.

### Conservation Status

*Tridacna noae* is not listed in FAO 208, and not evaluated in PCSD Resolution 15-521 and IUCN Red List. However, CITES listed all giant clam species including the *T. noae* in Appendix II (Table 2).

## DISCUSSION

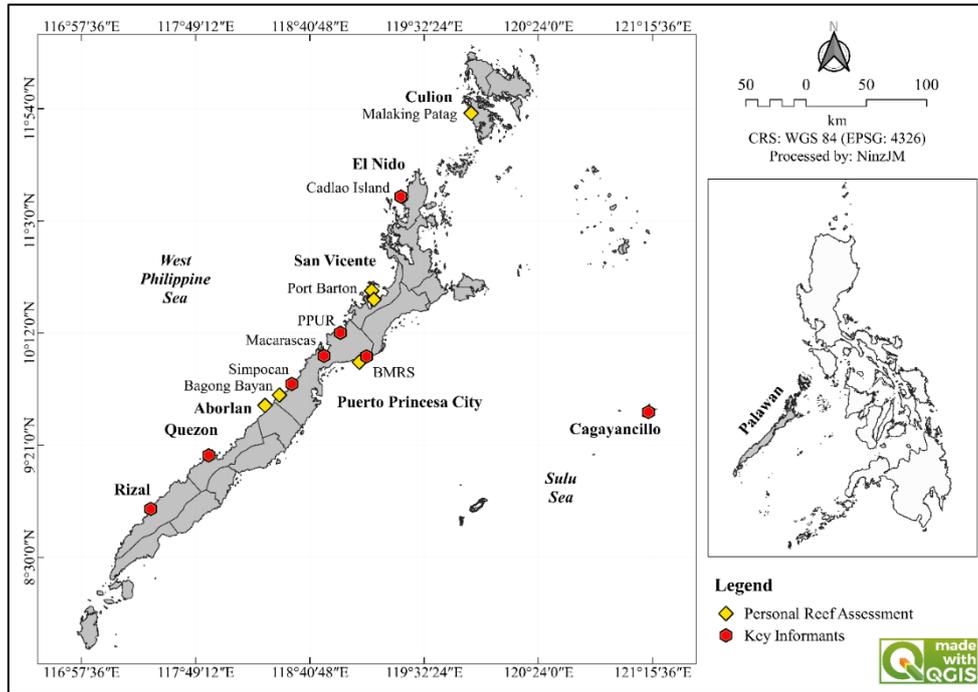
### Distribution Range of *Tridacna noae* in the Reefs of Palawan

The previous distribution range of *T. noae* in Palawan was only reported in the reefs of Port Barton, San Vicente (Ecube et al. 2019), and Malaking Patag, Culion (Mecha 2024). However, this study showed that *T. noae* can also be found in the central to southwest and eastern reefs of Palawan which provides an additional information on its distribution range in the entire province of Palawan and the Philippines (Lizano and Santos 2014). Currently, information on the distribution range of *T. noae* in the Philippines is still limited (Lizano and Santos 2014; Ecube et al. 2019; Mecha 2024; this study) although it is presumed to have a similar distribution range with *T. maxima*. Therefore, revisiting some surveyed sites with reports of *T. maxima* is needed for species revalidation. For example, the revalidation of the *T. maxima* population in various localities has been reported, where 42% of previously identified *T. maxima* was reclassified as *T. noae* in Papua New Guinea (Militz et al. 2015), while in Ningaloo Reef Marine Park, Australia, only *T. noae* are present with no individual *T. maxima* documented after the revisit in the park (Johnson et al. 2016).

On the other hand, a greater number of coastal areas with *T. noae* appeared on the west coast compared to the east despite the latter having more surveyed sites. The lesser degree of exploitation on the west coast compared to the heavily populated east coast of Palawan might be a factor for its absence in some surveyed areas. During bad weather, the majority of the residents living on the Palawan coast depends on the resources in shallow coastal areas for their livelihood and diet, which includes sea shells such as the giant clams (Ardines et al. 2020; Mecha and Dolorosa 2020; Mecha 2024). This is similar to the Kei Islands waters, Southeast of Indonesia within Indian ocean, where *T. noae* was only present in site with less human population than the surveyed sites with more human settlement near coastal areas (Triandiza et al. 2019). Additionally, Dolorosa et al. (2024) reported continuous decline of giant clams in Palawan, although high densities remained in some areas (Conales et al. 2015; Dano et al. 2020; Mecha and Dolorosa 2020). However, further assessments of

giant clams in various coastal areas are needed to understand better their distribution and population abundance, particularly in southern Palawan and other island municipalities. More data on the species

presence and density across the Philippines is also necessary to accurately evaluate its conservation status.



**Figure 2.** The current distribution range of *Tridacna noae* in Palawan. PPUR – Puerto Princesa Underground River; BMRS – Binduyan Marine Research Station.

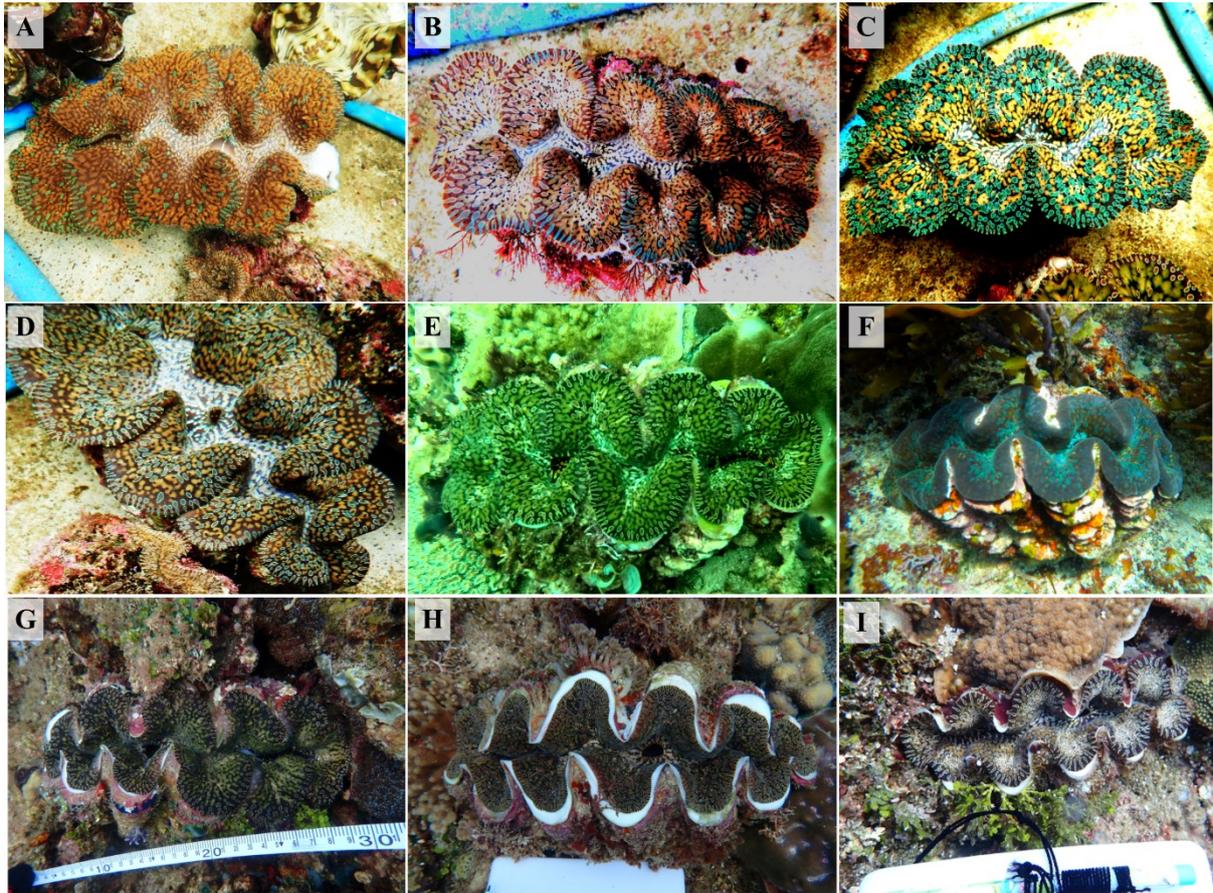
**Table 1.** Habitat characteristics of *Tridacna noae* in different reefs in Palawan.

Localities	Depth (m)	Habitat Characteristics
Malaking Patag, Culion	2	The <i>T. noae</i> were found partially buried in dead coral substrate surrounded by various macroalgae, such as <i>Sargassum</i> spp. and <i>Halimeda</i> spp. at reef flat.
Cadlao Island, El Nido	1	The <i>T. noae</i> was found partially buried in dead coral substrate surrounded by various macroalgae such as <i>Sargassum</i> spp. at reef slope. Some sub-massive live corals are also present in the area.
Cagayancillo	1	The <i>T. noae</i> was found partially buried in dead coral substrate surrounded by a few macroalgae such as <i>Sargassum</i> spp. at reef flat.
Port Barton, San Vicente	1	<i>Tridacna noae</i> were found partially buried in dead coral substrate and crevices of live hard coral surrounded by various macroalgae such as <i>Sargassum</i> spp., <i>Padina</i> spp. and some filamentous green algae near reef crest.
Binduyan Marine Research Station (BMRS), Puerto Princesa City	1	The <i>T. noae</i> were found partially buried in dead coral substrate surrounded by a few macroalgae such as <i>Sargassum</i> spp. at reef slope.
Puerto Princesa Underground River, Puerto Princesa City	2-4	<i>Tridacna noae</i> were found on top of a dead coral substrate next to a live coral near the reef crest.
Macarascas, Puerto Princesa City	3	The <i>T. noae</i> were found partially buried in dead coral substrate surrounded with various macroalgae such as <i>Sargassum</i> spp. at reef flat with nearby sub-massive live corals.
Simpocan, Puerto Princesa City	4	The <i>T. noae</i> were found partially buried in a dead coral substrate with various macroalgae such as <i>Sargassum</i> spp. and <i>Halimeda</i> spp. at reef flat with branching corals at the reef slope.
Bagong Bayan, Puerto Princesa City	3	The clam was found partially buried in dead coral substrate next to a live coral with <i>Sargassum</i> spp. at reef slope.

Localities	Depth (m)	Habitat Characteristics
Apurawan, Aborlan	5	The clam was found on top of dead coral substrate surrounded by <i>Sargassum</i> spp. at reef slope.
Panitian, Quezon	4	The <i>T. noae</i> was found partially buried in dead coral surrounded by live corals and a few macroalgae at reef flat.
Candawaga and Punta Baja, Rizal	5	The <i>T. noae</i> was found partially buried in dead corals surrounded by live corals and a few macroalgae at reef flat.



**Figure 3.** Images of *Tridacna noae* from various reefs of Palawan. A – Malaking Patag, Culion; B – Cadlao Island, El Nido; C – Cagayancillo; D-E – Port Barton, San Vicente; F – BMRS, PPC; G-L – PPUR, PPC; M-O – Simpocan, PPC.



**Figure 4.** Images of *Tridacna noae* from various reefs of Palawan. A-D – Macarascas, PPC; E – Bagong Bayan, PPC; F – Apurawan, Aborlan; G-H – Panitian, Quezon; I – Punta Baja, Rizal.

**Table 2.** The conservation status of eight species of giant clams in the Philippines. FAO – Food and Agriculture Organization; PCSD – Palawan Council for Sustainable Development; IUCN – International Union for Conservation of Nature; CITES – Convention on International Trade of Endangered Species.

Species of Giant Clams	FAO 208 (DA 2001)	PCSD Resolution 15-521 (PCSD 2017)	IUCN Red List of Threatened Species (2024)	CITES (2024)
<i>Hippopus porcellanus</i>	Endangered	Endangered	Least Concern	Appendix II
<i>Hippopus hippopus</i>	Endangered	Endangered	Least Concern	Appendix II
<i>Tridacna gigas</i>	Endangered	Endangered	Vulnerable	Appendix II
<i>Tridacna derasa</i>	Endangered	Endangered	Vulnerable	Appendix II
<i>Tridacna squamosa</i>	Endangered	Endangered	Least Concern	Appendix II
<i>Tridacna maxima</i>	Endangered	Endangered	Least Concern	Appendix II
<i>Tridacna noae</i>	Not Listed	Not Evaluated	Not Evaluated	Appendix II
<i>Tridacna crocea</i>	Endangered	Endangered	Least Concern	Appendix II

**Habitat Characteristics**

The reef flat and reef slope where it encountered in various coastal areas in Palawan are similar to the observations in Kavieng Lagoonal chain, Papua New Guinea (Militz et al. 2015), and Dongsha Atoll, South China Sea (Neo et al. 2018). In addition, the substrate characteristics (e.g. crevices of dead and live corals) and depth (e.g. 1-4 m) of habitat of *T. noae*

encountered in this study are also similar to the reports conducted in Indo-West Pacific region (Borsa et al. 2024; Marra-Biggs et al. 2022; Morejohn et al. 2023; Triandiza et al. 2019; Militz et al. 2015). This implies that *T. noae* are more common in outer reefs exposed to waves actions rather than the inner reef chains and most likely to be found in the abovementioned habitats. According to Dumas et al. (2014) and Millitz et al.

(2015), the larvae and juvenile of *T. noae* has active habitat selection similar to *T. maxima* utilizing chemosensory abilities that allow individuals to distinguish from favorable and unfavorable reef substrates. Majority of *T. noae* documented in this study were also found together with *T. maxima* and *Tridacna crocea* (Lamarck, 1819), suggesting that these giant clam species shared similar habitat selection in the reef (Neo et al. 2018; Mecha 2024). These habitat preferences of *T. noae* could be used as a reference for the conservation of its wild population and protection of the area (Militz et al. 2015; Morejohn et al. 2023). In addition, the presence of macroalgae in major reefs with *T. noae* in this study needs further investigation if it has a connection to the habitat preference of the clams since other studies did not include such information.

### Conservation Status

*Tridacna noae* was initially described as a variant of *T. maxima* by Rosewater (1965), which may explain its absence from the earliest list of protected species in FAO 208 (Table 2; DA 2001). However, the criteria or references used in FAO 208 for listing giant clam species remain unspecified. Regardless of being recognized as a distinct species in 2014 (Su et al. 2014; Neo et al. 2015), it has not been included in key conservation lists due to a lack of updates, particularly for newly recognized species like *T. noae*, which may not yet have been evaluated. As of 2024, the IUCN only recognizes seven giant clam species in the Philippines, with two listed as Vulnerable (VU) and five as Least Concern. Similarly, PCSD Resolution 15-521 (PCSD 2017) lists only these seven species as endangered. (Table 2). Despite this, CITES (2024) includes all species under the family Tridacninae in Appendix II due to the similarity of appearance principle, implying that species are listed because they closely resemble others subject to trade restrictions. This includes *T. noae*, which is not currently threatened with extinction but is listed to ensure that trade regulations prevent potential threats to its wild population.

Although the Philippine government prohibits the harvesting of giant clams, small species such as *T. maxima*, *T. noae*, and *T. crocea* are not only consumed as food (Ardines et al. 2020) but some are also collected for the aquarium industry due to their colorful mantle (Wabnitz et al. 2003; Fartherree 2023; Vogel and Hoeksema 2024). During the collection process, the burrowing species are forcibly extracted from either dead or live coral substrates which pose a threat to the coral reefs (Mecha 2024). In addition, while some reef areas recovered overtime (Abesamis et al. 2023), the general declining trend for coral cover in Palawan (Climaco et al. 2022; Dolorosa et al. 2023; Haworth et al. 2024) is an added threat to the giant clam species. Therefore, there is a need to update the

existing laws and strengthen the enforcement mechanisms to prevent the risk of localized extinction, especially the *T. maxima*, as its density data in the Philippines might be overestimated as this includes the *T. noae* from the previous giant clam assessments. Moreover, there is a need to protect the open access reef areas with *T. noae* in Palawan to conserve its remaining population in the wild. Currently, the hatchery propagation initiative for *T. noae* is ongoing in Palawan through the collaborative efforts of Western Philippines University (WPU) and the Malampaya Foundation Incorporation (MFI). This initiative would help conserve and protect the wild population of *T. noae* in the reefs of Palawan like the case of *T. gigas* (MFI 2020; Mecha and Dolorosa 2020).

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### ETHICAL CONSIDERATIONS

All Key Informants voluntarily shared the needed information and photos used in this study. No animals were captured or harmed during the conduct of this study.

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### REFERENCES

- Abesamis MR, Ang JL, Robles RC and Licuanan WY. 2023. Recovery of coral cover on inshore fringing reefs following mass coral bleaching in the Philippines. *Coral Reefs*, 42(1):99-104. <https://doi.org/10.1007/s00338-022-02322-y>
- Ardines R, Mecha NJM and Dolorosa R. 2020. Commonly gleaned macro-benthic invertebrates in a small offshore island of Cawili, Cagayancillo, Palawan, Philippines. *The Palawan Scientist*, 12: 102–125. <https://doi.org/10.69721/TPS.J.2019.11.1.09>

- ADB (Asian Development Bank). 2014. Regional state of the Coral Triangle—Coral Triangle marine resources: Their status, economics, and management. Mandaluyong City, Philippines: Asian Development Bank, 94pp.
- Borsa P, Fauvelot C, Tiavouane J, Grulois D, Rio M, Naguit A, Andrefouët S and Wabnitz C. 2014. Distribution of Noah's giant clam, *Tridacna noae*. Marine Biodiversity, 45: 339–344. <https://doi.org/10.1007/s12526-014-0265-9>
- Borsa P, Fauvelot C, Andrefouët S, Chai TT, Kubo H and Liu LL. 2015. On the validity of Noah's giant clam *Tridacna noae* (Röding, 1798) and its synonymy with Ningaloo giant clam *Tridacna ningaloo* Penny & Willan, 2014. Raffles Bulletin of Zoology, 63: 484–489.
- Climaco RB, Aludia GM, Mecha NJMF, Cornel ARB, Acebuque ANAM, Rodriguez JF, Miguel JA, Menardo ID and Dolorosa RG. 2022. Status of coral reefs, butterflyfishes, and benthic macro-invertebrates in Araceli and Dumaran, Palawan, Philippines. The Palawan Scientist, 14(2): 54-64. <https://doi.org/10.6972/TPS.J.2022.14.2.07>
- CITES (Conservation of International Trade of Endangered Species). 2024. Checklist of CITES Species. <https://checklist.cites.org>. Accessed on 20 January 2024.
- Conales SF, Bundal NA and Dolorosa RG. 2015. High density of *Tridacna crocea* in exposed massive corals proximate the ranger station of Tubbataha Reefs Natural Park, Cagayancillo, Palawan, Philippines. The Palawan Scientist, 7: 36-39. <https://doi.org/10.6972/TPS.J.2015.7.1.04>
- DA (Department of Agriculture). 2001. Conservation of rare, threatened and endangered fishery species. Philippines. Fisheries Administrative Order NO. 208, Series of 200. <https://www.bfar.da.gov.ph/wp-content/uploads/2021/04/FAO-No.-208-s.-2001.pdf>. Accessed on 24 January 2023.
- Daño JC, Villanueva EG and Dolorosa RG. 2020. High density of *Tridacna crocea* in Rita Island, Puerto Princesa City, Palawan, Philippines. The Palawan Scientist, 12: 159-163. <https://doi.org/10.6972/TPS.J.2020.12.1.11>
- Dolorosa RG, Climaco RB, Miguel JA, Aludia GM and Mecha NJ. 2023. Impact of super typhoon Odette on the reefs of northeastern Palawan, Philippines. Journal of Fisheries and Environment, 47(1):37-52.
- Dolorosa RG, Mecha NJ, Bano JD, Ecube KM, Villanueva EG, Cabaitan PC. 2024. Declining population of giant clams (Cardiidae: Tridacninae) in Palawan, Philippines. Ocean and Coastal Research, 72: e24010. <https://doi.org/10.1590/2675-2824072.22130>
- Dolorosa RG, Picardal RM, Conales SF. 2015. Bivalves and gastropods of Tubbataha Reefs Natural Park, Philippines. Check List, 11(1): 1506. <http://dx.doi.org/10.15560/11.1.1506>
- Dumas P, Tiavouane J, Senia J, Willam A, Dick L and Fauvelot C 2014. Evidence of early chemotaxis contributing to active habitat selection by the sessile giant clam *Tridacna maxima*. Journal of Experimental Marine Biology and Ecology, 452: 63–69. <https://doi.org/10.1016/j.jembe.2013.12.002>
- Ecube KMA, Villanueva EG, Dolorosa RG and Cabaitan PC. 2019. Notes on the first record of *Tridacna noae* (Röding, 1798) (Cardiidae: Tridacninae) in Palawan, Philippines. The Palawan Scientist, 11: 112-115. <https://doi.org/10.6972/TPS.J.2019.11.1.09>
- Ellis SC. 1997. Spawning and early larval rearing of giant clams (Bivalvia: Tridacnidae) No. 130. Waimanalo, Hawaii: Center for Tropical and Subtropical Aquaculture, California, USA. 52pp.
- Fartherree J. 2023. Giant Clams in the Reef Aquarium: Biology, Identification, and Care, Second Edition. Liquid Medium Publications, Tampa, Florida, USA. 196pp.
- Fauvelot C, Zuccon D, Borsa P, Grulois D, Magalon H, Riquet F, Andrefouët S, Berumen ML, Sinclair-Taylor TH, Gélín P, Behivoke F, ter Poorten JJ, Strong EE and Bouchet P. 2020. Phylogeographical patterns and a cryptic species provide new insights into Western Indian Ocean giant clams phylogenetic relationships and colonization history. Journal of Biogeography, 47: 1086-1105. <https://doi.org/10.1111/jbi.13797>
- Haworth BT, Cadigal GM, Zabala Jr. EC, Gonzales BJ, Jalover-Par CLS, Dolorosa RG, Gonzales-Plasus MM, Jontila JBS, Cabrestante Jr. MP, Bruce E and Baker E. 2024. Palawan (UNESCO Biosphere Reserve), Philippines: State of the Marine Environment 2024. Palawan Council for Sustainable Development Staff, Puerto Princesa City, Philippines. 130pp.
- IUCN (International Union for Conservation of Nature). 2024. The IUCN Red List of Threatened Species. Version 2023-1. <https://www.iucnredlist.org/>. Accessed on 20 January 2024.
- Johnson MS, Prince J, Brearley A, Rosser NL and Black R. 2016. Is *Tridacna maxima* (Bivalvia: Tridacnidae) at Ningaloo Reef, Western Australia? Molluscan Research, 36(4): 264-270. <https://doi.org/10.1080/13235818.2016.1181141>
- Lee LK, Neo ML, Hii KS, Gu H, Chen CA, Lim PT and Leaw CP. 2024. Vanishing giants: An assessment on the population status of giant clams across Malaysia. Regional Studies in Marine Science, 74: 103546. <https://doi.org/10.1016/j.rsma.2024.103546>
- Lizano AM and Santos MD. 2014. Updates on the status of giant clams *Tridacna* spp. and *Hippopus hippopus* in the Philippines using mitochondrial CO1 and 16S rRNA genes. Philippine Science Letters, 7: 187–200.
- Marra-Biggs P, Fatherree J, Green A and Toonen R. 2022. Range Expansion and First Observation of *Tridacna noae* in American Sāmoa. Ecology and Evolution, 12: e9635. <https://doi.org/10.1002/ece3.9635>
- Mecha NJMF. 2024. First record of Noah's clam *Tridacna noae* (Röding, 1798) in Culion: An addition to the distribution range in Palawan, Philippines. The Palawan Scientist, 16(1): 81-84. <https://doi.org/10.6972/TPS.J.2024.16.1.09>
- Mecha NJMF and Dolorosa RG. 2020. Searching the virtually extinct *Tridacna gigas* (Linnaeus, 1758) in the Reefs of Palawan, Philippines. The Philippine Journal of Fisheries 27(1): 1–18. <https://doi.org/10.31398/tjpf/27.1.2019-0005>
- MFI (Malampaya Foundation Incorporated). 2020. First of the country's hatchery-grown Philippines' true *Tridacna gigas* clams makes its home in north Palawan waters. <https://www.malampayafoundation.org/news/first-of-the-countrys-hatchery-grown-philippines-true->

- [tridacna-gigas-clams-makes-its-home-in-north-palawan-waters](#). Accessed on 24 January 2023.
- Militz TA, Kinch J and Southgate PC. 2015. Population Demographics of *Tridacna noae* (Röding, 1798) in New Ireland, Papua New Guinea. *Journal of Shellfish Research*, 34(2): 329–335. <https://doi.org/10.2983/035.034.0215>
- Morejohn K, Ainley L, Williamson J, Nevatte R, Liggins L, Cumbo VR and Gillings M. 2023. Genetic confirmation of *Tridacna noae* (Röding 1798) in the Cook Islands. *Coral Reefs*, 42(6): 1343–1350. <https://doi.org/10.1007/s00338-023-02432-1>
- Neo ML. 2023. A Field Guide to Giant Clams of the Indo-Pacific. World Scientific Publishing Co. Pte. Ltd. Singapore. 201pp.
- Neo ML, Eckman W, Vicentuan K, Teo SM and Todd PA. 2015. The ecological significance of giant clams in coral reef ecosystems. *Biological Conservation*, 181: 111–123. <https://doi.org/10.1016/j.biocon.2014.11.004>
- Neo ML, Liu LL, Huang D and Soong K. 2018. Thriving populations with low genetic diversity in giant clam species, *Tridacna maxima* and *Tridacna noae*, at Dongsha Atoll, South China Sea. *Regional Studies in Marine Science*, 24: 278–287. <https://doi.org/10.1016/j.rsma.2018.09.001>
- Neo ML and Low JKY. 2017. First observations of *Tridacna noae* (Röding, 1798) (Bivalvia: Heterodonta: Cardiidae) in Christmas Island (Indian Ocean). *Marine Biodiversity*, 48: 2183–2185. <https://doi.org/10.1007/s12526-017-0678-3>
- PCSD (Palawan Council for Sustainable Development). 2017. PCSD Resolution No. 15-52. Approving the 2014 Updated List of Terrestrial and Marine Wildlife in Palawan and their Categories Pursuant to Republic Act 9147, Otherwise known as the Wildlife Resources Conservation and Protection Act of 2001. <https://pcsd.gov.ph/pcsd-resolution-no-15-52/> Access on 20 January 2024.
- Rosewater J. 1965. The family Tridacnidae in the Indo-Pacific. *Indo-Pacific Mollusca*, 1: 347–396.
- Sandalo RM and Baltazar T. 1997. The Palawan biosphere reserve (Philippines). UNESCO (South Cooperation Program), Paris (France). Working Paper No. 19. 32pp.
- Su Y, Hung JH, Kubo H, Liu LL. 2014. *Tridacna noae* (Röding, 1798) – A valid giant clam species separated from *T. maxima* (Röding, 1798) by morphological and genetic data. *Raffles Bulletin of Zoology*, 62: 124–135.
- Triandiza T, Zamani NP, Madduppa H and Hernawan UE. 2019. Distribution and abundance of the giant clams (Cardiidae: Bivalvia) on Kei Islands, Maluku, Indonesia. *Biodiversitas*, 20(3): 884–892. <https://doi.org/10.13057/biodiv/d200337>
- UNESCO (United Nations Educational, Scientific and Cultural Organization). 2021. Palawan (Philippines). World Network of Island and Coastal Biosphere Reserves. <http://www.islandbiosphere.org/Contingut.aspx?IdPub=789>. Accessed on 20 January 2024.
- Vogel ML and Hoeksema BW. 2024. The role of aquaculture in the international trade of giant clams (Tridacninae) for the aquarium industry (2001–2019). *Aquaculture*, 583: 740563. <https://doi.org/10.1016/j.aquaculture.2024.740563>
- Wabnitz CCC, Taylor ML, Green E and Razak T. 2003. From Ocean to Aquarium: The Global Trade in Marine Ornamental Species. UNEP-WCMC, Cambridge, UK. 64pp.

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# Relative gene expression of calcium binding protein in *Dendrobium bigibbum* Lindl. symbiotically grown with basidiomycetes

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## ABSTRACT

*Dendrobium bigibbum* Lindl. is one of the native species of orchids in the Philippines. Its pharmaceutical potentials have already been explored in Asian countries. Meanwhile, its seed is one of the major constraints for seed germination and mycorrhizal association with compatible basidiomycetes influences the germination of the seeds. In the formation of mycorrhizal association, calcium signaling is one of the initial responses of the plants wherein calcium serves as secondary messenger which triggers physiological functions and changes in the plants and the fungal symbionts as well. In this article, the molecular expression of calcium binding protein was determined in two stages of developments (rhizoid and seedling stage). In vitro cultivation of *D. bigibbum* with selected basidiomycetes were extracted. Quantitative Reverse Transcriptase - Polymerase Chain Reaction was used to determine the Cycle Threshold values. Results revealed the upregulation of the calcium binding proteins which indicates the elevation of calcium concentration, thus, there is a presence of plant-fungal interaction.

**Keywords:** calcium, orchids, secondary messenger, symbiosis

## INTRODUCTION

Orchids is one of the diverse groups of ornamental plants with unique morphological characteristics to attract pollinators forming fungal association for its propagation. Also, most orchids species have medicinal and ornamental properties (Dressler 1993; Cox et al. 1998). *Dendrobium* is among the genus of Orchidaceae with diverse medicinal properties such as against angiogenic, diabetes, cataract, neurological diseases and other disease caused by pathogens (Da Silva and Ng 2017).

*Dendrobium* is a genus of orchids which can be epiphytic, lithophytic or terrestrial (Nontachaiyapoom et al. 2011). In a study of Bautista

and Valentino (2023), the size of *Dendrobium bigibbum* Lindl. embryo ranges from 30.5- 40.25  $\mu\text{m}$  in diameter. Accordingly, for the seed germination to proceed, orchidaceous mycorrhizal association is a necessity (Rasmussen and Rasmussen 2014; Smith and Read 2008).

Bautista and Valentino (2023), revealed that symbiotic association of *D. bigibbum* with the three species of basidiomycetes (*Volvariella volvacea* (Bull.) Singer, *Lentinus tigrinus* (Bull.) Fr., and *Pleurotus florida* Eger), enhanced its germination and growth. The calcium binding proteins responsible for the activation of calcium influx plays important role in the initial establishment of plant fungal symbiotic relationship. The calcium binding proteins are



activated during pre-symbiotic phase which as act marker genes for upstream signaling of calcium are activated and upregulated by fungal in the cortical regions of the plant (Herrbach et al. 2014; Mohanta and Bae 2015). Additionally, they regulate various physiological functions in plant pathogen interaction, plasma membrane mediated responses and plant perceive pathogen/ microbe associated molecular patterns (Luan and Wang 2021).

Transcription factors of calcium binding factors are known to be  $Ca^{2+}$ /calmodulin (CaM)-regulated transcription factors and both have a CaM binding domain which are known to mediate plant immunity (Galon et al. 2008). During symbiosis, the  $Ca^{2+}$ /CaM-dependent protein kinase (CCaMK) plays an essential role in the interpretation of symbiotic  $Ca^{2+}$  signaling in the nucleus for the establishment of symbiotic responses (Gleason et al. 2006; Yuan et al. 2017). Accordingly, calcium acts as a secondary messenger and core regulator during signal transduction following variation in biotic and abiotic factors which can be beneficial or antagonistic in nature cascading physiological and cellular responses (Dodd et al. 2010). The study aimed to determine the expression of calcium-binding proteins in *D. bigibbum* symbiotically grown with three selected fungi.

## METHODS

### In Vitro Propagation of *D. bigibbum*

Seeds from self-pollinated *D. bigibbum* capsule were used. The capsule was harvested 120 days after visible signs of successful pollination (with enlarged ovary and wilting of flowers). Knudson Orchid Medium (Morel Modification) with modifications (Bautista and Valentino 2023) was used as the growth medium for the in vitro culture of *D. bigibbum*. The seeds were allowed to germinate and develop until rhizoid and seedling stage.

### Maintenance of Fungal Inocula

Pure cultures of *V. volvacea*, *L. tigrinus*, and *P. florida* obtained from the culture collections of Center for Tropical Mushroom Research and Development were grown in a Potato Dextrose Agar (PDA) until the plates were fully colonized with mycelia.

### Co-culture Technique

Co-culture technique was carried out by laying filter paper strips in a plate fully colonized with fungal inoculum. The culture plates were then incubated for 30 days at  $25 \pm 2^\circ C$ , relative humidity of 23.0% with a photoperiod of 16/8-hours light/dark (Utami and Hariyanto 2019; Chen et al. 2020). For the seedling stage, twenty *D. bigibbum* protocorms with

first leaf (90 days after in vitro germination) were selected. These were seeded in plates fully colonized with fungal mycelia. Cultures were incubated for 45 days at  $25 \pm 2^\circ C$  with a photoperiod of 16/8-hours light/dark (Chen et al. 2020; Zhang et al. 2020).

### Morphological Characterization

The *D. bigibbum* were observed morphologically using dissecting microscope. The plant size was recorded and the appearance of *D. bigibbum* was also noted.

### RNA Extraction

Total RNA extraction of *D. bigibbum* during rhizoid and seedling stages were done using the RNeasy Plant Mini Kit (Qiagen, Germany) following the manufacturer's protocol. Fifty milligrams (50 mg) of *D. bigibbum* samples were grinded in liquid nitrogen. The powdered tissue was placed in a 2 mL microcentrifuge tubes and 500  $\mu L$  of RNA lysis buffer was added. It was then vortexed and transferred in a QIAshredder spin column (lilac). The lysate was cleared and adjusted to binding conditions, and was transferred to a RNeasy Mini spin column (pink). Contaminants were eliminated by adding 700  $\mu L$  RNA wash buffer, 500  $\mu L$  second RNA wash buffer with Ethanol. Centrifugation was done in each stage at 10,000 rpm for two minutes. After which, the RNA was eluted in 50  $\mu L$  RNase-free water and placed in a microcentrifuge tube. The extracted RNA was stored at  $-80^\circ C$  ultralow freezer (QIAGEN 2024).

### Detection of Total RNA

The hardened 1% agarose gel was placed into the gel tank filled with  $1 \times TAE$  buffer. Three  $\mu L$  of RNA samples were stained with 2x loading dye and were loaded into the agarose gel. The molecular ladder was also loaded in one of the wells of the gel for detection and quantification of the size of the molecule. The power supply was programmed and run at 1-5V/cm between electrodes for 30 minutes. After electrophoresis, the gel was removed from the tray and the excess buffer was drained. It was placed in a computer-controlled gel documentation system. The image was captured and exported into file (Lee et al. 2015).

### RNA Quantification

Ribonucleic Acid quantification was performed using photometric nucleic acid quantification using Multi Skan  $\mu$ drop plate. The  $\mu$ drop plate was cleaned with sterile distilled water and 2  $\mu L$  of RNA stock solution was placed into the wells of the low-measurement area of the  $\mu$ drop plate. The plate was loaded into the Multi skan instrument and the template was laid out using the Multi SkanIt software (Thermo Fisher Scientific Corporation 2015).

The absorbance at 260 nm was recorded and the amount of RNA was calculated using the formula:

$$\text{RNA concentration } (\mu\text{g/mL}) = \text{Abs}_{260} \times 40 \mu\text{g/mL} \times 20$$

Based from the computation the following concentration of RNA obtained were 461.952  $\mu\text{g/mL}$  (*D. bigibbum* with *V. volvacea*), 218.688  $\mu\text{g/mL}$  (*D. bigibbum* with *L. tigrinus*), 348.48  $\mu\text{g/mL}$  (*D. bigibbum* with *P. florida*) and 310.248  $\mu\text{g/mL}$  (asymbiotically grown *D. bigibbum*)

### Gene Expression Analysis

ViPrimePLUS One Step Taq RT-qPCR Green Master Mix I was used for qrt-PCR following the manufacturer's guide using the Step One instrument (Applied Biosystem, USA) Each reaction contained 5 $\mu\text{L}$  Taq One Step RT-qPCR Green Master Mix I, 1 $\mu\text{L}$  primer/probe mix, 2.5 $\mu\text{L}$  RNA (25ng), and 2 $\mu\text{L}$  nuclease free water. The primer used for calcium binding protein (Forward-GTCGTTGGAGAATACGAAGAG; Reverse-TGTGCGTCGTGAGATCCAGATAATG) while EF-1 $\alpha$  (Forward-TGTGGTCCCAATCATCCC; Reverse-ATAGGCTTGCTGGGAACC) served as the housekeeping gene. The 10  $\mu\text{L}$  of prepared master mix was loaded into a 0.2 PCR multiwell plate and sealing film was used to cover the multi-well plate to prevent evaporation when heated. The multi-well plate was loaded in the Step One Instrument RT-PCR platform and was set up based from the optimized standard cycling conditions EF-1 $\alpha$  served as the housekeeping 36 gene to normalize transcript abundance. Each RT-qPCR assay was replicated thrice. Finally, the relative gene expression was calculated using the Livak method or the conventional  $2^{-\Delta\Delta\text{CT}}$  method (Livak and Schmittgen 2001; Chen et al. 2020). Livak method for relative gene expression analysis assumes 100% and within 5% efficiencies of gene amplification of the target and reference genes. The Cycle threshold (Ct values) of the target and the reference genes were normalized using the formula  $\Delta\text{Ct}_{(\text{test})} = \text{Ct}_{(\text{target, test})} - \text{Ct}_{(\text{ref, test})}$ . Then, the  $\Delta\text{Cq}$  was normalized using the formula  $\Delta\Delta\text{Ct} = \Delta\text{Ct}_{(\text{test})} - \Delta\text{Ct}_{(\text{calibrator})}$ . Finally, the normalized gene expression was computed using the formula  $2^{-\Delta\Delta\text{Ct}}$ . Expression of gene is said to be upregulated when the computed fold change is statistically significantly higher as compared to the reference genes.

### Statistical Analysis

The study was laid out using completely randomized design. Test for difference was done using

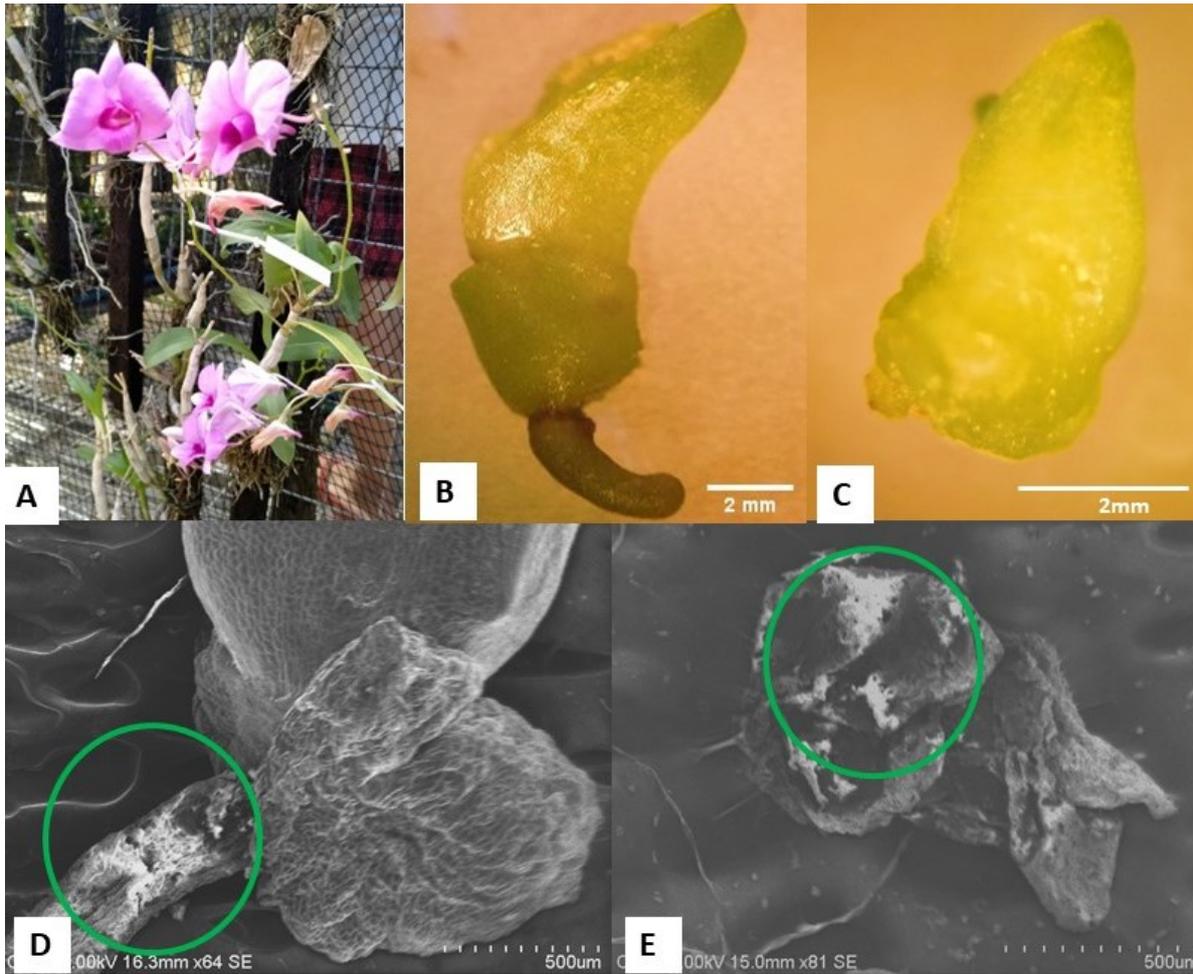
Analysis of Variance (ANOVA) and comparison among means by post-hoc test of homogenous subsets through Tukey's HSD test for the plant size and t-test for the expression of calcium binding protein. Significant level of difference was set at 0.05 level of significance.

## RESULTS

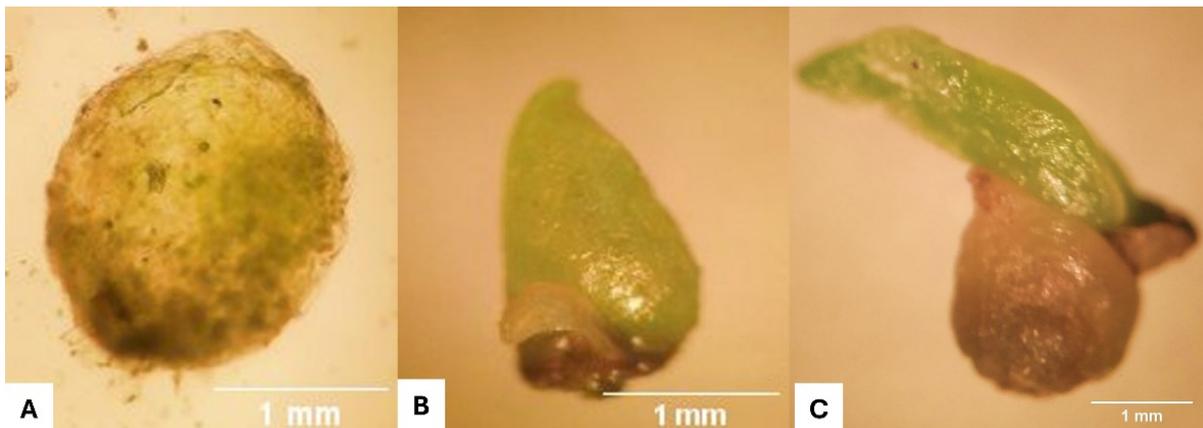
The expression of calcium binding protein (DoCML19- *Dendrobium*\_GLEAN\_10016982) was analyzed in rhizoid and seedling stages of *D. bigibbum* symbiotically grown with the selected basidiomycetes. During early stages of seed germination, the protocorm formation indicating the symbiotic association was observed. Additionally, protocorm responds to the fungal hyphae that enters the embryo at the basal end. As seen in Figure 1(D, E) via Scanning Electron Microscope, mycelial colonization is evident on the surface of the roots of the *D. bigibbum* seedlings and on the protocorm during the rhizoid stage.

In addition, morphological assessment of *D. bigibbum* in terms of plant size also revealed that *D. bigibbum* co-cultured with the selected fungi were significantly higher as compared to asymbiotically grown *D. bigibbum* both in rhizoid and seedling stages (Table 1). Morphologically and symbiotically grown *D. bigibbum* are vibrant green in color, intact leaf and roots (Figure 1). Meanwhile, as shown in Figure 2, asymbiotically grown *D. bigibbum* are dull green in color, and the lower parts of the protocorm is colored brown which is an indication of oxidation and early senescence of *D. bigibbum*. These coincide with the expression of calcium binding proteins. Presented in Figure 3 and Table 2 is the relative gene expression of calcium binding protein in *D. bigibbum*. In rhizoid stage, the expression of gene in terms of fold change was significant higher when co-cultured with *V. volvacea* (5.20), *L. tigrinus* (3.93) and *P. florida* (1.67) as compared to the asymbiotically cultured *D. bigibbum* (1.01). Similarly in seedling stage, upregulation of gene was recorded with 40.34, 28.70- and 5.83-fold change in *V. volvacea*, *P. florida* and *L. tigrinus*, respectively. Based from the statistical analysis, in both rhizoid and seedling stage, the calcium binding protein was upregulated when grown symbiotically as compared to asymbiotically grown *D. bigibbum*.

Upregulation of calcium-binding protein was observed in all *D. bigibbum* co-cultured with basidiomycetes both in rhizoid and seedling stage, however, relative expression is noticeably higher during seedling stage.



**Figure 1.** The *Dendrobium bigibbum*. A) *in situ*; B) seedling stage under dissecting microscope; C) rhizoid stage under dissecting microscope; D) seedling with fungal colonization on the root under scanning electron microscope; E) rhizoid stage with fungal colonization at the protocorm under scanning electron microscope.



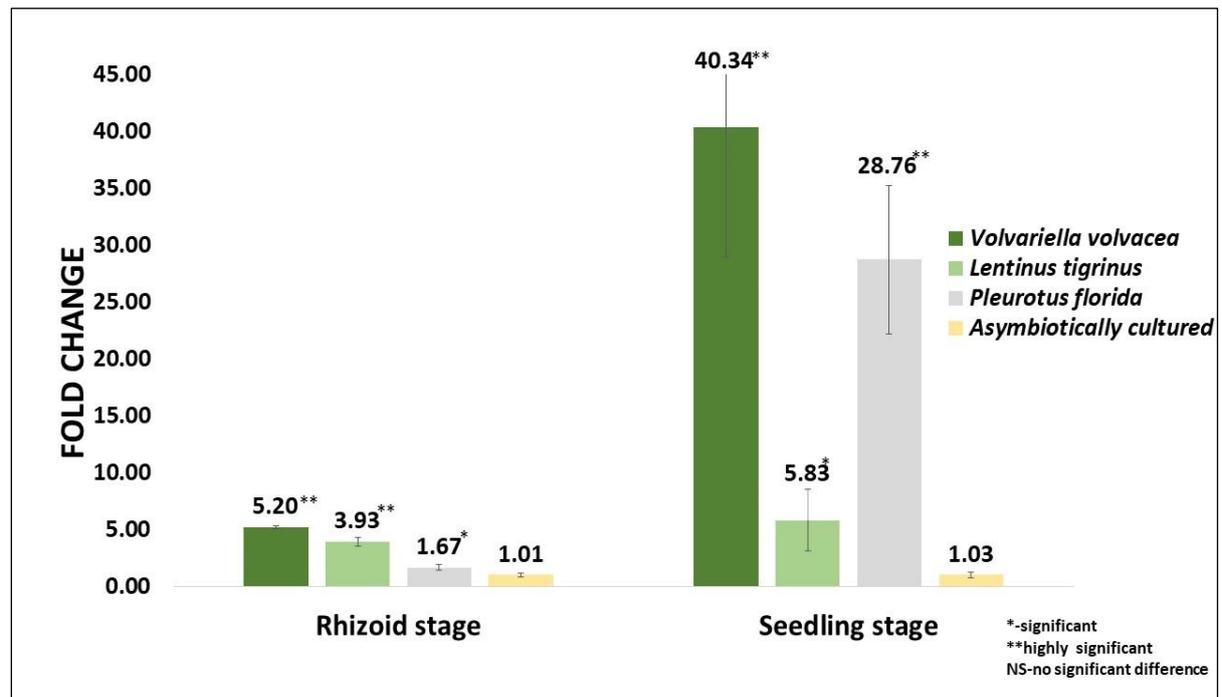
**Figure 2.** Asymbiotically grown *Dendrobium bigibbum*. A) germinated protocorm; B) rhizoid stage under dissecting microscope; C) seedling stage under dissecting microscope.

**Table 1.** Plant size of *Dendrobium bigibbum* in rhizoid and seedling stage (mm). \*Values are the Mean ± SD. Means within a column having the same letter of superscript are insignificantly different from each other at 0.05 level of significance using Tukey’s test.

Treatments	Rhizoid stage	Seedling stage
<i>D. bigibbum</i> co-cultured with <i>V. volvacea</i>	7.22 ± 0.932 <sup>a</sup>	18.59 ± 0.893 <sup>a</sup>
<i>D. bigibbum</i> co-cultured with <i>L. tigrinus</i>	4.35 ± 0.667 <sup>b</sup>	16.23.74 ± 0.594 <sup>a</sup>
<i>D. bigibbum</i> co-cultured with <i>P. florida</i>	5.13 ± 0.265 <sup>bc</sup>	14.32 ± 0.890 <sup>ab</sup>
Asymbiotically cultured in PDA	3.83 ± 0.989 <sup>c</sup>	12.86 ± 0.574 <sup>b</sup>

**Table 2.** Expression level of DOCML19 (calcium-binding protein) in rhizoid and seedling stage of *Dendrobium bigibbum*. \*Values are the Mean ± SD. Means within a column having \*\* are highly significant and \* are significantly different from the reference gene at 0.05 level of significance; † indicates upregulation of the DOCML19 gene.

Treatments	Rhizoid Stage	Seedling Stage
<i>D. bigibbum</i> co-cultured with <i>V. volvacea</i>	5.20 ± 0.120479 <sup>**†</sup>	40.34±11.38749 <sup>**†</sup>
<i>D. bigibbum</i> co-cultured with <i>L. tigrinus</i>	3.93 ± 0.414109 <sup>**†</sup>	5.83±2.685932 <sup>*†</sup>
<i>D. bigibbum</i> co-cultured with <i>P. florida</i>	1.670.24547 <sup>* †</sup>	28.76 ± 6.52658 <sup>**†</sup>
Asymbiotically grown <i>D. bigibbum</i>	1.01 ± 0.3509	1.030 ± 0.7841



**Figure 3.** Expression level of DOCML19 (calcium-binding protein) in rhizoid and seedling stage of *Dendrobium bigibbum*.

**DISCUSSION**

There are different stages for the formation of mycorrhizal association of orchids and fungi, such as the attraction of the symbiont, initial contact, initial fungal colonization, proliferation of the fungal hyphae within the orchid tissues, and colonization of the cortical cells. Mycorrhizal symbiosis produces signaling molecules, such as strigolactones from plant

roots, attracting fungi and stimulating hyphal branching (Akiyama et al. 2005; Kretzschmar et al. 2012; Valadares et al. 2012). Initial contact takes place when a compatible symbiont accesses the plant tissues with the formation of appressorium prior to hyphal penetration and fungal entry to the protocorm via the suspensor which occurs enzymatically (Smith and Read 2008). Lastly, the cortical cells will be colonized by the symbiont; this involves invagination of plant

plasma membrane, cytoskeletal rearrangements, nuclear change, other organelles change, peloton formation, and interfacial matrix formation (Perotto et al. 2014).

During this time, the change in cytoskeleton causes cortical microtubule depolarization, nuclear movement, and realignment to the peloton hyphae (Genre et al. 2005). Also, cytoplasmic changes trigger the breakdown of starch leading to metabolites' extensive membrane remodeling in colonized cells for plant metabolism and defense via the production of enzymes, such as polyphenol oxidase, peroxidase, and catalase, which are known as antioxidants (Valadares et al. 2012). Meanwhile, peloton functions to provide the nutrient transfer area between the symbionts where there is a high polyphenol oxidase activity for the breaking down of phytoalexins. There will then be a production of interfacial matrix by the orchids which is marked by the formation of callose, cellulose, and pectin around the collapsing pelotons, followed by septal disintegration. During these events, calcium act as secondary messenger (Bonfante 2001; Armstrong and Peterson 2002; Yi and Valent 2013).

Based from the studies of Dearnaley et al. (2007) and Chen et al. (2020), upregulation of calcium binding protein depicts the increase of cytosolic calcium which is an initial sign of infection or the host plant can be challenged by mutualistic or pathogenic partners. Accordingly, increase in calcium plays vital role in plant immunity by regulating the Salicylic acid biosynthesis via the positive regulation of isochorismate synthase 1(ICS1) and enhanced disease susceptibility 1(EDS1) (Dodd et al. 2010; Kudla et al. 2010; Seybold et al. 2014; Tsuda and Somssich 2015). Transcription factors of calcium binding factors are known to be Ca<sup>2+</sup>/calmodulin (CaM)- regulated transcription factors and both have a CaM binding domain which are known to mediate plant immunity (Galon et al. 2008; Kim et al. 2009; Wang et al. 2009; Zhang et al. 2010; Reddy et al. 2011; Bickerton and Pittman 2012).

During symbiosis, the Ca<sup>2+</sup>/CaM-dependent protein kinase (CCaMK) function in initiation of symbiotic responses by Ca<sup>2+</sup> signaling in the nucleus (Gleason et al. 2006; Yuan et al. 2017). In the process, Nod A factor which is responsible for the nodulation, activates the calcium binding proteins which in return induces the nodulation (Gleason et al. 2006). Similarly, the increment in calcium concentration can be attributed to the formation of root symbiosis, where the secondary metabolites, flavonoids such as luteolin and naringenin are being release for the biosynthesis of Nod factor (Moscatiello et al. 2010; Cui et al. 2019). Additionally, Reid et al. (2016, 2017) suggested that pre-interaction activity in symbiotic association, root-nodule symbiosis and cytokinin biosynthesis is regulated by Ca<sup>2+</sup> signaling.

According to Wang et al. (2020), calcium is regulated by GTPases during the process of symbiosis. Wherein, the calcium channels are activated the radical oxygen species that cascades calcium influx for plant immune responses. Moreover, as soon as interaction and recognition between the host plants and the microorganisms occur the intracellular calcium levels increase and minimal change in the calcium concentration could greatly affect the physiological activity and interaction of organisms (Harper and Harmon 2005; Charpentier et al. 2008; Mazars et al. 2009). Once the calcium level increased, it cannot be synthesized nor degraded thus the efflux and the influxes' reaction must maintain homeostasis (Lecourieux et al. 2002).

Meanwhile the difference on the concentration of calcium binding protein in seedling and rhizoid stage can be attributed to the specificity of calcium responses. Moreover, different species of orchids may interact with mycorrhizal association at different phases, some during the early phase of germination while others during the protocorm to adult phase of development (Rasmussen and Rasmussen 2014). Fungi that contribute to the development of orchids include the *Rhizoctonia*-like fungi complex, wood or litter-decomposing fungi, and fungal endophytes (Da Silva et al. 2015; Herrera et al. 2017). They serve as sources of minerals and nutrients in partially mycoheterotrophic orchids until the first green leaf emerges, while autotrophic orchids are less dependent on mycorrhizal association once the roots and leaves are formed (Herrera et al. 2017). Also, upon the host penetration of fungal symbiont, biosynthesis of hormones by the host plant in the infected tissues and the production of hormones by the infecting fungi may also take place, which aids in the development of the host (Robert-Seilaniantz et al. 2007). Fungal symbionts may have little effect on seed germination but may significantly increase the growth of orchids during the seedling stage. However, different fungal symbionts have also different effects on growth and development which can be attributed mainly due to development-dependent specificity (Zhang et al. 2020).

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## ETHICAL CONSIDERATIONS

The study is exempted from any ethics guidelines. Experimental animals are not involved in the study.

## DECLARATION OF COMPETING INTEREST

The author declares no conflict of interest.

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## REFERENCES

- Akiyama K, Matsuzaki K and Hayashi H. 2005. Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. *Nature*, 435: 824–827. <https://doi.org/10.1038/nature03608>
- Armstrong L and Peterson RL. 2002. The interface between the arbuscular mycorrhizal fungus *Glomus intraradices* and root cells of *Panax quinquefolius*: a Paris-type mycorrhizal association. *Mycologia*, 94: 587–595. <https://doi.org/10.1080/15572536.2003.11833187>
- Bautista NS and Valentino MJG. 2023. Symbiotic propagation of *Dendrobium bigibbum* Lindl. with selected saprophytic Basidiomycota. *Biodiversitas*, 24(6): 3519–3527. <https://doi.org/10.13057/biodiv/d240650>
- Bickerton PD and Pittman JK. 2012. Calcium signaling in plants. In: eLS. Chichester: John Wiley & Sons Ltd. <https://doi.org/10.1002/9780470015902.a0023722>
- Bonfante P. 2001. At the interface between mycorrhizal fungi and plants: the structural organization of cell wall, plasma membrane and cytoskeleton. In: Esser K and Hock B (eds). *The Mycota IX*. Berlin, Heidelberg: Springer-Verlag. 45–61 pp. [https://doi.org/10.1007/978-3-662-07334-6\\_4](https://doi.org/10.1007/978-3-662-07334-6_4)
- Charpentier M, Bredemeier R, Wanner G, Takeda N, Schleiff E and Parniske M. 2008. *Lotus japonicus* CASTOR and POLLUX are ion channels essential for perinuclear calcium spiking in legume root endosymbiosis. *Plant Cell*, 20: 3467–3479. <https://doi.org/10.1105/tpc.108.063255>
- Chen J, Yan B, Tang Y, Xing Y, Li Y, Zhou D and Guo S. 2020. Symbiotic and asymbiotic germination of *Dendrobium officinale* (Orchidaceae) respond differently to exogenous gibberellins. *International Journal of Molecular Sciences*, 21: 6104. <https://doi.org/10.3390/ijms21176104>
- Cox AV, Abdelhour DG, Bennett MD and Leitch IJ. 1998. Genome size and karyotype evolution in the slipper orchids (Cypripedioideae: Orchidaceae). *American Journal of Botany*, 85: 681–687. <https://doi.org/10.2307/2446538>
- Cui L, Guo F, Zhang J, Yang S, Meng J, Geng Y, Li X and Wan S. 2019. Synergy of arbuscular mycorrhizal symbiosis and exogenous Ca<sup>2+</sup> benefits peanut (*Arachis hypogaea* L.) growth through the shared hormone and flavonoid pathway. *Scientific Reports*, 9(1): 16281. <https://doi.org/10.1038/s41598-019-52630-7>
- Da Silva JAT and Ng TB. 2017. The medicinal and pharmaceutical importance of *Dendrobium* species. *Applied Microbiology and Biotechnology*, 101(6): 2227–2239. <https://doi.org/10.1007/s00253-017-8169-9>
- Da Silva JAT, Tsavkelova JA, Zeng EA, Ng TB, Parthibhan S, Dobránszki J, Cardoso JC and Rao MV. 2015. Symbiotic in vitro seed propagation of *Dendrobium*: fungal and bacterial partners and their influence on plant growth and development. *Planta*, 242: 1–22. <https://doi.org/10.1007/s00425-015-2301-9>
- Dearnaley JDW. 2007. Further advances in orchid mycorrhizal research. *Mycorrhiza*, 17: 475–486. <https://doi.org/10.1007/s00572-007-0138-1>
- Dressler RL. 1993. *Phylogeny and Classification of the Orchid Family*. Cambridge University Press, University of Cambridge. 330pp.
- Dodd AN, Kudla J and Sanders D. 2010. The language of calcium signaling. *Annual Review of Plant Biology*, 61: 593–620. <https://doi.org/10.1146/annurev-arplant-070109-104628>
- Galon Y, Nave R, Boyce JM, Nachmias D, Knight MR and Fromm H. 2008. Calmodulin-binding transcription activator (CAMTA) 3 mediates biotic defense responses in *Arabidopsis*. *Federation of European Biochemical Societies Letters*, 582: 943–948. <https://doi.org/10.1016/j.febslet.2008.02.037>
- Genre A, Chabaud M, Timmers T, Bonfante P and Barker DG. 2005. Arbuscular mycorrhizal fungi elicit a novel intracellular apparatus in *Medicago truncatula* root epidermal cells before infection. *The Plant Cell*, 17: 3489–3499. <https://doi.org/10.1105/tpc.105.035410>
- Gleason C, Chaudhuri S, Yang T, Munoz A, Pooviah BW and Oldroyd GED. 2006. Nodulation independent of rhizobia induced by a calcium-activated kinase lacking autoinhibition. *Nature*, 441(7097): 1149–1152. <https://doi.org/10.1038/nature04812>
- Harper JF and Harmon A. 2005. Plants, symbiosis and parasites: a calcium signaling connection. *Nature Reviews Molecular Cell Biology*, 6: 555–566. <http://dx.doi.org/10.1038/nrm1679>
- Herrbach V, Rembliere C, Gough C, Bensmihen S. 2014. Lateral root formation and patterning in *Medicago truncatula*. *Journal of Plant Physiology*, 171(3–4): 301–310. <https://doi.org/10.1016/j.jplph.2013.09.006>
- Herrera HR, Valadares D, Contreras D, Bashan Y and Arriagada C. 2017. Mycorrhizal compatibility and symbiotic seed germination of orchids from the Coastal Range and Andes in south central Chile. *Mycorrhiza*, 27: 175–188. <https://doi.org/10.1007/s00572-016-0733-0>
- Kim MC, Chung WS, Yun DJ and Cho MJ. 2009. Calcium and calmodulin mediated regulation of gene expression in plants. *Molecular Plant*, 2: 13–21. <https://doi.org/10.1093/mp/ssn091>
- Kretzschmar W, Kohlen J, Sasse L, Borghi M, Schlegel JB, Bachelier D, Reinhardt R, Bours HJ, Bouwmeester E and Martinoia E. 2012. A petunia ABC protein controls strigolactone- dependent symbiotic signalling and branching. *Nature*, 483: 341–344. <https://doi.org/10.1038/nature10873>
- Kudla J, Batistic O and Hashimoto K. 2010. Calcium signals: The lead currency of plant information processing. *The Plant Cell*, 22: 541–563. <https://doi.org/10.1105/tpc.109.072686>
- Lecourieux D, Mazars C, Pauly N, Ranjeva R and Pugin A. 2002. Analysis and effects of cytosolic free calcium increases in response to elicitors in *Nicotiana plumbaginifolia* cells. *Plant Cell*, 14: 2627–2641. <https://doi.org/10.1105/tpc.005579>
- Lee YI, Yang CK and Gebauer G. 2015. The importance of associations with saprotrophic non-Rhizoctonia fungi among fully mycoheterotrophic orchids is currently under-estimated: novel evidence from sub-tropical Asia. *Annals of Botany*, 116: 423–435. <https://doi.org/10.1093/aob/mcv085>
- Livak KJ and Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2<sup>-</sup>(Delta Delta C(T)) Method. *Methods*, 25: 402–408. <https://doi.org/10.1006/meth.2001.1262>
- Luan S and Wang C. 2021. Calcium signaling mechanisms across kingdoms. *Annual Review Cell and Developmental Biology*, 37: 311–340. <https://doi.org/10.1146/annurev-cellbio-120219-035210>
- Mazars C, Bourque S, Mithofer A, Pugin A and Ranjeva R. 2009. Calcium homeostasis in plant cell nuclei. *New Phytologist*, 181: 261–274. <https://doi.org/10.1111/j.1469-8137.2008.02680.x>
- Moscatiello R, Squartini A, Mariani P and Navazio L. 2010. Flavonoid-induced calcium signalling in rhizobium

- Leguminosarum* *bv. viciae*. *New Phytologist*, 188(3): 814–823. <https://doi.org/10.1111/j.1469-8137.2010.03411.x>
- Mohanta TK and Bae H. 2015. Functional genomics and signaling events in mycorrhizal symbiosis. *Journal of Plant Interactions*. 10(1): 21–40. <https://doi.org/10.1080/17429145.2015.1005180>
- Nontachaiyapoom S, Sasirat S and Manoch L. 2011. Symbiotic seed germination of *Grammatophyllum speciosum* Blume and *Dendrobium draconis* Rchb. f, native orchids of Thailand. *Scientia Horticulturae*, 130: 303–308. <https://doi.org/10.1016/j.scienta.2011.06.040>
- Perotto S, Rodda M, Benetti A, Sillo F, Ercole E, Rodda M, Girlanda M, Murat C and Balestrini R. 2014. Gene expression in mycorrhizal orchid protocorms suggests a friendly plant–fungus relationship. *Planta*, 239: 1337–1349. <https://doi.org/10.4161/15592324.2014.977707>
- Rasmussen HN and Rasmussen FN. 2014. Seedling mycorrhiza: a discussion of origin and evolution in Orchidaceae. *Botanical Journal of the Linnean Society*, 175: 313–327. <https://doi.org/10.1111/boj.12170>
- Reddy AS, Ali GS, Celesnik H and Day IS. 2011. Coping with stresses: roles of calcium- and calcium/calmodulin-regulated gene expression. *Plant Cell*, 23: 2010–2032. <https://doi.org/10.1105/tpc.111.084988>
- Reid D, Nadzieja M, Novák O, Heckmann AB, Sandal N and Stougaard J. 2017. Cytokinin biosynthesis promotes cortical cell responses during nodule development. *Plant Physiology*, 175(1): 361–375. <https://doi.org/10.1104/pp.17.00832>
- Reid DE, Heckmann AB, Novak O, Kelly S and Stougaard J. 2016. Cytokinin oxidase/dehydrogenase3 maintains cytokinin homeostasis during root and nodule development in *lotus japonicus*. *Plant Physiology*, 170(2): 1060–1074. <https://doi.org/10.1104/pp.15.00650>
- QIAGEN. 2024. RNeasy Kits for RNA Purification. <https://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/rna-purification/total-rna/rneasy-kits>.
- Robert-Seilaniantz A, Grant M and Jones JD. 2011. Hormone crosstalk in plant disease and defense: more than just jasmonate-salicylate antagonism. *Annual Review of Phytopathology*, 49: 317–343. <https://doi.org/10.1146/annurev-phyto-073009-114447>
- Seybold H, Trempel F, Ranf S, Scheel D, Romeis T and Lee J. 2014. Ca<sup>2+</sup> signaling in plant immune response: from pattern recognition receptors to Ca<sup>2+</sup> decoding mechanisms. *New Phytologist*, 204: 782–790. <https://doi.org/10.1111/nph.13031>
- Smith SE and Read DJ. 2008. *Mycorrhizal Symbiosis*, 3rd edition, Academic Press, San Diego, USA. <https://doi.org/10.1016/B978-0-12-370526-6.X5001-6>
- Thermo Fisher Scientific Corporation. 2015. *Thermo Scientific Multiskan GO User Manual*. <https://assets.thermofisher.com/TFS-Assets/LCD/manuals/D01521~.pdf>.
- Tsuda K and Somssich IE. 2015. Transcriptional networks in plant immunity. *New Phytologist*, 206: 932–947. <https://doi.org/10.1111/nph.13286>
- Utami ES and Hariyanto S. 2019. In vitro seed germination and seedling Development of a rare Indonesian native orchid *Phalaenopsis amboinensis*. *Scientifica*, 8105138. <https://doi.org/10.1155/2019/8105138>
- Valadares RBS, Pereira MM, Otero JT and Cardoso EJ. 2012. Narrow fungal mycorrhizal diversity in a population of the orchid *Coppensia doniana*. *Biotropica*, 44: 114–122. <https://doi.org/10.1111/j.1744-7429.2011.00769.x>
- Wang L, Tsuda K, Sato M, Cohen JD, Katagiri F and Glazebrook J. 2009. *Arabidopsis* CaM binding protein CBP60g contributes to MAMP-induced SA accumulation and is involved in disease resistance against *Pseudomonas syringae*. *PLoS Pathogens*, 5: e1000301. <https://doi.org/10.1371/journal.ppat.1000301>
- Wang R, He F, Ning Y and Wang GL. 2020. Fine-tuning of RBOH-mediated ROS signaling in plant immunity. *Trends Plant Science*, 25(11): 1060–1062. <https://doi.org/10.1016/j.tplants.2020.08.001>
- Yi M and Valent B. 2013. Communication between filamentous pathogens and plants at the biotrophic interface. *Annual Review of Phytopathology*, 51: 587–611. <https://doi.org/10.1146/annurev-phyto-081211-172916>
- Yuan P, Jauregui E, Du L, Tanaka K and Poovaiah BW. 2017. Calcium signatures and signaling events orchestrate plant–microbe interactions. *Current Opinions in Plant Biology*, 38: 173–183. <https://doi.org/10.1016/j.pbi.2017.06.003>
- Zhang Y, Xu S, Ding P, Wang D, Cheng YT, He J, Gao M, Xu F, Li Y, Zhu Z et al. 2010. Control of salicylic acid synthesis and systemic acquired resistance by two members of a plant-specific family of transcription factors. *Proceedings of the National Academy of Sciences of United States of America*, 107(42): 18220–18225. <https://doi.org/10.1073/pnas.1005225107>
- Zhang Y, Li YY, Chen XM, Guo XS and Lee YI. 2020. Effect of different mycobionts on symbiotic germination and seedling growth of *Dendrobium officinale*, an important medicinal orchid. *Botanical Studies*, 61: 2. <https://doi.org/10.1186/s40529-019-0278-6>

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## Biological properties of *Dendrobium crumenatum* Sw. leaves

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### ABSTRACT

*Dendrobium crumenatum* Sw. is one of the native species of orchids in the Philippines, which is known for the aesthetic value of its blossoms. Additionally, it is used in traditional medicine, but few studies have been conducted. The present study was carried out to determine the biological properties of ethanolic extracts from *D. crumenatum* leaves, such as antioxidant, antibacterial, antifungal, cytotoxicity, and teratogenicity. The phytochemical composition was determined using the thin-layer chromatography (TLC) method. Results revealed that essential oils, phenols, fatty acids, anthraquinones, coumarins, anthrones, tannins, flavonoids, and steroids were present in the ethanolic extract of *D. crumenatum* leaves. An antioxidant property of 584.9 ug/mg and a total phenolic content of 496.139 mg GAE/g were also detected. Moreover, antibacterial property as a protectant was recorded against *Escherichia coli* Escherich, Th. 1885 and *Staphylococcus aureus* Rosenbach, 1884. Meanwhile, no antifungal property was observed in the ethanolic extract of *D. crumenatum* leaves. Regarding cytotoxicity, the results revealed a moderately toxic effect with LC<sub>50</sub> value of 341.680 ppm. Lastly, the ethanolic extract of *D. crumenatum* leaves caused the lethal effect on zebrafish embryo, such as embryo coagulation and absence of heartbeat, while its teratogenic effects included severe growth retardation, yolk deformity, and delayed embryo development. Thus, *D. crumenatum* leaf extract contains phytochemical constituents with moderate toxicity, depicting its pharmaceutical potential.

**Keywords:** cytotoxicity, eradicator, phytochemical, protectant, teratogenicity

### INTRODUCTION

*Dendrobium* is a diverse and widespread group of flowering plants that belongs to the Orchidaceae family. With a total of 1,556 recognized species, it holds a significant position within this botanical family (Ram et al. 2021; Wang 2021). It is an epiphytic orchid native to the lowland tropics of Southeast Asia.

Its natural range extends from India and mainland Southeast Asia through the Malay Archipelago to the Philippines. In the Philippines, this species is one of the most commonly encountered orchids. It can be found growing on host trees at sea level up to about 500 meters in elevation (Kurniawan and Amelia 2021). Its ability to colonize disturbed habitats and its rapid growth contribute to its abundance in both natural



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forest settings and urban areas where host trees are abundant (Tabla and Vargas 2004). Due to its attractive and fragrant white flowers, it is widely cultivated and appreciated as an ornamental plant in the Philippines.

It is known in Asian countries as a traditional tonic since time immemorial, and it is also considered one of the 50 fundamental herbs used to treat various ailments (Cakova et al. 2017). Moreover, the stems of *Dendrobium* are beneficial to the stomach and the production of body fluids, nourishing yin, and clearing heat (Hu et al. 2012; Sliwinski et al. 2022). In the Philippines, it is used as a traditional medicine, macerated in coconut oil and applied as an ointment for skin diseases, wounds, ulcers, boils, and burns (Duenas-Lopez 2022). Moreover, Klongkumnuankarn et al. (2015) suggested that *Dendrobium* contains various bioactive compounds, including polysaccharides, alkaloids, flavonoids, and phenols, which may have antioxidant, anti-inflammatory, and immunomodulatory effects. Given the potential health benefits of *Dendrobium*, the study was carried out to address the research gap in exploring the potential of *Dendrobium crumenatum* Sw. leaves as a source of bioactive compounds for the development of novel pharmaceuticals and cosmetics. The present study focused on the antibacterial, antifungal, teratogenic, and cytotoxic properties of *D. crumentatum* leaf extracts.

## METHODS

### Collection and Extraction of *D. crumenatum* Leaves

The *D. crumenatum* plant (Figure 1) was gathered from Antipolo City, Philippines. Leaves free of disease were selected, washed with tap water, and then air-dried to ensure the complete removal of excess moisture. Subsequently, the dried leaves were grinded into a coarse powder using a blender. Twenty grams of the powdered leaves were extracted through a 72-hour maceration process using 100 mL of 95% ethanol at ambient temperature. The mixture was agitated for 15 minutes for every 12 hours. The solution was filtered using Whatman filter paper no. 1, and the solvent was removed via rotary evaporation (Chimsook 2016).

### Phytochemical Composition and Antioxidant Property of *D. crumenatum* Leaves

Phytochemical screening was carried out using thin-layer chromatography (TLC) on a 7 × 4 cm plate in a 16 mm ethyl acetate-chloroform (7:3) solution under UV light to visualize separation of



**Figure 1.** *Dendrobium crumenatum* on its natural habitat.

different compounds. Several reagents were also used for the detection of secondary metabolites, such as vanillin-sulfuric acid reagent (phenols, sterols, triterpenes, and essential oils), metallic potassium hydroxide (anthraquinones, coumarins, and anthrones), ferricyanide-ferric chloride reagent (phenolic compounds and tannins), Dragendorff's reagent (alkaloid), and antimony (III) chloride (flavonoids). The total phenolic content was determined using the Folin-Ciocalteu method, where gallic acid served as the standard. The total phenolic content in the ethanol extracts was quantified and expressed in terms of grams of gallic acid equivalents (gGAE) per 100 g of extract. Lastly, 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay was used for antioxidant detection. For the assay, a standard solution comprising 3 mL of DPPH and 100  $\mu$ L of methanol was placed in a test tube and incubated in complete darkness for 30 minutes. Absorbance was measured at 517 nm. The percentage of antioxidant activity or radical scavenging activity was calculated using the following formula:

$$\% \text{ Antioxidant activity} = [(Ac - As) \div Ac] \times 100,$$

where *Ac* represents the absorbance of the control (DPPH solution without extract), and *As* denotes the absorbance of the test sample (mixture of DPPH, ethanol, and crude extract). Catechin, a synthetic antioxidant, was used as a positive control.

### Bioassay of Antimicrobial Property

The antibacterial and antifungal properties of *D. crumenatum* were tested against *Escherichia coli* Escherich, Th. 1885; *Staphylococcus aureus*

Rosenbach, 1884; and *Aspergillus flavus* Link, 1809, following the protocol of Valentino et al. (2015) and Austria et al. (2017). Sterile distilled water served as the negative control, while streptomycin (for the antibacterial test) and nystatin (for the anti-fungal test) served as positive controls through the disc diffusion assay. For the eradicator test, paper discs were soaked in pure crude extracts and were seeded equidistantly in Mueller-Hilton agar plates streaked with cultures of *E. coli* and *S. aureus* in separate plates. For the protectant test, the filter paper discs were soaked with the test pathogens and seeded in plates flooded with leaf extracts. The zones of colonization (protectant) and zones of inhibition (eradicator) were measured at 12-hour and 24-hour observation intervals. For the antifungal test, plates with potato dextrose agar were flooded with crude extracts, and the paper discs were soaked in *A. flavus* with a standardized spore count of  $1.5 \times 10^6$  spores/mL, grown in potato dextrose broth. The zone of mycelial colonization was observed at 24 and 48 hours of incubation.

#### Cytotoxicity Assay Brine

The brine shrimp lethality test was performed using 10,000 ppm, 5,000 ppm, 1,000 ppm, and 500 ppm *D. crumenatum* extracts. Each well of an enzyme-linked immunosorbent assay (ELISA) plate contained ten nauplii, and the survival count was recorded at 6-, 12-, 18-, and 24-hour intervals. The efficacy of the active component was assessed using Abbot's formula.

#### Teratogenic Property of *D. crumenatum* Leaves

The methodology for assessing the teratogenicity of *D. crumenatum* leaf ethanolic extracts was adopted from Lindain et al. (2018). Zebrafish were acclimatized at  $26 \pm 1^\circ\text{C}$ , and spawning was induced in 12 hours of darkness. Fertilized eggs were collected, and morphological uniformity assessment was performed before teratogenicity assay. Three embryos were placed in each well of ELISA plates containing 3 mL of each concentration of *D. crumenatum* leaf ethanolic extract and a control, together with three fertilized eggs. Mortality, hatchability, and morphological abnormalities were examined and determined using a compound microscope at 12-, 24-, 36- and 48-hours post-treatment, and were photo-documented using a digital camera. The number of heartbeats and the  $\text{LC}_{50}$  were also recorded.

## RESULTS

### Phytochemical Composition and Antioxidant Property of *D. crumenatum* Leaves

The examination of phytochemical composition involved TLC analysis for the identification of secondary metabolites within the ethanolic extract of *D. crumenatum* leaves. As

presented in Table 1, essential oils, phenols, fatty acids, anthraquinones, coumarins, anthrones, tannins, flavonoids, and steroids were detected in the *D. crumenatum* leaf extract. For the total phenolic content, *D. crumenatum* leaf ethanolic extract contained 496.139 mg GAE/g and an antioxidant property of  $584.9 \mu\text{g mL}^{-1}$  (Table 2).

**Table 1.** Phytochemical composition of *Dendrobium crumenatum* leaf ethanolic extract.

Phytochemicals	Ethanolic Extract
Essential oils	+
Triterpenes	-
Sterols	-
Phenols	+
Fatty acids	+
Sugars	-
Anthraquinones	+
Coumarins	+
Anthrones	+
Tannins	+
Flavonoids	+
Steroids	+
Alkaloids	-
Amino acids	-

### Antimicrobial Potential of *D. crumenatum* Leaves

For the eradicator test against *E. coli* (Table 3), no zone of inhibition was observed, while against *S. aureus*, zones of inhibition measuring 10.17 mm and 9.80 mm at 12 and 24 hours, respectively, were recorded. As a protectant (Table 4 and Figure 2), reduction in zones of colonization was recorded for both *E. coli* (10.45 mm and 16.22 mm at 12 and 24 hours) and *S. aureus* (8.15 mm and 8.47 mm at 12 and 24 hours, respectively). For the antifungal property, no inhibition of mycelial growth was observed in *A. flavus* after 24 to 48 hours of incubation (Figure 3).

**Table 2.** Total phenolic content and radical scavenging *Dendrobium crumenatum* leaf ethanolic extract. \*Wavelength 517 nm BK UV 1000 Spectrophotometer \*Abs DPPH = 0.212.

Sample Description	Total Phenolic Content (mg GAE/g)	Radical Scavenging Activity ( $\mu\text{g/mL}$ )
<i>D. crumenatum</i>	496.139	584.9
Catechin (control)	-	781.8

### Cytotoxicity of *D. crumenatum* Leaves

The cytotoxicity of the ethanolic extract of *D. crumenatum* leaves was assessed via the brine shrimp lethality assay. The mean and percentage mortality of brine shrimp (*Artemia salina* Linnaeus, 1758) nauplii exposed to various extract concentrations for 24 hours are detailed in Table 5. Maximum mean mortality

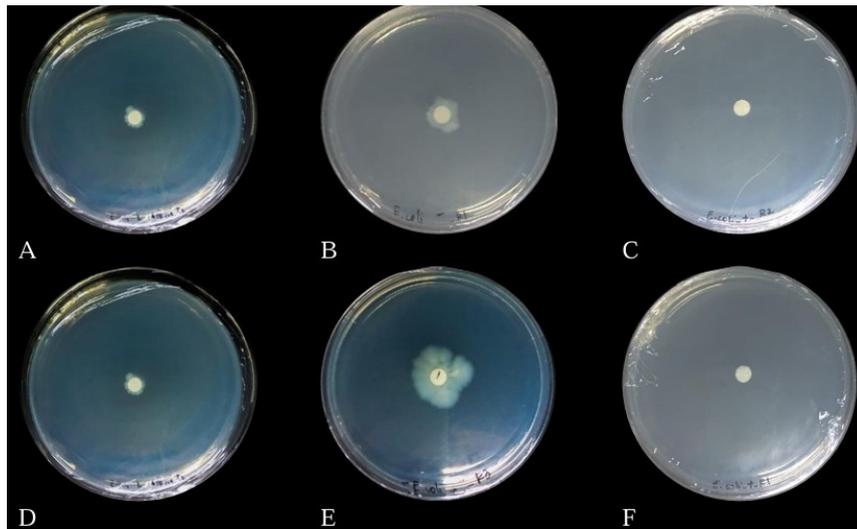
(100%) occurred at 10,000 ppm and 5,000 ppm, whereas the minimum mean mortality (66.67%) was observed at 500 ppm. Statistical analysis revealed no significant differences between 10,000 ppm and 5,000 ppm, while 1,000 ppm was comparable to both higher concentrations. Significant differences were noted among all concentrations compared to the control. The LC<sub>50</sub> was estimated at 341 ppm.

**Teratogenicity of *D. crumenatum***

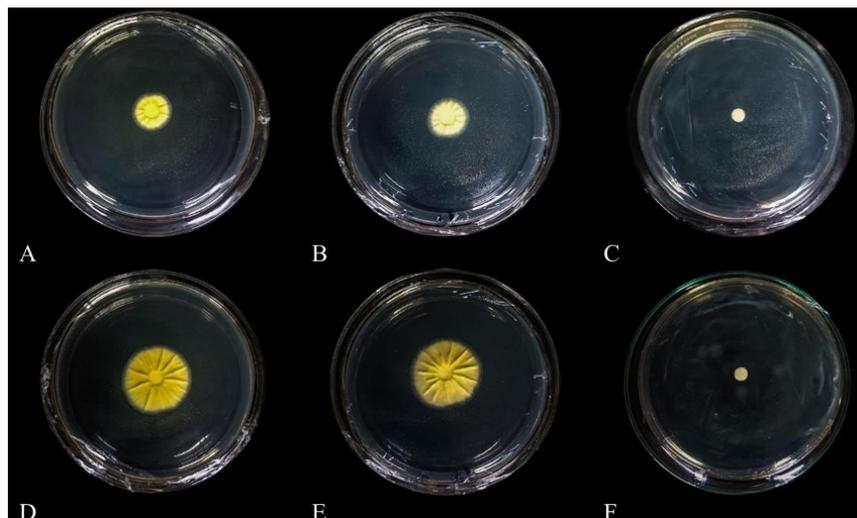
For the teratogenicity of *D. crumenatum*, several parameters were evaluated which include hatchability, heartbeat rate, morphological defects,

teratogenic effects, and mortality rates of *Danio rerio* (Hamilton, 1822) embryos.

**Hatchability of *D. rerio* embryos.** Hatching usually occurs within 48 to 72 hours post-treatment application (hpta). Table 6 shows the mean hatchability percentages of embryos exposed to different concentrations of leaf extract after 48 hours. Embryos in embryo water and 500 ppm solutions had the highest mean hatchability at 100%, while in 5,000 ppm and 10,000 ppm, embryos did not hatch. This may be due to premature embryo arrest and delayed development, indicating sublethal effects of the extract.



**Figure 2.** Antibacterial activity of *Dendrobium crumenatum* as protectant (A) 12 hours of incubation, ethanolic extract; (B) 12 hours of incubation, negative control; (C) 12 hours of incubation, positive control; (D) 24 hours of incubation, ethanolic extract; (E) 24 hours of incubation, negative control; and (F) 24 hours of incubation, positive control against *Escherichia coli*.



**Figure 3.** Antifungal activity of *Dendrobium crumenatum* (A) 24 hours of incubation, ethanolic extract; (B) 24 hours of incubation, negative control; (C) 24 hours of incubation, positive control; (D) 48 hours of incubation, ethanolic extract; (E) 48 hours of incubation, negative control; and (F) 48 hours of incubation, positive control against *Aspergillus flavus*.

**Table 3.** Mean diameter of zone of inhibition (mm) affected by the treatments against *Escherichia coli* and *Staphylococcus aureus* after 12 and 24 hours of incubation. Values are presented as mean  $\pm$  sd, means having the same letter of superscript in the same column are not significantly different from each other at 5% level of significance using Tukey's test.

Treatment	Diameter of Zones of Inhibition			
	<i>Escherichia coli</i>		<i>Staphylococcus aureus</i>	
	12 hours	24 hours	12 hours	24 hours
Ethanol extract	0.00 <sup>b</sup> $\pm$ 0.00	0.00 <sup>b</sup> $\pm$ 0.00	10.17 <sup>b</sup> $\pm$ 4.49	9.80 <sup>b</sup> $\pm$ 4.16
Positive control	29.05 <sup>a</sup> $\pm$ 0.80	27.8 <sup>a</sup> $\pm$ 0.99	33.20 <sup>a</sup> $\pm$ 3.34	31.78 <sup>a</sup> $\pm$ 0.71
Negative control	0.00 <sup>b</sup> $\pm$ 0.00	0.00 <sup>b</sup> $\pm$ 0.00	0.00 <sup>b</sup> $\pm$ 0.00	0.00 <sup>b</sup> $\pm$ 0.00

**Table 4.** Mean diameter of zone of bacterial colonization (mm) of *Escherichia coli* and *Staphylococcus aureus* against *Dendrobium crumenatum* leaves ethanol extract after 12 and 24 hours of incubation. Values are presented as mean  $\pm$  sd, means having the same letter of superscript in the same column are not significantly different from each other at 5% level of significance using Tukey's test.

Treatment	Diameter of Zones of Colonization			
	<i>Escherichia coli</i>		<i>Staphylococcus aureus</i>	
	12 hours	24 hours	12 hours	24 hours
Ethanol extract	10.45 <sup>b</sup> $\pm$ 2.05	16.22 <sup>b1</sup> $\pm$ 2.3	8.15 <sup>b</sup> $\pm$ 0.39	8.47 <sup>b</sup> $\pm$ 0.23
Positive control	0.00 <sup>a</sup> $\pm$ 0.00	0.00 <sup>a</sup> $\pm$ 0.00	0.00 <sup>a</sup> $\pm$ 0.00	0.00 <sup>a</sup> $\pm$ 0.00
Negative control	13.13 <sup>c</sup> $\pm$ 0.76	22.43 <sup>c</sup> $\pm$ 0.15	9.28 <sup>c</sup> $\pm$ 0.65	11.98 <sup>c</sup> $\pm$ 1.98

**Table 5.** Mean percentage mortality of *Artemia salina* embryos after 12, 24, 36 and 48 hours of exposure to different concentrations of *Dendrobium crumenatum* Swartz leaves ethanolic extract. Values are presented as mean  $\pm$  sd of three zebrafish embryos, means having the same letter of superscript in the same column are not significantly different from each other at 5% level of significance using Tukey's test.

Concentration (ppm)	Mortality Rate (%)			
	12 hpta	24 hpta	36 hpta	48 hpta
0.00	0.00 <sup>b</sup> $\pm$ 0.00	0.00 <sup>b</sup> $\pm$ 0.00	0.00 <sup>b</sup> $\pm$ 0.00	0.00 <sup>b</sup> $\pm$ 0.00
500	0.00 <sup>b</sup> $\pm$ 0.00			
1000	0.00 <sup>b</sup> $\pm$ 0.00			
5000	25.00 <sup>ab</sup> $\pm$ 50.00			
10000	100.00 <sup>a</sup> $\pm$ 0.00			

**Table 6.** Mean percentage hatchability of *Dendrobium rerio* embryo after 48 hours of exposure to different concentration of *Dendrobium crumenatum* Swartz leaves ethanol extract. Values are presented as mean  $\pm$  sd of three zebrafish embryos, means having the same letter of superscript in the same column are not significantly different from each other at 5% level of significance using Tukey's test.

Concentration (ppm)	Hatchability	Heartbeat Rate
0.00	100.00 <sup>a</sup> $\pm$ 0.00	168.44 <sup>a</sup> $\pm$ 6.71
500	100.00 <sup>a</sup> $\pm$ 0.00	171.11 <sup>a</sup> $\pm$ 18.73
1000	88.89 <sup>a</sup> $\pm$ 19.25	118.67 <sup>b</sup> $\pm$ 20.78
5000	0.00 <sup>b</sup> $\pm$ 0.00	0.00 <sup>c</sup> $\pm$ 0.00
10000	0.00 <sup>b</sup> $\pm$ 0.00	0.00 <sup>c</sup> $\pm$ 0.00

**Heartbeat rate of *D. rerio* embryos.** The heartbeat, a vital physiological indicator, was monitored in *D. rerio* embryos during the pharyngula stage while exposed to different concentrations of *D. crumenatum* ethanol extract. As presented in Table 6, higher concentrations led to adverse effects, including early coagulation and developmental delays, with no heartbeat observed in embryos exposed to 10,000 ppm after 36 hours of incubation, which may be attributed to early coagulation evident at 12 hpta. Similarly, embryos exposed to 5,000 ppm exhibited delayed developmental growth, precluding heartbeat recording.

As shown in Table 6, lower concentrations resulted in increased heartbeat rates compared to the control, but a reduction was noted at 1,000 ppm. Overall, exposure to *D. crumenatum* ethanol extract resulted in a dose-dependent reduction in heartbeat rate. These underscore the escalating toxicity with increasing concentrations of *D. crumenatum* ethanol extract on *D. rerio* embryos. In contrast, control embryos exhibited a mean heartbeat of 168.44, while those exposed to 500 ppm demonstrated a significantly higher mean heartbeat of 171.1, higher than the control but lower than the 1,000 ppm group, which recorded a mean heartbeat of 118.67. Consequently, exposure to

varying concentrations of *D. crumenatum* ethanolic extract resulted in a reduction in heartbeat rate.

**Morphological abnormalities and teratogenicity effects.** The ethanolic extract from *D. crumenatum* induced morphological abnormalities and teratogenic effects at various developmental stages (Table 7). Embryos exposed to 1,000 ppm and 500 ppm exhibited yolk sac edema and limited pigmentation, with more severe effects at the higher concentration. Growth retardation was observed at 5,000 ppm, while coagulation, indicating lethal effects, occurred at 10,000 ppm. Control groups showed no abnormal morphological traits (Figure 4).

**Mortality of *D. rerio* embryos.** The mortality rates of embryos following various durations of exposure are delineated in Table 8, with mortality indicated by embryo coagulation. Findings reveal that concentrations of 10,000 ppm and 5,000 ppm resulted in 100% and 25% mortality, respectively, within 12 hours. Conversely, concentrations ranging from 0 to 1,000 ppm exhibited no mortality.

Utilizing probit analysis, the median lethal concentration (LC<sub>50</sub>) of the ethanolic extract derived from *D. crumenatum* leaves in zebrafish embryos was 6,984.314 ppm. This outcome is attributed to the presence of phytochemicals, which, despite displaying diverse functional properties such as antibacterial and antioxidant effects, can also exert toxic effects on developing embryos.

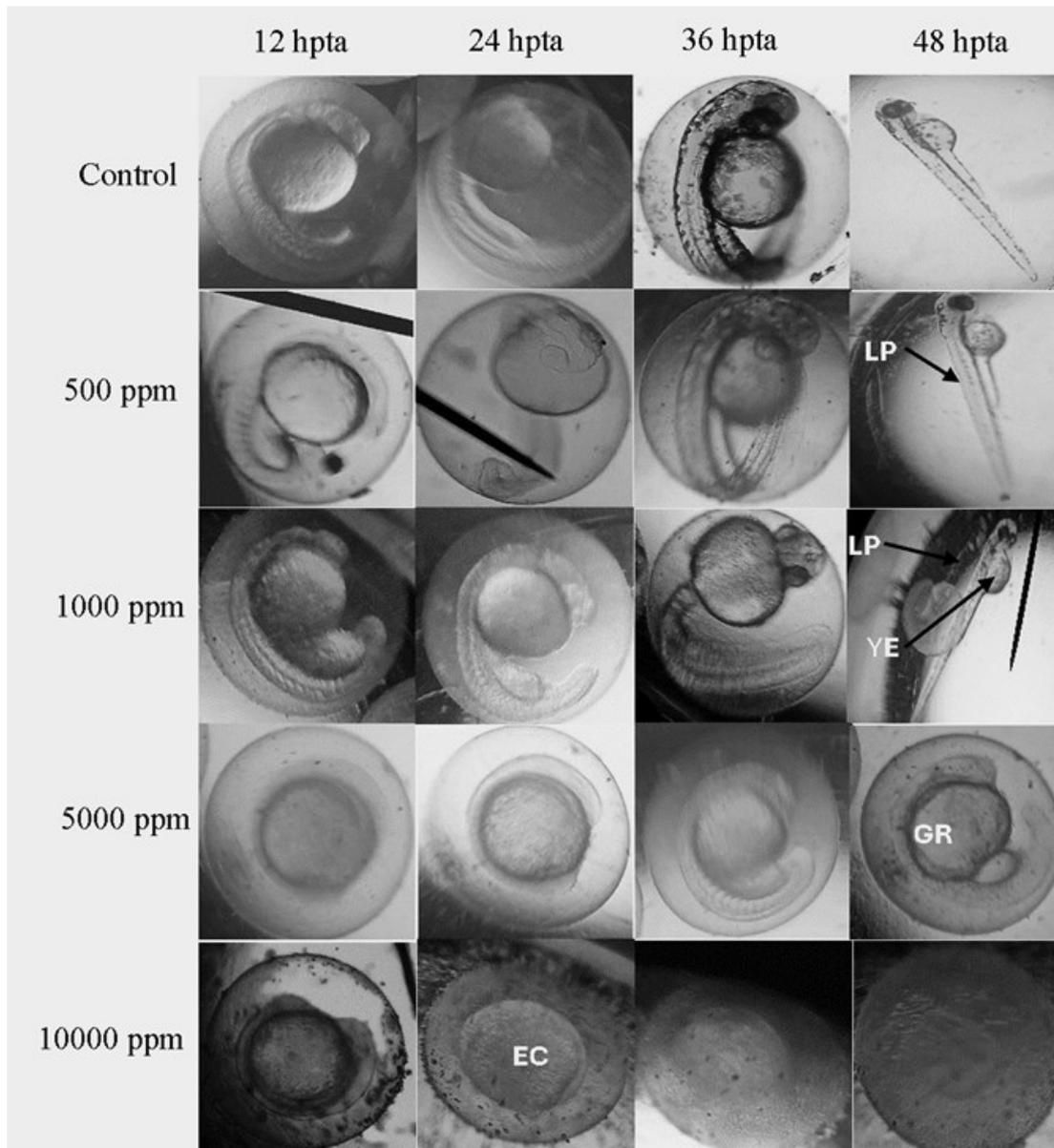
## DISCUSSION

Phytochemicals are secondary metabolites produced by plants to which their medicinal properties can be attributed (Larayetan et al. 2019; Gorlenko et al. 2020). These include saponins, flavonoids, alkaloids, phenols, essential oils, steroids, lignins, and tannins, all with known biological activities (Lam et al. 2015; Li et al. 2022). In recent years, the antimicrobial, antiparasitic, anticancer, antioxidant, and cytotoxic activities of these phytochemicals have been greatly explored and are continuously being established (Lin et al. 2018; Meitei et al. 2019). Accordingly, phenolic compounds and flavonoids are natural sources of antioxidant activities, eliminating reactive oxygen species that cause various diseases (Mazid et al. 2011; Paudel et al. 2019; Madjid et al. 2020).

Phytochemicals derived from plants constitute integral components in the process of drug development. The utilization of certain medicinal plants has facilitated the identification and isolation of therapeutic agents used in addressing various human ailments (Paudel et al. 2019). Essential oils, from aromatic plants like *Margotia gummifera* (Desf.) Lange, *Schinus areira* L., *Lavandula angustifolia* Mill, *Matricaria chamomilla* L., and *Cordia verbenacea* D.C., serve as valuable resources for aromatherapy, particularly for inhalation therapy, and present potential as complementary and alternative treatments for chronic obstructive pulmonary disease.

**Table 7.** Teratogenic effects of various concentrations of ethanolic extract of *Dendrobium crumenatum* Sw. leaves at 12, 24, 36 and 48 hours of exposure (+ = present; - = absent).

<i>Dendrobium crumenatum</i>	Toxological Endpoints	Time of Exposure (hours)	Concentration (%)				
			0	500	1000	5000	10000
Ethanol extract	Lethal		0	500	1000	5000	10000
	Coagulation	12	-	-	-	-	-
		24	-	-	-	-	+
		36	-	-	-	-	+
		48	-	-	-	-	+
	Teratogenic						
	Little pigmentation	12	-	-	-	-	-
		24	-	-	-	-	-
		36	-	-	-	-	-
		48	-	+	-	-	-
	Growth retardation	12	-	-	-	-	-
		24	-	-	-	-	-
		36	-	-	-	-	-
		48	-	-	-	+	-
	Yolk sac edema	12	-	-	-	-	-
		24	-	-	-	-	-
		36	-	-	+	-	-
		48	-	-	+	-	-



**Figure 4.** Morphological development of zebrafish embryos exposed to the different stages with different concentrations of *Dendrobium crumenatum* Swarts leaves ethanolic extract after 12, 24, 36 and 48 hours of exposure. Lethal effects: embryo coagulation (EC) 24 hpta in 10000 ppm; growth retardation (GR) 48 hpta in 5000 ppm; teratogenic effects: yolk sac edema (YE) and little pigmentation (LP) at 48 hpta in 1000 ppm and little pigmentation at 500 ppm at 48 hpta.

**Table 8.** Mean percentage mortality of *Dendrobium rerio* embryos after 12, 24, 36 and 48 hours of exposure to different concentrations of *Dendrobium crumenatum* leaves ethanolic extract. Values are presented as mean  $\pm$  sd of three zebrafish embryos, means having the same letter of superscript in the same column are not significantly different from each other at 5% level of significance using Tukey's test.

Concentration (ppm)	12 hpta	24 hpta	36 hpta	48 hpta
0.00	0.00 <sup>b</sup> $\pm$ 0.00	0.00 <sup>b</sup> $\pm$ 0.00	0.00 <sup>b</sup> $\pm$ 0.00	0.00 <sup>b</sup> $\pm$ 0.00
500	0.00 <sup>b</sup> $\pm$ 0.00			
1000	0.00 <sup>b</sup> $\pm$ 0.00			
5000	25.00 <sup>ab</sup> $\pm$ 0.00			
10000	100.00 <sup>a</sup> $\pm$ 0.00			

Their efficacy in managing lung-related inflammation underscores their therapeutic significance (Zhang et al. 2022). Additionally, phenolic compounds, including tannins and flavonoids, are recognized for their antioxidant properties in both medicinal and culinary plants (Dai and Mumper 2010).

Based on the study of Ramesh et al. (2019), several genera of the family Orchidaceae are considered medicinal orchids. The detection of anthraquinones and anthrones in the ethanolic extract of *D. crumenatum* is noteworthy due to their diverse biological activities, including anticancer and antimicrobial properties (Fouillaud et al. 2016). Moreover, the presence of coumarins in *D. crumenatum* suggests therapeutic applications in managing inflammatory conditions, cardiovascular diseases, and oxidative stress-related ailments (Venugopala et al. 2013). The presence of steroids in *D. crumenatum* further underscores the plant's potential in treating inflammatory conditions, autoimmune disorders, and cancer (Sandrasagaran et al. 2014). Phenolic acids are known for their antioxidant properties, both direct and indirect, through the induction of protective enzymes and regulation of signaling pathways (Kumar and Goel 2019; Zhang et al. 2022). Variations in antioxidant activity among *Dendrobium* species are due to differences in species, isolated constituents or fractions, extraction techniques, and assay methodologies (Chimsook 2016).

The antibacterial potential is attributed to phytochemicals such as flavonoids, tannins, fatty acids, and alkaloids. These substances destabilize cellular membranes by integrating into lipid bilayers, leading to membrane disruption and potential bacterial inhibition (Desbois et al. 2010; Cushnie et al. 2014; Agustini et al. 2020; Zhang et al. 2022). Tannins exhibit a range of physiological impacts, including anti-inflammatory, secretion-reducing, antibacterial, and antiparasitic properties (Zhang et al. 2022). According to Cushnie et al. (2014), flavonoids and alkaloids have been recognized for their diverse therapeutic benefits, including their roles as antihypertensive, anti-rheumatic agents, antimicrobial, and antioxidants. In a study by Desbois et al. (2010) free fatty acids (FFA) showed potent biological activity, with the ability to kill or inhibit bacterial growth by destabilizing cellular membranes. Moreover, FFAs are used to defend against many parasitic and pathogenic bacteria, with various applications in medicine, agriculture, and food preservation. They function by integrating into and altering lipid bilayers, thus disrupting the membranes' barrier properties. This action can lead to membrane fusion, resulting in the release and aggregation of substances within the membranes. In the present study, the protectant property of *D. crumenatum* against *S. aureus* and *E. coli* was depicted. This indicates that it

can be applied to healthy host cells prior to infection to combat microbial colonization (Mehta et al. 2020). Protectant is also known as preventive agent and is commercially used as antibacterial protection for disinfection, often as a component of antibacterial cleansers. They are only effective before the occurrence of bacterial pathogens (Namukobe et al. 2021).

Plant extracts with  $LC_{50} < 1,000$  ppm as toxic and those with  $LC_{50} > 1,000$  ppm as non-toxic. The teratogenic activity of *D. crumenatum* can also be attributed to the anticancer properties of its phytochemical constituents. Some of the compounds in orchids with anticancer properties include erianin (Petpiroon et al. 2017; Liu et al. 2019), phoyunnanin (Phiboonchaiyanan et al. 2018), erianthridin (Boonjing et al. 2021), ephemeranhol (Nonpanya et al. 2020), gigantol (Cai et al. 2021); denbinobin (Wang et al. 2022) and, dendrofalconerol (Petpiroon et al. 2017). Their actions include suppressing the signaling pathways for the growth and regulation of cancer cells (Bhummaphan and Chanvorachote 2015; Treesuwan et al. 2018; Luo et al. 2019; Aksorn et al. 2021), as well as migration and invasion, which lead to cancer cell death (Yang et al. 2023). Erianin was first isolated from *Dendrobium* sp. of Orchidaceae, with known active components with antitumor potentials (Zhang et al. 2019; Li et al. 2023; Deng et al. 2024). Its antitumor property is due to its cell apoptosis-promoting activity, which induces cell cycle arrest, apoptosis and autophagy, while inhibiting angiogenesis (Zhou et al. 2009; Li et al. 2019; Yi and Lan 2020). According to Petpiroon et al. (2017), phoyunnanin, from *D. venustum*, has anti-migration and epithelial-mesenchymal transition-suppression activities caused by reduced alpha v and beta 3 integrins and focal adhesion kinase/ protein kinase signals. Another active phenol, erianthridin, suppresses migration and invasion of all non-small cell lung cancer cells (H460) through actin and matrix metalloproteinase inhibition (Pothongsrisist et al. 2021). Moreover, denbinobin has the ability to downregulate the expression of decoy receptor-3, and it functions together with the Fas ligand, causing a reduction in pancreatic adenocarcinoma (Magwere 2009). Lastly, dendrofalconerol also possesses anti-migratory activity against metastatic cancer cells via expression of integrin  $\beta 1$  and integrin  $\alpha 4$  (Pengpaeng et al. 2015).

Further studies are necessary to verify the findings of the study. High-Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC) may be used for the quantification of the phytochemicals detected. Meanwhile, the Minimum Inhibitory Concentration (MIC) for the antibacterial property of *D. crumenatum* must be performed. Lastly, cytotoxicity tests using cancer cell lines can be conducted for further utilization of *D. crumenatum*.

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## ETHICAL CONSIDERATIONS

The study is exempted from any ethics guidelines.

## DECLARATION OF COMPETING INTEREST

The authors declare that there are no competing interests to any authors.

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## REFERENCES

- Agustini V, Simaremare E, Gunawan E, Awom J and Wopi S. 2020. Phytochemical screening, antibacterial and cytotoxic activity of *Dendrobium lasianthera* from Papua. *Jurnal Biologi Papua*, 12(2): 93-101. <https://doi.org/10.31957/jbp.1289>
- Aksorn N, Losuwannarak N, Tungsukruthai S, Roytrakul S and Chanvorachote P. 2021. Analysis of the protein-protein interaction network identifying c-met as a target of gigantol in the suppression of lung cancer metastasis. *Cancer Genomics & Proteomics*, 18(3): 261-272. <https://doi.org/10.21873/cgp.20257>
- Austria KC, Waing KGD and Valentino MJG. 2017. Antioxidant and antibacterial potentials of *Bambusa blumeana* J. A. and J. H. Schultes and *Bambusa vulgaris* Schrad.ex Wendl. shoot extracts. *International Journal of Biology, Pharmacy and Allied Sciences*, 6(11): 2175-2188.
- Bhummaphan N and Chanvorachote P. 2015. Gigantol suppresses cancer stem cell-like phenotypes in lung cancer cells, *Evidence-Based Complement and Alternative Medicine*, 2015: 836564. <https://doi.org/10.1155/2015/836564>
- Boonjing S, Pothongsrisit S, Wattanathamsan O, Sritularak B and Pongrakhananon V. 2021. Erianthridin induces non-small cell lung cancer cell apoptosis through the suppression of extracellular signal-regulated kinase activity. *Planta Medica*, 87(4): 283-293. <https://doi.org/10.1055/a-1295-8606>
- Cai Y, Hao Y, Xu H, Chen K and Ren B. 2021. Gigantol inhibits cell proliferation and induces apoptosis by regulating DEK in non-small cell lung cancer. *Experimental and Therapeutic Medicine*, 22(5): 1317. <https://doi.org/10.3892/etm.2021.10752>
- Cakova V, Bonte F and Lobstein A. 2017. *Dendrobium*: sources of active ingredients to treat age-related pathologies. *Aging and Disease*, 8(6): 827-849. <https://doi.org/10.14336/ad.2017.0214>
- Chimsook T. 2016. Phytochemical screening, total phenolic content, antioxidant activities and cytotoxicity of *Dendrobium signatum* leaves. *MATEC Web of Conferences*, 62: 03005. <https://doi.org/10.1051/mateconf/20166203005>
- Cushnie TPT, Cushnie B and Lamb AJ. 2014. Alkaloids: An overview of their antibacterial, antibiotic-enhancing and antivirulence activities. *International Journal of Antimicrobial Agents*, 44(5): 377-386. <https://doi.org/10.1016/j.ijantimicag.2014.06>
- Dai J and Mumper RJ. 2010. Plant phenolics: extraction, analysis and their anti oxidant and anticancer properties. *Molecules*, 15(10): 7313-7352. <https://doi.org/10.3390/molecules15107313>
- Deng Y, Zhong L, Zhao Y, Wan P, Zhang Y, Liao Y, Zhang H and Liu B. 2024. Erianin suppresses the development of acute myeloid leukemia via PPARα and regulating PI3K/AKT signaling pathways [Preprint]. *Research Square*, <https://doi.org/10.21203/rs.3.rs-5279493/v1>
- Desbois AP and Smith VJ. 2010. Antibacterial free fatty acids: activities, mechanisms of action and biotechnological potential. *Applied Microbiology and Biotechnology*, 85(6): 1629-1642. <https://doi.org/10.1007/s00253-009-2355-3>
- Duenas-Lopez MA. 2022. *Dendrobium crumenatum* (pigeon orchid) (p. 44838223) [Dataset]. *CABI Compendium*. <https://doi.org/10.1079/cabicompendium.44838223>
- Fouillaud M, Venkatachalam M, Girard-Valenciennes E, Caro Y and Dufosse L. 2016. Anthraquinones and derivatives from marine-derived fungi: structural diversity and selected biological activities. *Marine Drugs*, 14(4): 64. <https://doi.org/10.3390/md14040064>
- Gorlenko CL, Kiselev HY, Budanova EV, Zamyatnin AA and Ikryannikova LN. 2020. Plant secondary metabolites in the battle of drugs and drug-resistant bacteria: new heroes or worse clones of antibiotics? *Antibiotics*, 9(4): 170. <https://doi.org/10.3390/antibiotics9040170>
- Hu J, Fan W, Dong F, Miao Z and Zhou J. 2012. Chemical components of *Dendrobium chrysostom*. *Chinese Journal of Chemistry*, 30(6): 1327-1330. <https://doi.org/10.1002/cjoc.201100670>
- Klongkumnuankarn P, Busaranon K, Chanvorachote P, Sritularak B, Jongbunprasert V and Likhitwitayawuid K. 2015. Cytotoxic and antimigratory activities of phenolic compounds from *Dendrobium brymerianum*. *Evidence-Based Complementary and Alternative Medicine*, 1-9. <https://doi.org/10.1155/2015/350410>
- Kumar N and Goel N. 2019. Phenolic acids: natural versatile molecules with promising therapeutic applications. *Biotechnology Reports*, 24: e00370. <https://doi.org/10.1016/j.btre.2019.e00370>
- Kurniawan FY and Amelia DE. 2021. Chlorophyll and carotenoid level comparisons of pigeon orchid (*Dendrobium crumenatum*) in water and light stress treatment. *Indonesian Journal of Science and Education*, 5(1): 44-48.

- Lam YW, Ng TB, Yao RJR, Shi J, Xu K, Sze SCW and Zhang KY. 2015. Evaluation of chemical constituents and important mechanism of pharmacological biology in *Dendrobium* plants. Evidence-Based Complementary and Alternative Medicine, 1–25. <https://doi.org/10.1155/2015/841752>
- Larayetan R, Ololade ZS, Ogunmola OO and Ladokun A. 2019. Phytochemical constituents, anti oxidant, cytotoxicity, antimicrobial, antitrypanosomal, and antimalarial potentials of the crude extracts of *Callistemon citrinus*. Evidence-Based Complementary and Alternative Medicine, 1–14. <https://doi.org/10.1155/2019/5410923>
- Li G, Zhang H, Lai H, Liang G, Huang J, Zhao F, Xie X and Peng C. 2023. Erianin: A phytoestrogen with therapeutic potential. Frontiers in Pharmacology, 14: 1197056. <https://doi.org/10.3389/fphar.2023.1197056>
- Li C, Sun X, Song Z, Sun J, Li Y, Wang N, Zhang D, Ye F and Dai J. 2022. Chemical constituents from the stems of *Dendrobium gratiosissimum* and their biological activities. Phytochemistry, 201: 113260. <https://doi.org/10.1016/j.phytochem.2022.113260>
- Li S, Xu HX, Wu CT, Wang WQ, Jin W, Gao HL, Li H, Zhang SR, Xu JZ, Qi ZH, Ni QX, Yu XJ and Liu L. 2019. Angiogenesis in pancreatic cancer: current research status and clinical implications. Angiogenesis, 22: 15–36. <https://doi.org/10.1007/s10456-018-9645-2>
- Lindain JMG, Dulay RMR and Valentino MJG. 2018. Antibacterial and teratogenic activity of *Eleusine indica* leaves extracts. Journal of Biodiversity and Environmental Sciences, 13(4): 131–140.
- Lin HY, Chang TC, Chang ST and Tradit J. 2018. A review of anti oxidant and pharmacological properties of phenolic compounds in *Acacia confuse*. Journal of Traditional and Complementary Medicine, 8(4): 443–450. <https://doi.org/10.1016/j.jtcme.2018.05.002>
- Liu YT, Hsieh MJ, Lin JT, Chen G, Lin CC, Lo YS, Chuang YC, His YT, Chen MK and Chou MC. 2019. Erianin induces cell apoptosis through ERK pathway in human nasopharyngeal carcinoma. Biomedicine & Pharmacotherapy, 111: 262–269. <https://doi.org/10.1016/j.biopha.2018.12.081>
- Luo Y, Ren Z, Du B, Xing S, Huang S, Li Y, Lei Z, Li D, Chen H, Huang Y, et al. 2019. Structure Identification of ViceninII extracted from *Dendrobium officinale* and the reversal of TGF- $\beta$ 1-induced epithelial-mesenchymal transition in lung adenocarcinoma cells through TGF- $\beta$ /Smad and PI3K/Akt/mTOR signaling pathways. Molecules, 24(1): 144. <https://doi.org/10.3390/molecules24010144>
- Madjid AMO, Sanni A and Lagnika L. 2020. Chemical diversity and pharmacological properties of genus *Acacia*. Asian Journal of Applied Sciences, 13(2): 40–59. <https://doi.org/10.3923/ajaps.2020.40.59>
- Magwere T. 2009. Escaping immune surveillance in cancer: is denbinobin the panacea? British Journal of Pharmacology, 157(7): 1172–1174. <https://doi.org/10.1111/j.1476-5381.2009.00286.x>
- Mazid M, Khan TA and Mohammad F. 2011. Role of secondary metabolites in defense mechanisms of plants. Biology and Medicine, 3: 232–249. <https://api.semanticscholar.org/CorpusID:13943474>
- Mehta R, Dhruv S, Kaushik V, Sen KK, Khan NS, Abhishek A, Dixit AK and Tripathi VN. 2020. A comparative study of antibacterial and antifungal activities of extracts from four indigenous plants. Bioinformation, 16(3): 267–273. <https://doi.org/10.6026/97320630016267>
- Meitei AL, Pamarthi RK, Kumar R, Bhutia NT, Rai D, Kiran P, Singh AK, Gazmer R and Singh DR. 2019. *Dendrobium nobile* orchid in traditional medicine - a phytochemical analysis. Indian Journal of Horticulture, 76(3): 557–560. <https://doi.org/10.5958/0974-0112.2019.00090.2>
- Namukobe J, Sekandi P, Byamukama R, Murungi M, Namboozee J, Ekyibentenga Y, Nagawa CB and Asiimwe S. 2021. Antibacterial, antioxidant, and sun protection potential of selected ethno medicinal plants used for skin infections in Uganda. Tropical Medicine and Health, 49: 49. <https://doi.org/10.1186/s41182-021-00342-y>
- Nonpanya N, Prakhongcheep O, Petsri K, Jitjaicham C, Tungsukruthai S, Sritularak B and Chanvorachote P. 2020. Ephemeranthol A suppresses epithelial to mesenchymal transition and FAK-Akt signaling in lung cancer cells. Anticancer Research, 40(9): 4989–4999. <https://doi.org/10.21873/anticancer.14502>
- Paudel MR, Chand M and Pant B. 2019. Assessment of anti oxidant and cytotoxic activities of extracts of *Dendrobium crepidatum*. Biomolecules, 9(9): 478. <https://doi.org/10.3390/biom9090478>
- Pengpaeng P, Sritularak B and Chanvorachote P. 2015. Dendrofalconerol A suppresses migrating cancer cells via EMT and integrin proteins. Anticancer research, 35(1): 201–205.
- Petpiroon N, Sritularak B and Chanvorachote P. 2017. Phoyunnanin E inhibits migration of non-small cell lung cancer cells via suppression of epithelial-to-mesenchymal transition and integrin  $\alpha$ v and integrin  $\beta$ 3. BMC Complementary and Alternative Medicine, 17(1): 553. <https://doi.org/10.1186/s12906-017-2059-7>
- Phiboonchaiyanan PP, Petpiroon N, Sritularak B and Chanvorachote P. 2018. Phoyunnanin E induces apoptosis of non-small cell lung cancer cells via p53 activation and down-regulation of surviving. Anticancer Research, 38(11): 6281–6290. <https://doi.org/10.21873/anticancer.12984>
- Pothongsrisit S, Arunrungvichian K, Hayakawa Y, Sritularak B, Mangmool S and Pongrakhananon V. 2021. Erianthridin suppresses non-small-cell lung cancer cell metastasis through inhibition of Akt/mTOR/p70<sup>S6K</sup> signaling pathway. Scientific reports, 11(1): 6618. <https://doi.org/10.1038/s41598-021-85675-8>
- Ramesh T, Koperuncholan M, Praveena R, Ganeshkumari K, Vanithamani J, Muruganantham P and Renganathan P. 2019. Medicinal properties of some *Dendrobium* orchids – a review. Journal of Applied and Advanced Research, 4(4): 119–128. <https://doi.org/10.21839/jaar.2019.v4i4.72>
- Ram AT, Shamina M and Pradeep AK. 2021. *Dendrobium crumenatum* (Orchidaceae): a new record for mainland India. Journal of Botany, 12(3): 45–48. <https://doi.org/10.1111/jb.12345>

- Sandrasagar U, Subramaniam S and Murugaiyah V. 2014. New perspective of *Dendrobium crumenatum* orchid for antimicrobial activity against selected pathogenic bacteria. *Pakistan Journal of Botany*, 46(2): 719-724.
- Sliwinski T, Kowalczyk T, Sitarek P and Kolanowska M. 2022. Orchidaceae-derived anticancer agents: a review. *Cancers*, 14(3): 754. <https://doi.org/10.3390/cancers14030754>
- Tabla VP and Vargas CF. 2004. Phenology and phenotypic natural selection on the flowering time of a deceit-pollinated tropical orchid, *Myrmecophila christinae*. *Annals of Botany*, 94(2): 243-250. <https://doi.org/10.1093/aob/mch134>
- Treesuwan S, Sritularak B, Chanvorachote P and Pongrakhananon V. 2018. Cypripedin diminishes an epithelial-to-mesenchymal transition in non-small cell lung cancer cells through suppression of Akt/GSK-3 $\beta$  signaling. *Scientific reports*, 8(1): 8009. <https://doi.org/10.1038/s41598-018-25657-5>
- Valentino MJG, Ganado LS, Ganado MR and Undan JR. 2015. Phytochemical screening and bioassay of the anti-microbial activity of three species of bamboo in Nueva Ecija, Philippines. *Advances in Environmental Biology*, 9(24): 389-396.
- Venugopala K, Venugopala R and Odhav B. 2013. Review on natural coumarin lead compounds for their pharmacological activity. *BioMed Research International*, 2013: 963248. <https://doi.org/10.1155/2013/963248>
- Wang G, Wang J, Deng Y, Qin L, He Y and Tan D. 2022. Chemical constituents and nutritional health functions of *Dendrobium nobile*: a review. *Food Science and Technology*, 42: e84522. <https://doi.org/10.1590/fst.84522>
- Wang YH. 2021. Traditional uses and pharmacologically active constituents of dendrobium plants for dermatological disorders: a review. *Natural Products and Bioprospecting*, 11(5): 465–487. <https://doi.org/10.1007/s13659-021-00305-0>
- Yang W, Chen D, Ji Q, Zheng J, Ma Y, Sun H, Zhang Q, Zhang J, He Y and Song T. 2023. Molecular mechanisms underlying the anticancer property of *Dendrobium* in various systems of the human body: a review. *Biomedicine & Pharmacotherapy*, 165: 115223. <https://doi.org/10.1016/j.biopha.2023.115223>
- Yi X and Lan X. 2020. LC-MS/MS method for the determination of erianin in rat plasma: application to a pharmacokinetic study. *Biomedical Chromatography*, 34(7): e4826. <https://doi.org/10.1002/bmc.4826>
- Zhang Y, Zhang Q, Wei F and Liu N. 2019. Progressive study of effects of erianin on anticancer activity. *OncoTargets and Therapy*, 12: 5457–5465. <https://doi.org/10.2147/OTT.S200161>
- Zhang N, Bian Y and Yao L. 2022. Essential oils of *Gardenia jasminoides* J. Ellis and *Gardenia jasminoides* f. *longicarpa* Z.W. Xie & M. Okada flowers: chemical characterization and assessment of anti-inflammatory effects in alveolar macrophage. *Pharmaceutics*, 14(5): 966. <https://doi.org/10.3390/pharmaceutics14050966>
- Zhou H, Yang B, Hong M and Sheng L. 2009. Liquid chromatographic-mass spectrometry analysis and pharmacokinetic studies of erianin for intravenous injection in dogs. *Arzneimittelforschung*, 59(3): 141–145. <https://doi:10.1055/s-0031-1296377>

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# New record of a rare moray eel, *Enchelycore kamara* Böhlke & Böhlke, 1980 (Anguilliformes: Muraenidae), from the Philippines

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## ABSTRACT

A single specimen (957 mm total length) of the rare moray eel *Enchelycore kamara* Böhlke & Böhlke, 1980, was collected from the Puerto Princesa New Public Market, Palawan, Philippines, representing the largest recorded size for the species and its first record in Philippine waters. A brief description, fresh photographs, and a COI sequence were provided for this specimen. The distribution range of *E. kamara* was also updated based on additional specimens, sequences, and underwater photographs obtained from online public sources.

**Keywords:** DNA barcoding, maximum size, Muraeninae, Pacific Ocean, taxonomy

## INTRODUCTION

Muraenidae are one of the most diverse families in the order Anguilliformes, comprising around 230 species across 16 genera in two subfamilies worldwide (Fricke et al. 2025). Located in the Coral Triangle, the Philippines holds remarkable marine biodiversity; nevertheless, moray eels are difficult to detect due to their reclusive habits, leading to ongoing discoveries of undescribed species and new records in this region, with more than 80 species reported (e.g. Huang et al. 2021, 2023a, b, 2024; Cabebe-Barnuevo et al. 2023).

Members of the genus *Enchelycore* Kaup, 1856 are medium- to large-sized piscivorous morays,

characterized by long, arched jaws that meet only at the tips, and large fangs that remain visible when the mouth is closed (Smith and Böhlke 2022). Another proposed characteristic is their elongated, tapering jaws, which narrow the anterior part of the head, quantified by the interorbital width ( $\leq 10\%$  of head length in *Enchelycore* vs.  $> 10\%$  in most other genera) (Huang et al. 2022b). Currently, 13 *Enchelycore* species are considered valid, with three species distributed in the Atlantic and 10 species occurring in the Indo-Pacific (Smith 2012; Mohapatra et al. 2017). According to FishBase (Froese and Pauly 2024; <https://www.fishbase.org/>) and Global Biodiversity Information Facility (GBIF; <https://www.gbif.org/>) records, only two species—*Enchelycore bayeri*



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(Schultz, 1953) and *Enchelycore schismatorhynchus* (Bleeker, 1853)—have been reported from Philippine waters to date (Froese and Pauly 2024).

*Enchelycore kamara* Böhlke & Böhlke, 1980 is a rare moray with only seven voucher specimens reported in the literature, including six from the Line Islands and Palau (type series) and one from the Ryukyu Islands (Böhlke and Böhlke 1980; Shibukawa et al. 2007). A non-specimen-based record was also reported from Guam (Myers 1999), and it is therefore considered a widespread species in the central and western Pacific. It is believed to be a small to medium-sized moray, as the known maximum size of this species is 530 mm, while the remaining known specimens are relatively smaller, ranging from 201.8 to 388 mm (Böhlke and Böhlke 1980; Shibukawa et al. 2007).

During our Palawan ichthyological survey conducted in 2023, a specimen of *E. kamara* was collected from the Puerto Princesa New Public Market. This is a large mature female (957 mm total length), significantly larger than the previous size record, and represents the first confirmed record of this species in Philippine waters. Furthermore, some unpublished records can be attributed to *E. kamara*, including specimens from Mindoro, Cebu, and Bohol, Philippines, housed in the Smithsonian National Museum of Natural History (USNM) collection; GBIF specimen records from Queensland, Australia, and the Admiralty Islands, Papua New Guinea; and an underwater photograph from Raja Ampat, Indonesia, obtained via iNaturalist (<https://www.inaturalist.org>). This study aims to provide supplementary biological and distributional information on this rare moray.

## METHODS

A fresh specimen was collected from the Puerto Princesa New Public Market, Palawan, Philippines. It was immediately photographed, and a piece of muscle tissue was obtained from a small incision on the belly near the anus and preserved in 95% ethanol. The sex was determined by dissection and direct observation of the gonad type. The specimen was then fixed in 10% formalin. After fixation in 10% formalin, it was soaked in 20%, 40%, and 60% ETOH, before it has been transferred to 70% ethanol for long-term preservation. This specimen was transported to Taiwan for further examination and was deposited in the Department of Oceanography, National Sun Yat-sen University, Kaohsiung (DOS).

Morphometrics were recorded as percentages of head length (HL) or total length (TL) following the definitions in Böhlke et al. (1989). The vertebral formula was given as the number of pre-dorsal fin, pre-

anal fin, and total vertebrae, counted from radiographs following the definitions in Böhlke (1982). Teeth and head pores were examined under a stereomicroscope using the terminology in Smith et al. (2019).

A partial fragment (680 bp) of the cytochrome *c* oxidase subunit I (COI) gene was amplified from extracted genomic DNA via polymerase chain reaction (PCR) using the primers FishF2 (5'-TCG ACT AAT CAT AAA GAT ATC GGC AC-3') and FishR2 (5'-ACT TCA GGG TGA CCG AAG AAT CAG AA-3') (Ward et al. 2005). The PCR thermal cycling conditions were as described in Huang et al. (2021). The PCR products were Sanger-sequenced from both ends and were assembled in Molecular Evolutionary Genetics Analysis (MEGA) version 12 (Kumar et al. 2024). The assembled sequence was then submitted to GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>).

Additional sequences of *Enchelycore* species were obtained from BOLD Systems (Ratnasingham et al. 2024; <https://boldsystems.org/>) and GenBank to construct a maximum likelihood (ML) tree in MEGA, using the best-fitting Tamura-Nei +  $\Gamma$  + *I* substitution model and a bootstrap analysis with 1,000 iterations (Felsenstein 1985; Tamura and Nei 1993). Two morays from the subfamily Uropterygiinae—*Uropterygius mactanensis* Huang, Balisco, Evacitas & Liao, 2023 and *Uropterygius hades* Huang, Hibino, Balisco & Liao, 2024—were used as outgroups in the ML analysis, as the two subfamilies of Muraenidae (Muraeninae and Uropterygiinae) have been shown to be genetically reciprocally monophyletic in previous studies (e.g. Reece et al. 2010; Tang and Fielitz 2013). The genetic distance was also calculated using the Kimura 2-Parameter (K2P) model (Kimura 1980) in MEGA.

## RESULTS

### *Enchelycore kamara* Böhlke & Böhlke, 1980 (Figures 1–4, Table 1)

*Enchelycore kamara* Böhlke & Böhlke, 1980: 173 (Tongareva Atoll, Line Islands). Holotype, USNM 221161.

### Material Examined

One specimen: DOS 09957, 957 mm TL, mature female, purchased from Puerto Princesa New Public Market, Palawan, Philippines, 07 November 2023, collected by W.C. Huang and R.A. Balisco, with GenBank COI accession number PV257807.



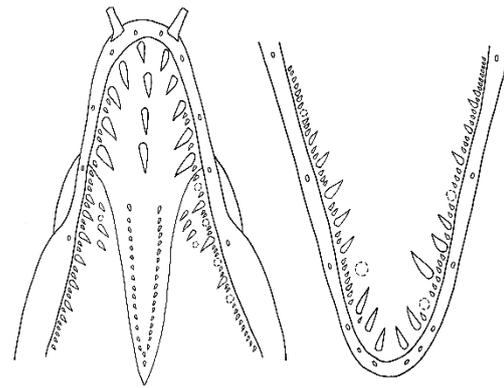
**Figure 1.** *Enchelycore kamara*, DOS 09957, 957 mm total length, Palawan, Philippines, fresh coloration.



**Figure 2.** *Enchelycore kamara* (indicated by arrow), DOS 09957, 957 mm total length, Palawan, Philippines, fresh coloration. Photo taken at the Puerto Princesa New Public Market, sold alongside *Gymnothorax javanicus* (Bleeker, 1859).

#### Brief Description of DOS 09957

Proportions were measured as percentage of TL: tail length 50.3; preanal length 49.7; trunk length 34.6; head length 15.2; predorsal length 17.3; body depth at gill opening 6.9; body depth at anus 6.0. Proportions were measured as percentage of HL: predorsal length 114.5; length of upper jaw 37.8; length of lower jaw 38.4; interorbital width 7.7; snout length 14.1; eye diameter 7.8. Vertebral counts were pre-dorsal fin vertebrae 15; pre-anal fin vertebrae 63; total vertebrae 138 (Table 1).



**Figure 3.** Dentition of *Enchelycore kamara*, DOS 09957, 957 mm total length. Upper jaw (left) and lower jaw (right). Dotted circles represent the sockets of missing teeth.



**Figure 4.** Dentition of *Enchelycore kamara*, DOS 09957, 957 mm total length, showing the arrangement and various sizes of teeth.

Body was moderately elongate and robust (Figures 1 and 2). The anus was located at mid-length of body. The dorsal fin was moderately high, originating slightly posterior to the gill opening. The anal fin was low, its origin immediately behind anus. Jaws were subequal, narrow, tapering, highly arched, and not completely closed; teeth were always visible. The snout was long and somewhat pointed. Eyes were moderate in size, positioned above mid-length of upper jaw, with a narrow interorbital space. The anterior nostril was slender and tubular; posterior nostril was oval with a slightly raised rim, positioned above and slightly posterior to anterior margin of eye. There were three supraorbital pores, four infraorbital pores, six preoperculo-mandibular pores, and two branchial pores.

**Table 1.** Morphometric measurements, teeth, and vertebral counts of *Enchelycore kamara*. Abbreviations: HL, head length; TL, total length. <sup>a</sup> count based on the dentition illustration in Shibukawai et al. (2007); <sup>b</sup> count based on the dentition illustration of the holotype in Böhlke and Böhlke (1980); <sup>c</sup> based on data from Smith (2012).

Source	This study	Shibukawai et al. (2007)	Böhlke and Böhlke (1980)
	DOS 09957	NSMT-P 75543	Types (ANSP, CAS, USNM)
	n = 1	n = 1	n = 6
TL (mm)	957	201.8	204–530
% TL			
Tail length	50.3	51.9	50–53
Preanal length	49.7	48.1	47–50
Trunk length	34.6	32.6	-
Head length	15.2	15.5	14–15
Predorsal length	17.3	16.0	16–19
Body depth at gill opening	6.9	5.5	5–6
Body depth at anus	6.0	5.9	4–5
% HL			
Predorsal length	114.5	103.2	-
Length of upper jaw	37.8	40.9	37–40
Length of lower jaw	38.4	40.9	-
Snout length	14.1	14.9	14–16
Interorbital width	7.7	-	8–10
Eye diameter	7.8	10.2	9–10
Teeth			
Intermaxillary-peripheral	5	4–5 <sup>a</sup>	5 <sup>b</sup>
Intermaxillary-median	3	3 <sup>a</sup>	3 <sup>b</sup>
Maxillary-outer	25–29	18–20	19–26
Maxillary-inner	4	4	4–5 <sup>b</sup>
Vomerine	35	ca. 24	11–19 + 3–7
Dentary	37–39	31	27–34
Vertebrae			
Pre-dorsal fin	15	13	14–16
Pre-anal fin	63	60	60–64 <sup>c</sup>
Total	138	137	138–143

**Dentition** (Figures 3 and 4). Tooth size varied, and edges were not serrated. Intermaxillary teeth were arranged in three rows, all long, triangular canines of similar size; five peripheral teeth were present on each side, with some tiny teeth in spaces between larger teeth; three depressible median teeth were observed. Maxillary teeth were biserial anteriorly and uniserial posteriorly; the outer row consisted of seven larger and 18–22 smaller teeth arranged alternately, with larger teeth decreasing in size posteriorly while smaller teeth remained the same size; the inner row had four pointed teeth, not significantly larger than those in the outer row. Vomerine teeth numbered 35 small conical teeth, mostly biserial, narrowing into a single row at the posterior end. Dentary teeth were mostly in uniserial arrangement, with three significantly larger canines anteriorly, followed by another long canine in the inner row flanked by six–seven small teeth, the remaining teeth

were similar to the maxillary teeth, with larger and smaller teeth arranged alternately; totaling 37–39 dentary teeth on each side.

**Coloration** (Figures 1 and 2). The ground color was pale yellowish to light brown, covered with numerous small dark-brown spots over the body and fins. Spots were smaller than the eye and were distributed more densely dorsally than ventrally, with the densest spots on the tips of the jaws and tail, forming a darker coloration. The inner skin of the oral cavity was darker than the body, without spots. The mouth corner and gill opening were not significantly darker. The iris was reddish brown. The preserved coloration was similar to that of the fresh specimen, except slightly faded.

#### Distribution

This species was previously known from the central and western Pacific, including the Line Islands,

Tuamotus, Queensland, Admiralty Islands, Raja Ampat, Palau, Guam, the Ryukyu Islands, and now from the Philippines (Figure 5).

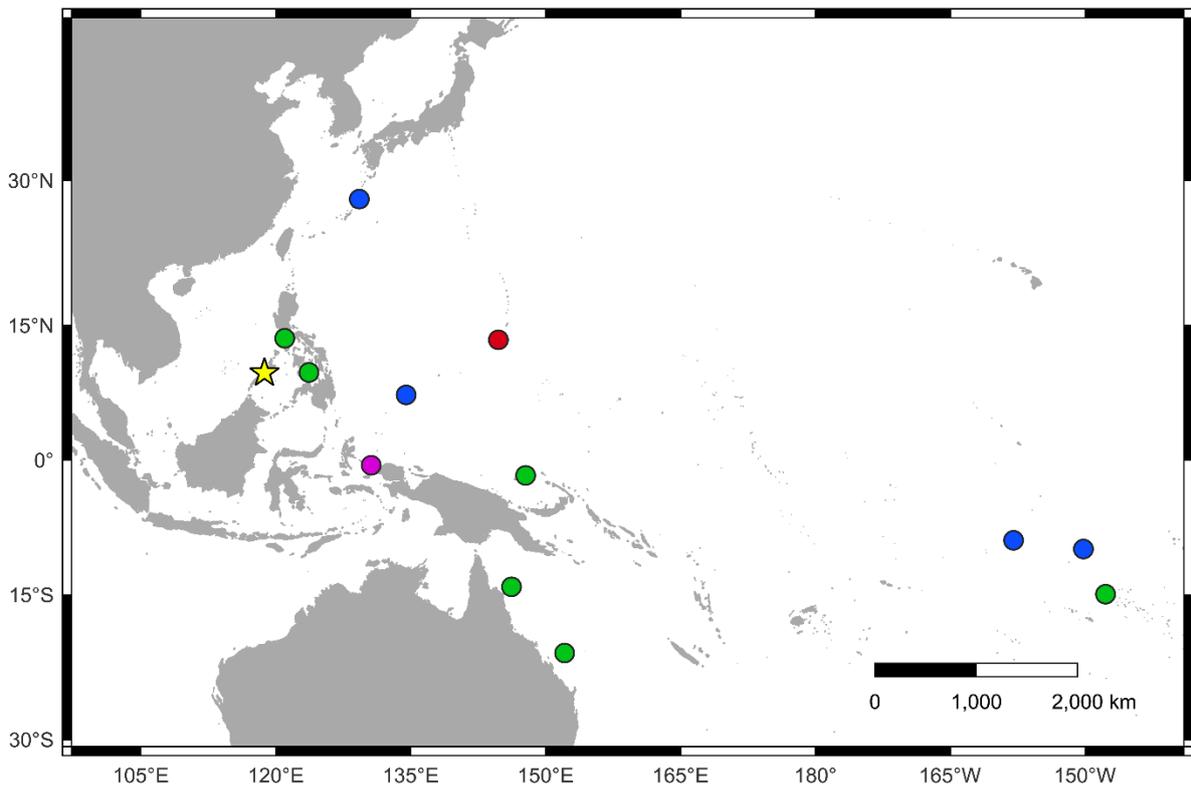
**Molecular Analysis**

An unidentified Muraenidae sequence (BOLD sequence ID: LIDMA867-11) from Tuamotus, French Polynesia, clustered with DOS 09957 and was identified as *E. kamara*. According to the topology of the ML tree, *E. kamara* was most closely related to *E. schismatorhynchus*, with a 16.9% K2P genetic distance (Figure 6).

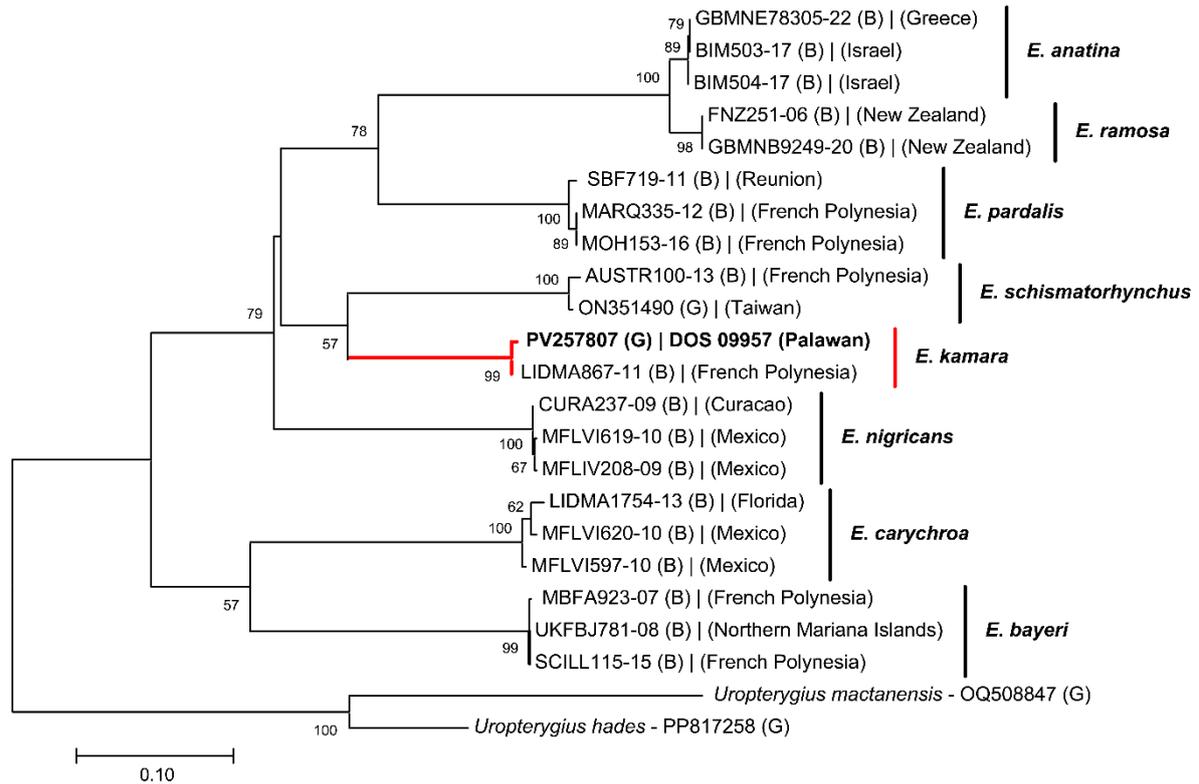
**DISCUSSION**

Most morphological characters of the *E. kamara* specimen from Palawan align with those described by Böhlke and Böhlke (1980) and Shibukawa et al. (2007). However, notable differences

were observed, including greater body depths and smaller eyes (Table 1), which may be attributed to allometric growth (Huang et al. 2022a). Its narrow interorbital width (7.7% of HL) also supports the perspective of Huang et al. (2022b) that this measurement can be used as a diagnostic characteristic for the genus *Enchelycore*. Interestingly, significantly more teeth were observed on the outer maxilla, vomerine, and dentary in DOS 09957 than in smaller specimens (Table 1), leading to the hypothesis that larger *E. kamara* individuals possess more teeth. However, this observation contrasts with *E. schismatorhynchus*, in which larger individuals (949 mm TL) have fewer maxillary and dentary teeth and fewer tooth rows than smaller ones (510–642 mm TL) (Huang et al. 2022b). Although sexual dimorphism in the dentition of morays has been reported in many species (e.g. Hatooka 1986; Smith et al. 2008; Huang et al. 2019, 2023a), changes in tooth number with body size have yet to be thoroughly studied. Further investigation is needed.



**Figure 5.** Distribution of *Enchelycore kamara*. Colors represent different sources: yellow star, this study; blue dot, literature with voucher specimens; green dot, unpublished specimens or sequences; red dot, non-specimen-based literature record; pink dot, iNaturalist record.



**Figure 6.** Maximum-likelihood tree of available cytochrome *c* oxidase subunit I sequences (636 bp) for *Enchelycore* species, obtained from BOLD Systems and GenBank. *Uropterygius mactanensis* and *U. hades* of the subfamily Uropterygiinae are used as outgroups. Numerals beside the internal branches represent bootstrap values; values below 50 are not shown. (B) = sequence from BOLD Systems; (G) = sequence from GenBank.

*Enchelycore kamara* is the only species in the genus that exhibits small dark spots scattered across its pale-yellowish body and fins. Another distinctive characteristic is its posteriorly positioned dorsal fin origin, which originates behind the gill opening, unlike in most other species where the dorsal fin originates above or before the gill opening, except for *Enchelycore propinqua* Mohapatra, Smith, Mohanty, Mishra & Tudu, 2017. Despite these distinguishable characteristics, *E. kamara* is still occasionally misidentified due to the limited information available about the species. For instance, two specimens identified as *Gymnothorax* sp., USNM 435509 from Cebu and USNM 435625 from Bohol, along with fresh photographs and private COI sequences on BOLD Systems, were confirmed as *E. kamara* based on their color patterns and sequence similarities. As a counterexample, a photograph record of *E. kamara* from Guam in a recent study (Figure 3F in Myers et al. 2025) was actually a misidentification of *Gymnothorax fimbriatus* (Bennett, 1832).

Moreover, the species most closely related to *E. kamara* on BOLD Systems is *Gymnothorax minor*

(Temminck & Schlegel, 1846), with 87% COI sequence similarity. This is not surprising, as previous studies (e.g. Tang and Fielitz 2013; Smith et al. 2019) have shown that *Enchelycore* is a polyphyletic genus. This may be due to the genetic markers used, which might not accurately reflect deeper phylogenetic relationships among genera within Muraenidae. Alternatively, the genus *Enchelycore* may need to be more precisely redefined, since many of its diagnostic characteristics appear to have evolved multiple times (Smith et al. 2019). For now, the researchers tentatively follow the current classification, which places the species *kamara* within the genus *Enchelycore*. Further studies are needed to clarify their phylogenetic relationships.

This study provides additional information on the rare muraenid *E. kamara*, including fresh photographs, a COI sequence, and updated details on its maximum size and distribution range, all of which are crucial for its accurate identification and management.

## FUNDING

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## ETHICAL CONSIDERATIONS

Wildlife gratuitous permit (No. 2023-02) and export permit (No. 2023-013) were obtained from the Palawan Council for Sustainable Development Staff (PCSDS) before specimen collection and transport.

## DECLARATION OF COMPETING INTEREST

The authors declare that there are no competing interests among the authors.

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## REFERENCES

- Böhlke EB, McCosker JE and Böhlke JE. 1989. Family Muraenidae. In: Böhlke EB (ed). Fishes of the Western North Atlantic. Memoirs of the Sears Foundation for Marine Research, 1 (part 9). Yale University, New Haven, pp. 104-206. <https://doi.org/10.12987/9781933789323-010>
- Böhlke EB. 1982. Vertebral formulae for type specimens of eels (Pisces: Anguilliformes). Proceedings of the Academy of Natural Sciences of Philadelphia, 134: 31-49.
- Böhlke JE and Böhlke EB. 1980. *Enchelycore kamara*: a new moray from the tropical Pacific Ocean. Proceedings of the Academy of Natural Sciences of Philadelphia, 132: 173-175.
- Cabebe-Barnuevo RA, Obar EAA, Penuela DFA, Motomura H, Babaran RP and Malay MCD. 2023. Two new records of moray eels representing genera *Gymnothorax* and *Strophidon* (Actinopterygii: Anguilliformes: Muraenidae) from the Philippines. Acta Ichthyologica et Piscatoria, 53: 217-226. <https://doi.org/10.3897/aiep.53.108838>
- Felsenstein J. 1985. Confidence limits on phylogenies: an approach using bootstrap. Evolution, 39: 783-791. <https://doi.org/10.2307/2408678>
- Fricke R, Eschmeyer WN and Fong JD. 2025. Eschmeyer's Catalog of Fishes: Genera/Species by Family/Subfamily.

- <http://researcharchive.calacademy.org/research/ichthyology/catalog/SpeciesByFamily.asp>. Accessed on 11 March 2025.
- Froese R and Pauly D. (eds). 2024. FishBase. World Wide Web electronic publication. [www.fishbase.org](http://www.fishbase.org). Accessed on 11 March 2025.
- GBIF (Global Information Facility). 2025. <https://www.gbif.org/>. Accessed on 11 March 2025.
- GenBank. 2025. GenBank Overview. <https://www.ncbi.nlm.nih.gov/genbank/>. Accessed on 11 March 2025.
- Hatooka K. 1986. Sexual dimorphism found in teeth of three species of moray eels. Japanese Journal of Ichthyology, 32(4): 379-386. <https://doi.org/10.11369/jji1950.32.379>
- Huang WC, Balisco RA, Evacitas FC and Liao TY. 2023a. Description of a new moray eel (Anguilliformes: Muraenidae) from the Philippines, with a preliminary assessment of genetic and morphological variations of the *Uropterygius concolor* species complex. Journal of Fish Biology, 103(3): 593-602. <https://doi.org/10.1111/jfb.15472>
- Huang WC, Chen HM and Liao TY. 2019. Revalidation of a moray eel, *Gymnothorax mucifer* Snyder, 1904 (Teleostei: Anguilliformes: Muraenidae), with a revised distribution. Zootaxa, 4559(1): 151-165. <https://doi.org/10.11646/zootaxa.4559.1.6>
- Huang WC, Chen HM, Tseng YC and Liao TY. 2022a. Assessing phenovariant species pairs of moray eels: Hybridization, introgression, and morphology. Zoologica Scripta, 51(6): 676-694. <https://doi.org/10.1111/zsc.12560>
- Huang WC, Hibino Y, Balisco RA and Liao TY. 2024. Description of a new uniformly brown estuarine moray eel (Anguilliformes, Muraenidae) from the Central Indo-Pacific Ocean. In: Ho HC, Russell B, Hibino Y and Lee MY (eds). Biodiversity and taxonomy of fishes in Taiwan and adjacent waters. ZooKeys, 1220: 15-34. <https://doi.org/10.3897/zookeys.1220.129685>
- Huang WC, Liao TY and Tan HH. 2023b. *Uropterygius cyamommatus*, a new moray eel (Anguilliformes: Muraenidae) from anchialine caves in Christmas Island, Australia, and Panglao Island, the Philippines. Raffles Bulletin of Zoology, 71: 268-278. <https://doi.org/10.26107/RBZ-2023-0021>
- Huang WC, Loh KH and Chen HM. 2022b. *Gymnothorax poikilospilus*, a new moray eel (Teleostei: Anguilliformes: Muraenidae) from Penghu Islands, western Taiwan. Zootaxa, 5189(1): 87-102. <https://doi.org/10.11646/zootaxa.5189.1.11>
- Huang WC, Smith DG, Loh KH and Liao TY. 2021. Two new moray eels of genera *Diaphenchelys* and *Gymnothorax* (Anguilliformes: Muraenidae) from Taiwan and the Philippines. Zoological Studies, 60: 24. <https://doi.org/10.6620/ZS.2021.60-24>
- Kimura M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution, 16: 111-120. <https://doi.org/10.1007/BF01731581>
- Kumar S, Stecher G, Suleski M, Sanderford M, Sharma S and Tamura K. 2024. MEGA12: Molecular Evolutionary Genetic Analysis version 12 for adaptive and green computing. Molecular Biology and Evolution, 41(12): msae263. <https://doi.org/10.1093/molbev/msae263>
- Mohapatra A, Smith DG, Mohanty SR, Mishra SS and Tudu PC. 2017. *Enchelycore propinqua* sp. nov., a new moray eel (Anguilliformes: Muraenidae: Muraeninae) from the Indian Coast. Zootaxa, 4319(3): 595-599. <https://doi.org/10.11646/zootaxa.4319.3.11>
- Myers RF. 1999. Micronesian reef fishes: a comprehensive guide to the coral reef fishes of Micronesia, 3rd revised and expanded edition. Coral Graphics, Barrigada, Guam, 330pp.
- Myers RF, Burdick DR, Mundy BC, Lindfield SJ, Tibbatts B and Donaldson TJ. 2025. New and recent records of fishes from

- the Mariana Islands, western Pacific Ocean. *Journal of the Ocean Science Foundation*, 42: 15-90. <https://doi.org/10.5281/zenodo.14750902>
- Ratnasingham S, Wei C, Chan D, Agda J, Agda J, Ballesteros-Mejia L, Ait Boutou H, El Bastami ZM, Ma E, Manjunath R, et al. 2024. BOLD v4: A Centralized Bioinformatics Platform for DNA-Based Biodiversity Data. In: DeSalle R (ed). *DNA barcoding: Methods and protocols*. Springer US, New York, pp. 403-441. [https://doi.org/10.1007/978-1-0716-3581-0\\_26](https://doi.org/10.1007/978-1-0716-3581-0_26)
- Reece JS, Bowen BW, Smith DG and Larson A. 2010. Molecular phylogenetics of moray eels (Muraenidae) demonstrates multiple origins of a shell-crushing jaw (*Gymnomuraena*, *Echidna*) and multiple colonizations of the Atlantic Ocean. *Molecular Phylogenetics and Evolution*, 57(2): 829-835. <https://doi.org/10.1016/j.ympev.2010.07.013>
- Shibukawa K, Takata Y and Shinohara G. 2007. First record of a rare moray, *Enchelycore kamara* (Actinopterygii: Anguilliformes: Muraenidae), from Amami-oshima Island, Ryukyu Islands, Japan. *Species Diversity*, 12(3): 187-192. <https://doi.org/10.12782/specdiv.12.187>
- Smith DG and Böhlke EB. 2022. Muraenidae. In: Heemstra PC, Heemstra E, Ebert DA, Holleman W and Randall JE (eds). *Coastal fishes of the western Indian Ocean. Volume 2*. South African Institute for Aquatic Biodiversity, Makhanda, pp. 37-79.
- Smith DG, Bogorodsky SV, Mal AO and Alpermann TJ. 2019. Review of the moray eels (Anguilliformes: Muraenidae) of the Red Sea, with description of a new species. *Zootaxa*, 4704(1): 1-87. <https://doi.org/10.11646/zootaxa.4704.1.1>
- Smith DG, Brokovich E and Einbinder S. 2008. *Gymnothorax baranesi*, a new moray eel (Anguilliformes: Muraenidae) from the Red Sea. *Zootaxa*, 1678(1): 63-68. <https://doi.org/10.11646/zootaxa.1678.1.4>
- Smith DG. 2012. A checklist of the moray eels of the world (Teleostei: Anguilliformes: Muraenidae). *Zootaxa*, 3474(1): 1-64. <https://doi.org/10.11646/zootaxa.3474.1.1>
- Tamura K and Nei M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, 10: 512-526. <https://doi.org/10.1093/oxfordjournals.molbev.a040023>
- Tang KL and Fielitz C. 2013. Phylogeny of moray eels (Anguilliformes: Muraenidae), with a revised classification of true eels (Teleostei: Elopomorpha: Anguilliformes). *Mitochondrial DNA*, 24(1): 55-66. <https://doi.org/10.3109/19401736.2012.710226>
- Ward RD, Zemlak TS, Innes BH, Last PR and Hebert PD. 2005. DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, 360: 1847-1857. <https://doi.org/10.1098/rstb.2005.1716>

**ROLE OF AUTHORS:** WCH and RATB collected and processed the specimen, with RATB handling the collection and transport permits. WCH photographed, examined, and sequenced the specimen. Both authors contributed to the preparation of the manuscript and approved the final version.

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# Enhancing seed germination of *Abelmoschus esculentus* (L.) Moench and *Ipomoea aquatica* Forssk. through atmospheric pressure plasma-activated distilled water

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## ABSTRACT

Seed priming is a pre-planting activity that has been found to enhance seed vigor and improve seed germination, particularly in species with poor or inconsistent germination rates or in deteriorated or poorly stored seeds. Several priming agents have been investigated, and one emerging priming agent is plasma-activated water (PAW). This study aimed to determine the effects of atmospheric pressure plasma (APP)-activated distilled water on the germination and seedling characteristics of *Abelmoschus esculentus* (L.) Moench and *Ipomoea aquatica* Forssk. Distilled water was treated with APP for 1, 3, and 5 minutes. Electrical conductivity (EC), pH, resistivity, total dissolved solids (TDS), oxidation-reduction potential, contact angle, temperature, and surface tension were determined. Further, germination tests were conducted to determine the effect of pre-soaking seeds in PAW on germination and seedling characteristics. As exposure time (ET) to APP increased, the pH, EC, and TDS of PAW also increased, while resistivity decreased. No definite effects were observed on the other physical parameters. *Abelmoschus esculentus* and *I. aquatica* showed a greater increase in seed area after pre-soaking in PAW for 8 h compared to the control. Further, PAW-treated seeds of both species exhibited higher percent water imbibition compared to the control. Regardless of ET, PAW-treated seeds had higher germination percentage, germination rate index, faster germination, and shorter germination time. Seedlings of PAW-treated seeds developed longer roots, heavier root and shoot biomass, and a higher seedling vigor index. These findings provide evidence that PAW is a potential seed priming treatment for *A. esculentus* and *I. aquatica*. The researchers propose that pre-soaking seeds in plasma-activated water exposed to APP for as little as one minute can significantly enhance germination rates and improve seedling traits.

**Keywords:** atmospheric pressure plasma jet, plasma agriculture, seed invigoration, seed technology, vegetables

## INTRODUCTION

High and uniform germination is one of the primary factors that need to be considered to attain

cropping sustainability and success (Reed et al. 2022). The importance of high-quality seed germination responses contributes directly to crop production and food security (Finch-Savage and Bassel 2016).



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Therefore, pre-planting activities like seed germination and treatment, as well as the selection of seed varieties, are important factors that need to be investigated for efficient and sustainable food production (Lamichhane et al. 2018). Farmers frequently face challenges with low seed germination, which can be attributed to various factors such as improper storage conditions, unfavorable climatic influences, pathogen infestations, reduced seedling vigor, and infections caused by seed-borne pathogens (Jose et al. 2023). Low seed germination rates and diminished seedling vigor can be effectively addressed through various enhancement techniques, with seed priming emerging as a particularly promising approach (Devika et al. 2021).

Seed priming is the conditioning for seeds through controlled hydration at a level that allows activation of pregerminative metabolic activity but not sufficient to proceed to radicle emergence (Raj and Sheeja 2019). It is commonly done to hasten germination and to improve the uniformity of seedling growth (Zulfiqar 2021). Priming also improves plant tolerance to drought and other extreme conditions (Hussain et al. 2022). Multiple techniques and materials have been used as priming agents. Among the priming agents are essential oils, chemical salts, plant extracts, bacterial metabolites, and treated water or water exposed to physical processes such as radiation, sonication, and plasma, such as atmospheric plasma (Bafoil et al. 2018; Bera et al. 2022).

Plasma activated water (PAW) is currently considered an emerging priming agent. The PAW can be produced by exposing water to cold plasma discharge from an atmospheric pressure plasma (APP) jet for a specific amount of time. Exposure of water to plasma causes changes in the physical characteristics of water such as pH, electric conductivity (EC), and total dissolved solids (TDS). The interaction of plasma and water causes increased amounts of reactive oxygen and nitrogen species (RONS), which have been reported to improve germination, growth, and development of plants, increase plant tolerance to diseases, delay ripening, and promote reinvigoration, among others (Adhikari et al. 2020). Numerous studies have been conducted to examine the effects of PAW. However, in the Philippines, research on this topic remains limited, particularly in crops such as *Abelmoschus esculentus* (L.) Moench and *Ipomoea aquatica* Forssk. Since plant species exhibit varying responses to treatments, it is essential to document these differences. Therefore, this study was conducted to explore the germination of these two species, providing a foundation for future research and further investigation.

By observing and documenting the effects of PAW on germination and seedling growth in *A. esculentus* and *I. aquatica*, the results of this study can contribute to the development of newer seed invigoration techniques that may improve crop production, minimize seed losses, and promote adoption of eco-friendly technologies. Plasma technology is recognized as an eco-friendly innovation due to its avoidance of harmful chemicals and pollutants, making it a sustainable alternative for various applications. Further, plasma treated water can still be recycled without drastic changes in its physical properties (Sajib et al. 2020).

The general objective of the study was to determine the effects of APP-treated distilled water on the germination of seeds, as well as the seedling characteristics of *A. esculentus* and *I. aquatica*. Specifically, the study aimed to physically characterize distilled water treated with APP at varying exposure time (ET), to correlate different physical properties of PAW with ET, and to evaluate the effect of presoaking seeds with distilled water treated with APP at varying ET on germination and seedling characteristics.

## METHODS

### Preparation of Distilled Plasma-activated Water

The schematic representation of the APP device used in this study is shown in Figure 1. The electrodes were two 0.5 mm diameter tungsten (W) rods enclosed in a custom-built glass tube with a 14 mm outside diameter sealed with rubber (Figure 1). The spacing between the W rods was approximately 10 mm, and they were connected to a 10 kV, 30 mA neon light power supply. The upper part of the custom-built glass tube had two openings as gas inlets. One was sealed with a rubber stopper, while the other was connected to an air compressor that delivered 55 L min<sup>-1</sup>. Distilled water (200 mL) was exposed to APP with a nozzle-to-water distance at approximately 5 cm at 0, 1, 2, 3, 4, and 5 min for the preliminary measurements and 0, 1, 3, and 5 min for the seed application.

### Characterization of Distilled Plasma-activated Water

For the measurements of physical properties of distilled water treated with APP at various exposure times, three trials were conducted. Physical parameters measured included pH, temperature (T), electrical conductivity (EC), resistance, total dissolved solids (TDS), contact angle (CA), and surface tension (ST). A handheld automatic water quality meter (YY-

1010) was used to measure pH, T ( $^{\circ}\text{C}$ ), TDS (TDS), EC ( $\mu\text{s cm}^{-1}$ ), and R ( $\text{M}\Omega$ ). The resolution and accuracy of pH, EC, TDS, T, and R were  $0.01 \pm 0.02$  pH,  $1 \mu\text{s cm}^{-1} \pm 2\%$  full-scale temperature,  $1 \text{ ppm} \pm 2\%$  full-scale temperature,  $0.1^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ , and  $0.1 \text{ M}\Omega \pm 1\%$  full scale, respectively. Other parameters such as CA and ST were measured using Ossila goniometer. A 1 cc syringe was used to drop PAW onto cleaned glass slides.

### Preparation of Seed Treatments

The experiments were arranged in a completely randomized design (CRD) with three replications and two subsamples each for the germination test to determine seed germination and seedling characteristics. One hundred seeds were used for each subsample for a total of 2,400 seeds per species. Further, for seedling characteristics, 10 seedlings were measured per subsample, with a total of 300 seedlings per species. There were three pre-soaking treatments (APP-treated distilled water with 1-, 3- and 5- min exposure durations) and a control treatment (untreated distilled water). Distilled water was purchased commercially. Seeds of *A. esculentus* and *I. aquatica*, sourced from commercial seed stores in Quezon City, Philippines, were used in the study. Seeds of *A. esculentus* and *I. aquatica* were obtained from Condor Quality seeds last 2023.

### Presoaking, Germination Tests, and Seedling Characterization

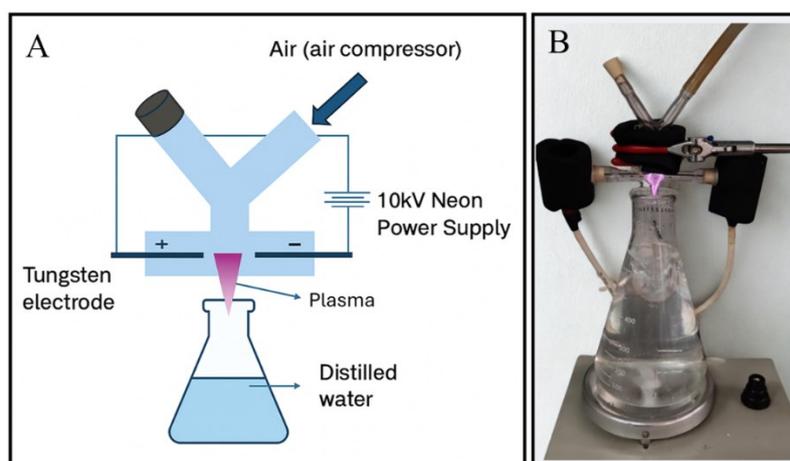
The seeds were subjected to pre-germination analysis using the method published by Adhikari et al. (2021). Ten seeds per subsample were used for each species. Seed weight was measured initially using a digital analytical balance and then seeds were soaked in treatments for 8 h. After soaking, the seeds were blot- and air-dried for 5 min. The final weights of the

seeds were determined. Percent water imbibition was calculated using the formula in Table 1. Further increase in seed area was measured using a dissecting microscope and ImageJ®, where initial (before soaking) and final (after 8 h soaking) seed areas were measured using the formula in Table 1.

A separate batch of seeds was used for the germination tests. Seeds were soaked in treatments for 12 h and air-dried for 8 h before being subjected to a germination test using the top-of-paper method (Rao et al. 2006). A day after sowing, manual counting of germinated seeds was done, characterized by radicle protrusion. Observation was continued until the 7<sup>th</sup> day of germination. Germination percentage (GP%), germination rate index (GRI), mean germination time (MGR), and germination speed (GS) were computed. The formulas for these germination parameters are shown in Table 1.

**Percent germination (%) (GP).** One hundred seeds per subsample for three replicates were sown in moistened paper towels placed in plastic tubs. Seeds were placed equidistantly on the moist paper towels, which were sprayed with 6-7 mL of distilled water using an atomizer during the 14-day testing period to keep the setup moist. Counting of seedlings was done daily for 14 days. A higher germination percentage indicated higher seed viability.

**Germination rate index (GRI).** This parameter combines both germination percentage and germination time (Tadros et al. 2011). For GRI, a higher value indicates higher vigor and more uniform, earlier germination (Bhadra et al. 2024). Daily counting of germinated seeds was performed from day 1 to day 14. Further, the higher germination rate index indicates higher seed vigor which specifically means earlier yet uniform germination.



**Figure 1.** (A) Schematic of the atmospheric pressure plasma jet set-up (Park et al. 2013; Malapit and Baculi 2021) and (B) actual distilled water treatment.

**Table 1.** Summary of the formulas used in the study.

Parameter	Formula
%Imbibition	$((\text{Seed wt. }_f - \text{Seed wt. }_i) / \text{Seed wt. }_i) \times 100$
%Increase in Seed Area	$((\text{Seed area }_f - \text{Seed area }_i) / \text{Seed area }_i) \times 100$
Germination Percentage	$((\text{Total no. of seedlings}) / (\text{No. of seeds sown})) \times 100$
Germination Rate Index	$n/1 + \dots + n/14; n = \text{no. of seedlings}$
Mean Germination Time	$\sum(\text{Daily no. of germinated seeds} \times \text{no. days from beginning of test}) / (\text{Total germinated seed at final count})$
Germination Speed	$(\text{Total no. of seedlings at Day }_i / \text{Day count }_f\text{first} + \text{Total no. of seedlings at Day }_f / \text{Day count }_f\text{final})$
Vigor Index I	$(\text{Root length} + \text{Shoot length}) \times \text{Germination percentage}$

**Mean germination time (MGT).** Mean germination time measured the time it took for the seed to germinate, focusing on the day at which most seeds had germinated. A lower MGT indicated a shorter time for germination (Orchard 1977).

**Germination speed (GS).** This parameter represented the rate of germination in terms of the total germinated seeds in a given time frame. A higher germination speed indicated faster germination (Abbasi Khalaki et al. 2019). The data from daily germination were used to calculate GS (Table 1).

**Seedling growth (root and shoot length and weight, number of secondary roots).** Seedling growth was measured by selecting 10 seedlings per subsample. Root and shoot lengths (cm) were measured using a ruler, while root and shoot weights (g) were obtained using an analytical balance to determine fresh weight.

**Vigor Index II.** Vigor Index II (VII) was calculated by determining the sum of root and shoot lengths and multiplying by germination percentage. A higher seed vigor index indicated that the seed lot was more vigorous. The VII was considered important factor providing insight into seed stress tolerance. Seed vigor pertains to uniformity of seed germination (Ranmeechai et al. 2022). Higher vigor index indicated higher seedling quality and computed using the formula (Table 1).

**Statistical Analysis**

The data on the physical parameters of PAW were collected in five replications with three subsamples per replication. The average of each physical parameter was plotted against the treatment (exposure time). Further, other parameters are presented in Table 1. Moreover, the mean values of different physical parameters of PAW-treated water were determined. The data on the physical parameters

of PAW were analyzed using Analysis of Variance (ANOVA) after meeting assumptions of normality and homogeneity of variance. A Kruskal-Wallis test, a non-parametric method, was employed to assess significant differences in oxidation-reduction potential, followed by Dunn’s test for pairwise comparisons. Additionally, the relationship between physical parameters (that exhibited normality and homogeneity of variance) and exposure time was examined using Pearson correlation analysis and was visualized with the *ggcorrplot* package in R (Wei and Simko 2024).

Seed germination and seedling characteristics data were analyzed using Analysis of Variance (ANOVA) after meeting assumptions of normality and homogeneity of variance. Analysis was conducted through Jamovi ® software to determine whether treatment or exposure time of APP-treated distilled water had an effect on pre-germination, germination, and seedling characteristics of *A. esculentus* and *I. aquatica*. Pairwise mean comparisons were performed using the least significant difference (LSD) test at a 5% level of significance.

**RESULTS**

**Characteristics of Plasma Activated Water (PAW)**

The characteristics of PAW in terms of acidity, total dissolved solids, electric conductivity, and resistivity were plotted against the exposure time to APP, as shown in Figure 2. Specifically, pH measured the acidity and basicity of the material. Total dissolved solids, on the other hand, represented the total amount of solids dissolved in water. The solids included soluble hydrogen carbonate ions, chloride salts, and non-volatile solids, among others. Further, electric conductivity referred to the ability of the

material to pass the flow of electric current, while resistivity measured how strongly a material resisted the electric current. The data showed that the acidity increased as the exposure time of distilled water to APP was prolonged.

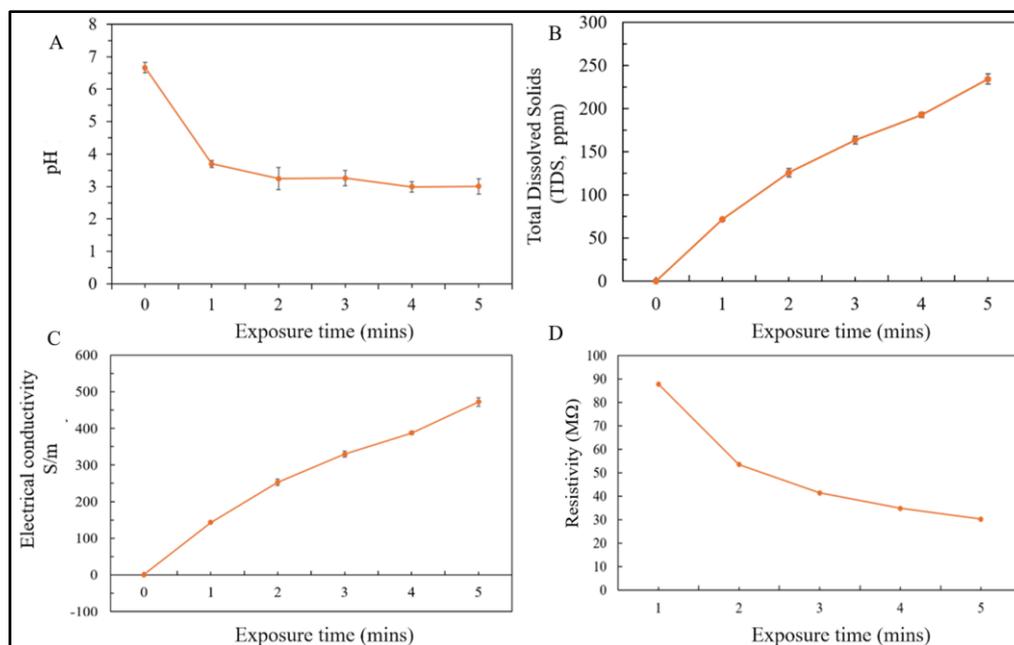
The pH underwent a significant reduction from 6.67 to 3.67 within just 1 minute of exposure to APP (Figure 2A). However, the rate of pH changes progressively decreased with prolonged exposure durations. In terms of electric conductivity, distilled water increased its EC as ET to APP was prolonged from  $2 \mu\text{s cm}^{-1}$  to  $126 \mu\text{s cm}^{-1}$  to  $459.33 \mu\text{s cm}^{-1}$ . This pattern was similarly observed in total dissolved solids, where TDS increased in distilled water as the exposure time to APP was prolonged (from 0 to 228.33 ppm). However, in terms of resistivity, there was a decrease in resistivity as the exposure time of distilled water to APP was extended. Correlational analysis of these characteristics was conducted to determine the relationships of these parameters across increasing exposure times (Figure 2).

Other physical parameters of distilled water treated with APP at varying exposure times are presented in Table 2. No significant differences were observed in temperature, contact angle, and surface tension of water treated or exposed to APP at varying durations. Further, significant differences were observed in the oxidation reduction potential (ORP) of PAW compared to the control. The highest ORP was observed in distilled water exposed to APP for 4 to 5

minutes (646–662 V). Specifically, there was almost a 50% increase in oxidation-reduction potential when distilled water was exposed to APP for 1 minute and a gradual increase thereafter.

### Correlational Analysis of Different PAW Physical Parameters and Exposure Time to APP

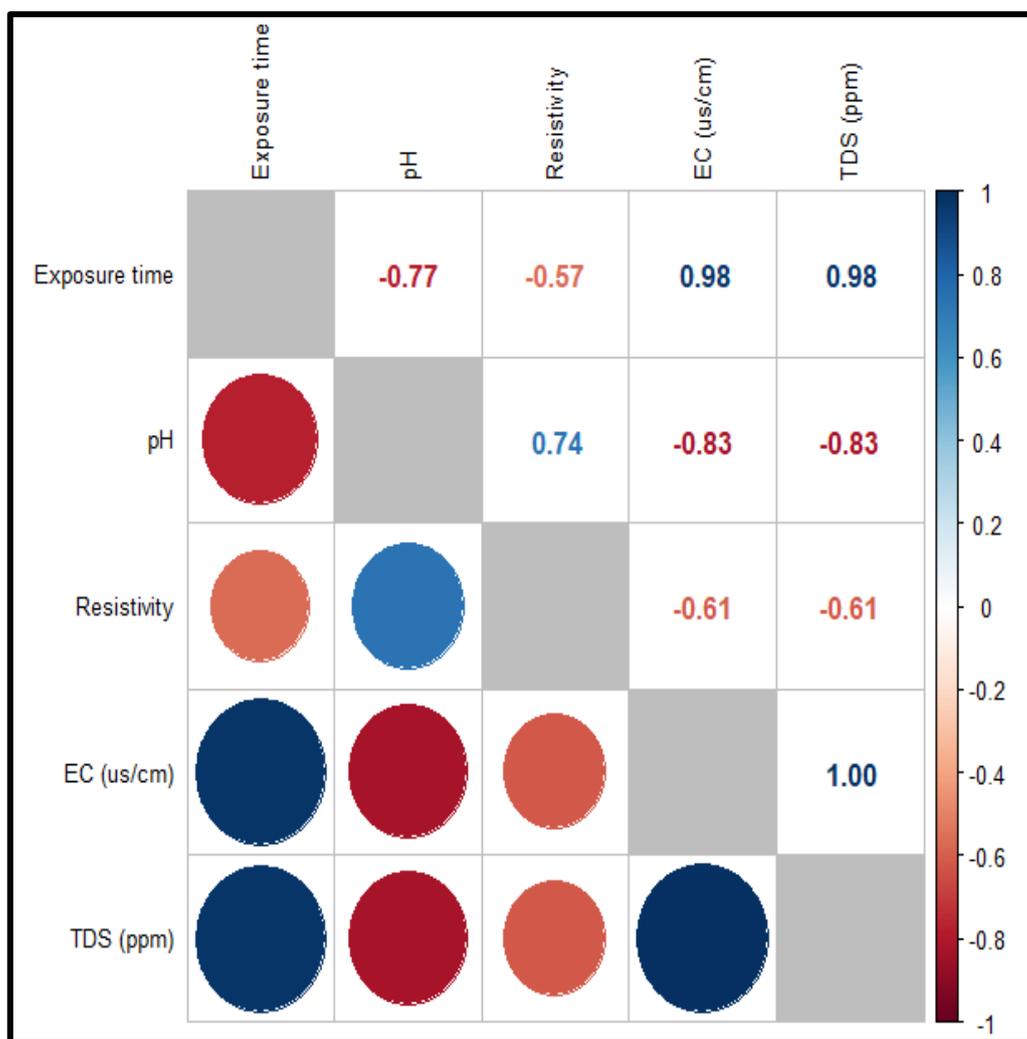
Correlation analysis of four physical parameters of treated distilled water and exposure time to atmospheric pressure plasma revealed significant relationships among pH, R, EC, and TDS when correlated with exposure time (ET) to APP. A significant correlation was observed on pH, EC, TDS, and R when correlated with ET to APP. A strong negative correlation was observed between the pH of treated water and ET ( $P = 0.000$ ,  $r = -0.77$ ), while a moderate negative correlation was observed when R was correlated with ET. However, a very strong positive correlation was observed when EC and TDS were correlated with ET ( $P = 0.000$ ,  $r = 0.98$ ;  $P = 0.000$ ,  $r = 0.98$ , respectively). Physical properties of APP-treated distilled water were also correlated with each other. R had a strong negative correlation with EC ( $P = 0.000$ ,  $r = -0.61$ ) and TDS ( $P = 0.000$ ,  $r = -0.61$ ). However, it had a strong positive correlation with pH ( $P = 0.000$ ,  $r = 0.74$ ). Moreover, a very strong negative correlation was observed when pH was correlated with EC ( $P = 0.000$ ,  $r = -0.83$ ) and TDS ( $P = 0.000$ ,  $r = -0.83$ ) (Figure 3).



**Figure 2.** Physical characteristics—(A) pH, (B) total dissolved solids, (C) electric conductivity, and (D) resistivity—of plasma activated water plotted against the exposure time of distilled water to atmospheric pressure plasma.

**Table 2.** Other physical characteristics of distilled water treated with atmospheric pressure plasma in varying exposure times. Different superscript letters indicate the significant differences in mean values at  $P < 0.05$ .

Exposure Times (min)	Temperature (°C)	Oxidation-Reduction Potential (V)	Contact Angle (°)	Surface Tension
0	30.61 ± 2.35	196.33 ± 14.93 <sup>c</sup>	39.07 ± 0.97	299.16 ± 38.98
1	30.53 ± 2.26	382.67 ± 59.34 <sup>b</sup>	37.94 ± 0.96	305.03 ± 4.48
2	30.72 ± 2.12	462.00 ± 2.52 <sup>b</sup>	39.67 ± 0.66	296.90 ± 16.04
3	30.83 ± 2.42	569.67 ± 1.16 <sup>a</sup>	38.38 ± 0.94	308.12 ± 16.04
4	31.06 ± 2.43	646.00 ± 14.34 <sup>a</sup>	40.64 ± 0.61	304.4 ± 15.11
5	31.07 ± 2.43	662.00 ± 3.05 <sup>a</sup>	39.25 ± 1.01	332.12 ± 19.40
<b>P value</b>	<b>0.977</b>	<b>&lt; 0.001</b>	<b>0.884</b>	<b>0.40</b>



**Figure 3.** The correlogram shows correlation coefficients for selected physical parameters pH, resistivity, electric conductivity, and total dissolved solids of distilled water treated with atmospheric pressure plasma at varying exposure times. Variables with more intense colors and larger sizes indicate stronger correlations, with blue signifying positive correlations, whereas red indicates negative correlations.

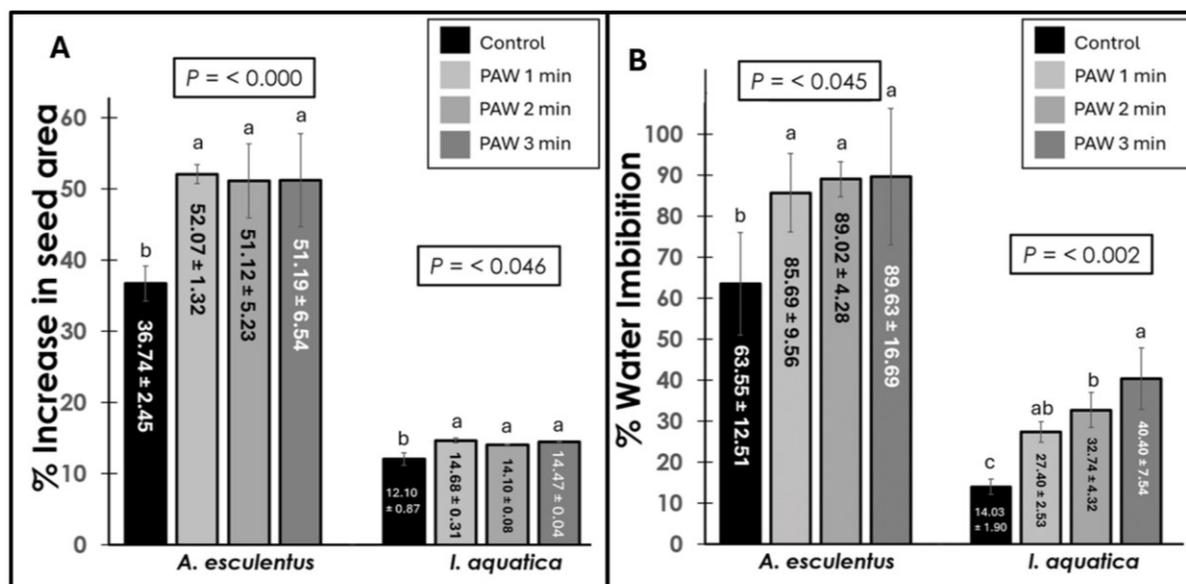
### Effect of PAW on Germination, and Seedling Characteristics

Significant differences were observed in the response of *A. esculentus* seeds soaked in distilled water treated with APP at various exposure times. For *A. esculentus*, seeds treated with PAW were larger than the control regardless of ET (Figure 4). This was similar to *I. aquatica*, where PAW-treated seeds showed a higher increase in seed area compared to the control (Figure 5). Water uptake or seed water imbibition is a vital step to initiate germination. Imbibition or water uptake occurred when seeds were exposed to water. Water imbibition promoted enzyme hydration, which in turn activated enzymes. Important hydrolytic enzymes included proteases, amylases and lipase, which played a role in the breakdown of food sources in the seed (Joshi 2018). Water uptake or imbibition was observed through swelling and softening of the testa. Figure 5 showed the morphological swelling and softening of the testa across three species. As shown in Figure 1, PAW significantly increased water absorption in *I. aquatica* ( $P = 0.00$ ) and *A. esculentus* ( $P = 0.046$ ) when compared to the control (distilled, untreated). Moreover, similar responses were observed in seeds treated with PAW regardless of the PAW's ET to APP.

The effects of PAW treatment on seed germination are presented in Table 3. In all selected germination parameters, GP, GRI, MGT, and GS, significant differences were observed in the germination response of seeds for *A. esculentus* and *I. aquatica*. Higher germination rates were observed in seeds of *A. esculentus* and *I. aquatica* treated with

PAW compared to the control (untreated, distilled). This indicated that PAW promoted the germination of seeds. In terms of the germination rate index or seed vigor index, higher GRI was observed in seeds treated with PAW when compared to the control. The germination rate index determined the uniformity and earliness of seed germination. A higher GRI meant that more seeds germinated earlier. Higher GRI was consistently observed in PAW-treated seeds than in untreated seeds, indicating that PAW increased seed vigor.

Seedling characteristics of *A. esculentus* and *I. aquatica* whose seeds were treated with distilled water exposed to APP at varying durations are presented in Table 4. Significant differences were observed in root length, weight, shoot weight, and vigor index I were observed in *A. esculentus* and *I. aquatica*, except for shoot length, while in wheat, all parameters showed significant differences across treatments. In *A. esculentus* and *I. aquatica*, roots were longer in seeds treated with distilled water exposed to APP for 1 minute. Consistently, the shortest root length was observed in the control. Further, in terms of root weight, the heaviest roots were observed in treated seeds. Moreover, in terms of shoot length, similar shoot lengths were observed in *A. esculentus* and *I. aquatica* across treatments. Furthermore, in terms of shoot weight, seedlings derived from PAW-treated seeds had heavier shoots than the control. Lastly, in terms of vigor index I, seeds treated with distilled water exposed to APP for 1 minute consistently had the highest seed vigor value across two species.



**Figure 4.** Percentage increase in seed area and percentage of water imbibition. Different letters indicate significant differences in mean values at  $P < 0.05$ .



**Figure 5.** Representative micrographs (40X) of *Abelmoschus esculentus* and *Ipomoea aquatica* seeds before (Dry) and after imbibition with plasma-activated water (Imbibed).

**Table 3.** Germination characteristics (germination percentage (GP), germination rate index (GRI), mean germination time (MGT), mean germination rate (MGR) and germination speed (GS) of *A. esculentus* and *I. aquatica* seeds across plasma-activated water treatments. Different letters in superscript indicate significant differences in mean values at  $P < 0.05$ .

Crop Species	Treatments	Germination Characteristics			
		GP (%)	GRI	MGT	GS
<i>Abelmoschus esculentus</i>	Control	54.29 ± 2.86 <sup>b</sup>	6.01 ± 0.49 <sup>b</sup>	3.55 ± 0.35 <sup>a</sup>	4.03 ± 0.57 <sup>b</sup>
	PAW (1 min)	93.33 ± 1.65 <sup>a</sup>	23.63 ± 1.29 <sup>a</sup>	1.98 ± 0.23 <sup>b</sup>	7.63 ± 0.79 <sup>a</sup>
	PAW (3 min)	94.29 ± 2.85 <sup>a</sup>	24.46 ± 1.05 <sup>a</sup>	1.81 ± 0.18 <sup>b</sup>	7.75 ± 0.31 <sup>a</sup>
	PAW (5 min)	88.56 ± 2.86 <sup>a</sup>	23.63 ± 0.64 <sup>a</sup>	1.77 ± 0.15 <sup>b</sup>	7.57 ± 0.54 <sup>a</sup>
	<i>P</i> value	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>
<i>Ipomoea aquatica</i>	Control	92.38 ± 5.95 <sup>b</sup>	9.49 ± 1.05 <sup>b</sup>	3.71 ± 0.19 <sup>b</sup>	5.88 ± 0.92 <sup>b</sup>
	PAW (1 min)	96.19 ± 3.30 <sup>a</sup>	18.39 ± 0.67 <sup>a</sup>	2.23 ± 0.10 <sup>a</sup>	10.76 ± 0.01 <sup>a</sup>
	PAW (3 min)	96.19 ± 1.65 <sup>a</sup>	21.86 ± 1.39 <sup>a</sup>	1.97 ± 0.22 <sup>a</sup>	10.91 ± 0.47 <sup>a</sup>
	PAW (5 min)	99.05 ± 1.65 <sup>a</sup>	19.96 ± 1.08 <sup>a</sup>	2.30 ± 0.04 <sup>a</sup>	10.39 ± 0.30 <sup>a</sup>
	<i>P</i> value	<b>0.023</b>	<b>0.000</b>	<b>0.010</b>	<b>0.000</b>

**Table 4.** Root (Rt) and shoot (St), length (L) and weight (Wt), and seedling vigor index (VII) of *Abelmoschus esculentus* and *Ipomoea aquatica* seedlings across plasma-activated water treatments. Different superscript letters indicate significant differences in mean values at  $P < 0.05$ .

Crop	Treatments	RtL (cm)	RtWt (g)	StL (cm)	StWt (g)	VII
<i>Abelmoschus esculentus</i>	Control	6.27 ± 0.57 <sup>c</sup>	0.01 ± 0.000 <sup>c</sup>	11.50 ± 0.10	0.28 ± 0.06 <sup>c</sup>	963.90 ± 41.17 <sup>c</sup>
	PAW (1 min)	10.0 ± 0.86 <sup>a</sup>	0.04 ± 0.001 <sup>a</sup>	12.97 ± 0.50	0.43 ± 0.05 <sup>ab</sup>	2223.33 ± 2.14 <sup>a</sup>
	PAW (3 min)	9.83 ± 0.35 <sup>ab</sup>	0.077 ± 0.006 <sup>a</sup>	12.93 ± 1.50	0.57 ± 0.071 <sup>a</sup>	2125.05 ± 19.78 <sup>ab</sup>
	PAW (5 min)	8.67 ± 0.55 <sup>c</sup>	0.077 ± 0.015 <sup>c</sup>	13.23 ± 0.31	0.36 ± 0.053 <sup>bc</sup>	1941.33 ± 137.86 <sup>b</sup>
	<i>P</i> value	<b>0.000</b>	<b>0.000</b>	<b>0.108</b>	<b>0.007</b>	<b>0.000</b>
<i>Ipomoea aquatica</i>	Control	7.633 ± 0.45 <sup>c</sup>	0.015 ± 0.00 <sup>c</sup>	11.33 ± 0.72	0.14 ± 0.02 <sup>c</sup>	1963 ± 191.65 <sup>c</sup>
	PAW (1 min)	13.27 ± 0.68 <sup>a</sup>	0.043 ± 0.00 <sup>a</sup>	12.3 ± 1.01	0.37 ± 0.05 <sup>a</sup>	2263 ± 82.99 <sup>a</sup>
	PAW (3 min)	9.83 ± 0.35 <sup>ab</sup>	0.044 ± 0.01 <sup>a</sup>	10.87 ± 0.81	0.33 ± 0.04 <sup>ab</sup>	2088 ± 137.56 <sup>ab</sup>
	PAW (5 min)	8.1 ± 0.2 <sup>b</sup>	0.037 ± 0.02 <sup>b</sup>	11.3 ± 0.27	0.29 ± 0.03 <sup>b</sup>	1921 ± 63.24 <sup>b</sup>
	<i>P</i> value	<b>0.001</b>	<b>0.000</b>	<b>0.101</b>	<b>0.001</b>	<b>0.000</b>

## DISCUSSION

### Characteristics of PAW and Correlation of Different Physical Parameters and Exposure Time to APP

The results of this correlational study corroborate the findings of Sivachandiran and Khacef (2017), who observed a drop in the pH of distilled water from 7.0 to 3.65 during the first minutes of exposure to APP. They also observed an increase in the EC of distilled water after 15 minutes of exposure. This serves as clear evidence of the accumulation of active ions in PAW. A significant increase in the number of active substances like Reactive Nitrogen Species (RNS) and Reactive Oxygen Species (ROS) was observed in plasma-activated water due to the dissolution of ionizing air in water. Various studies have reported similar findings (Benstaali et al. 1998; Oehmigen et al. 2010; Lukes et al. 2012). Sivachandiran and Khacef (2017) further explained that in gas-water environments, like the treatment set-up in this study, the increase in acidity can be attributed to the formation of  $\text{HNO}_2$  and  $\text{HNO}_3$  acids. In terms of total dissolved solids, Chou and Ting (2023) and Rathore (2022) indicated that ROS and RNS dissolve when they come in contact with distilled water. These dissolved reactive species increase both TDS and EC in distilled water exposed to APP. Chou and Ting (2023) also emphasized that the increase in TDS of distilled water exposed to APP indicates successful plasma activation of the water. Through plasma activation, some compounds were generated, as evidenced by the increasing TDS of PAW, such as hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), ozone ( $\text{O}_3$ ), nitrite ( $\text{NO}_2^-$ ), and nitrates ( $\text{NO}_3^-$ ). Furthermore, hydroxyl radicals (OH), peroxyxynitrite ( $\text{ONOO}^-$ ), and nitric oxide (NO) are among the typically produced substances, as reported by Wong et al. (2023). Rathore (2022) also observed an increase in dissolved species with increasing exposure time of distilled water to APP.

Resistivity on the other hand, is the ability of a material to resist electric conductivity. Figure 2D shows that R decreases with prolonged exposure to APP. Since there is an increased accumulation of electrically conducting substances in PAW, such as ROS and RONS, as evidenced by the increasing EC and TDS (Figure 2B and 2C), R decreases. The accumulation of ions in the treated water makes it less resistant to EC, hence lowering R.

### Effect of PAW on Germination and Seedling Characteristics

The high concentration of RONS in treated distilled water might have caused a higher imbibition rate due to the weakening of the testa, which allowed

greater water absorption. Acid treatment in seeds is commonly used to break dormancy by softening the seed coats, which PAW might have done to the seeds of *A. esculentus* and *I. aquatica*. This likely resulted in more rapid and higher imbibition rates compared to the control. Kučerová et al. (2019) reported that in wheat, increased water uptake was observed when wheat seeds were presoaked in PAW. Another study noted that PAW containing ROS improves seed germination through testa softening due to its acidic nature, while another study found that PAW treatment increased testa pore size, thereby enhancing water uptake (Priatama et al. 2022; Tonmitr and Yonesu 2023).

Guragain et al. (2023) explained that RONS in PAW, such as  $\text{NO}_2$  and  $\text{H}_2\text{O}_2$  may interact with seeds that generally have hydrophobic testa. These RONS interact with the waxy surface of the testa, effectively stripping away the wax. This interaction promotes seed hydrophilicity, initiating quicker water penetration and absorption during the imbibition stage that speed up germination. They also reported that seeds of *Hordeum vulgare* L., *Fagopyrum esculentum* Moench., and *Brassica nigra* (L.) W.D.J. Koch had higher imbibition rates than those soaked in untreated distilled water (Guragain et al. 2021).

The differences in germination characteristics of seeds presoaked in PAW and those soaked only in distilled water can be attributed to the physicochemical parameters of PAW. Studies have shown that the presence of nitric oxide in PAW may have enhanced germination in different plant species such as *Solanum lycopersicum* L., *Capsicum annum* L., *Glycine max* (L.) Merr., and *H. vulgare* (Sivachandiran and Khacef 2017; Guragain et al. 2021; Khan et al. 2023). Pandey et al. (2023) further noted that PAW contains nitrogen compounds important to agricultural production.

Moreover, the presence of various nitrogen species, particularly  $\text{NO}_3^-$ , stimulates germination by breaking seed dormancy. The RONS in PAW may provide a positive signal that eases dormancy and influences the abscisic/gibberellic acids pathway, which is mainly responsible for germination (Guragain et al. 2021). Khan et al. (2023) elaborated that when  $\text{NO}_3^-$  is reduced to  $\text{NO}_2^-$  through nitrate reductase, amino acids may be produced that are vital in plant development, especially during germination. Furthermore, ROS absorbed by the seed during imbibition triggered increased respiration, which initiated sugar oxidation. However, the effect of different PAW exposed to APP at varying ET was found to be insignificant in the germination percentage of *A. esculentus* and *I. aquatica*. Similar responses were observed on seeds of *H. vulgare* and *B. nigra*.

treated with PAW for 5 and 10 minutes (Guragain et al. 2021).

Sivachandiran and Khacef (2017) reported that PAW-treated seeds had shorter shoots than untreated ones. Fan et al. (2020) also observed shorter shoot lengths in PAW-treated mungbean seedlings. Moreover, Sukhani et al. (2021) obtained a similar result in their study on algal growth. They observed higher biomass yield in *Chlorella vulgaris* Beijerinck grown in PAW (Sukhani et al. 2021). In dicots, it is not recommended to have seedlings with leggy shoots, or excessively long shoots, because such shoots are weak allocate energy to elongation rather than in biomass accumulation, making them prone to bending. The increase in biomass accumulation in seedlings is more desirable, which was observed in seedlings derived from PAW-treated seeds.

Kučerová et al. (2019) supported the findings of this study as they observed stimulated growth in wheat seedlings watered with PAW. Moreover, Priatama et al. (2022) reported that in *Arabidopsis*, non-thermal plasma treatment improved germination and long-term growth, as evidenced by increased seedling biomass, enhanced production of antioxidants and phytohormones, and promoted expression of drought tolerance genes. They also stated that the high concentrations of nitrate and nitrite contributed significantly to the improvement of plants irrigated with PAW compared to those plants irrigated with untreated water. Since nitrogen is essential for plant growth and metabolism, APP's ability to increase nitrate and nitrite concentration in water suggests that PAW can act as a nitrogen fertilizer. Given that nitrogen is the backbone of metabolism, plant organ development in the test species improved under PAW treatment compared to the control. Despite not having variable responses across treatments on shoot length, shoots were heavier on PAW-treated plants. Song et al. (2023) reported that PAW-treated peanut seeds had improved biomass-related morphological traits, particularly hypocotyl growth. At the cellular level, they observed enlarged parenchyma cells in the hypocotyl pith of PAW-treated seeds. Further, qRT-PCR showed upregulation of auxin response and growth-related genes such as AhGI 75, AhCUC3-65, and AhARF5. The upregulation of these genes promotes cell division, thereby increasing biomass.

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## GENERATIVE AI STATEMENT

The authors did not use generative AI tools while writing the paper.

## ETHICAL CONSIDERATIONS

The study was exempted from any ethics guidelines. Human respondents and experimental animals were not involved in the study. However, safety protocols in handling plasma were strictly observed.

## DECLARATION OF COMPETING INTEREST

The authors declare that there are no competing interests among the authors.

## ACKNOWLEDGMENTS

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## REFERENCES

- Abbasi Khalaki M, Ghorbani A and Dadjou F. 2019. Influence of nano-priming on *Festuca ovina* seed germination and early seedling traits under drought stress, in laboratory condition. *ECOPERSIA*, 7(3): 133-139.
- Adhikari B, Adhikari M and Park G. 2020. The effects of plasma on plant growth, development, and sustainability. *Applied Sciences*, 10(17): 6045. <https://doi.org/10.3390/app10176045>
- Adhikari B, Dhital PR, Ranabhat S and Poudel H. 2021. Effect of seed hydro-priming durations on germination and seedling growth of bitter melon (*Momordica charantia*). *PLoS ONE*, 16(8): e0255258. <https://doi.org/10.1371/journal.pone.0255258>
- Bafoil M, Jemmat A, Martinez Y, Merbahi N, Eichwald O, Dunand C and Yousfi M. 2018. Effects of low temperature plasmas

- and plasma activated waters on Arabidopsis thaliana germination and growth. *PLoS ONE*, 13(4): e0195512. <https://doi.org/10.1371/journal.pone.0195512>
- Benstaali B, Moussa D, Addou A and Brisset JL. 1998. Plasma treatment of aqueous solutes: Some chemical properties of a gliding arc in humid air. *European Physical Journal Applied Physics*, 4: 171-179. <https://doi.org/10.1051/epjap:1998258>
- Bera K, Dutta P and Sadhukhan S. 2022. Seed priming with non-ionizing physical agents: plant responses and underlying physiological mechanisms. *Plant Cell Reports*, 41(1): 53–73. <https://doi.org/10.1007/s00299-021-02798-y>
- Bhadra M, Mondal S, Das A and Bandyopadhyay A. 2024. Gibberellic acid treatment improves seed germination and seedling establishment in *Tinospora cordifolia* (Willd.) Hook. F. and Thoms. *Journal of Applied Biology & Biotechnology*, 12(4): 128-135. <https://doi.org/10.7324/JABB.2024.158607>
- Chou YJ and Ting Y. 2023. Plasma-activated water regulated transcriptome gene expression leading to high germination and growth of mung beans. *Chemical and Biological Technologies in Agriculture*, 10: 146. <https://doi.org/10.1186/s40538-023-00497-2>
- Devika OS, Singh S, Sarkar D, Bamwal P, Suman J and Rakshit A. 2021. Seed priming: A potential supplement in integrated resource management under fragile intensive ecosystems. *Frontiers in Sustainable Food Systems*, 5: 654001. <https://doi.org/10.3389/fsufs.2021.654001>
- Fan L, Xiufang L, Yunfang M and Qisen X. 2020. Effects of plasma-activated water treatment on seed germination and growth of mung bean sprouts. *Journal of Taibah University for Science*, 14(1): 823-830. <https://doi.org/10.1080/16583655.2020.1778326>
- Finch-Savage WE and Bassel GW. 2016. Seed vigour and crop establishment: extending performance beyond adaptation. *Journal of Experimental Botany*, 67(3): 567–591. <https://doi.org/10.1093/jxb/erv490>
- Guragain RP, Baniya HB, Shrestha B, Guragain DP and Subedi DP. 2023. Improvements in germination and growth of sprouts irrigated using plasma activated water (PAW). *Water*, 15(4): 744. <https://doi.org/10.3390/w15040744>
- Guragain RP, Prakash S, Pradhan SP, Baniya HB, Pandey BP, Basnet N, Sedhai B, Dhungana S, Chhetri GK, Joshi UW et al. 2021. Impact of Plasma-Activated Water (PAW) on Seed Germination of Soybean. *Journal of Chemistry*, 2021: 7517052. <https://doi.org/10.1155/2021/7517052>
- Hussain S, Ali B and Saqib M. 2022. Chapter 15—Seed priming to enhance salt and drought stress tolerance in plants: Advances and prospects. In: Shanker AK, Shanker C, Anand A and Maheswari M. (eds). *Climate Change and Crop Stress*. Academic Press, 441-464. <https://doi.org/10.1016/B978-0-12-816091-6.00012-2>
- Jose J, Mercado M and de Guzman L. 2023. Germinability and vigor of partially-aged corn (*Zea mays* L.) seeds presoaked in Sampaguita (*Jasminum sambac* [L.] Aiton) flower extract and essential oil. *International Journal of Agricultural Technology*, 19(4): 1553-1556.
- Joshi R. 2018. Role of Enzymes in Seed Germination. *International Journal of Creative Research Thoughts*. 6(2): 1481- 1485.
- Khan M, Ali S, Al Azzawi TNI and Yun BW. 2023. Nitric Oxide Acts as a Key Signaling Molecule in Plant Development under Stressful Conditions. *International Journal of Molecular Sciences*, 24(5): 4782. <https://doi.org/10.3390/ijms2405478>
- Kučerová K, Henselová M, Slováková L and Hensel K. 2019. Effects of plasma activated water on wheat: Germination, growth parameters, photosynthetic pigments, soluble protein content, and antioxidant enzymes activity. *Plasma Processes and Polymers*, 16(3): e1800131. <https://doi.org/10.1002/ppap.201800131>
- Lamichhane JR, Debaeke P, Steinberg C, You MP, Barbetti MJ and Aubertot JN. 2018. Abiotic and biotic factors affecting crop seed germination and seedling emergence: a conceptual framework. *Plant Soil*, 432(1-2): 1–28. <https://doi.org/10.1007/s11104-018-3780-9>
- Lukes P, Brisset J-L and Locke BR. 2012. Biological Effects of Electrical Discharge Plasma in Water and in Gas–Liquid Environments. In: Parvulescu VI, Magureanu M and Lukes P (eds). *Plasma Chemistry and Catalysis in Gases and Liquids*. Wiley-VCH Verlag GmbH & Co., Germany, pp. 309-352. <https://doi.org/10.1002/9783527649525.ch8>
- Malapit GM, and Baculi RQ. 2022. Bactericidal efficiency of silver nanoparticles deposited on polyester fabric using atmospheric pressure plasma jet system. *The Journal of The Textile Institute*, 113(9):1878-1886. <https://doi.org/10.1080/00405000.2021.1954426>
- Oehmigen K, Hähnel M, Brandenburg R, Wilke Ch, Weltmann KD and von Woedtke Th. 2010. The role of acidification for antimicrobial activity of atmospheric pressure plasma in liquids. *Plasma Processes and Polymers*, 7(3-4): 250–257. <https://doi.org/10.1002/ppap.200900077>
- Orchard T. 1977. Estimating the parameters of plant seedling emergence. *Seed Science Technology*, 5: 61-9.
- Pandey S, Jangra R, Ahlawat K, Mishra R, Mishra A, Jangra S, and Prakash R. 2023. Selective generation of nitrate and nitrite in plasma activated water and its physicochemical parameters analysis. *Physics Letters A*. 474:128832.
- Pandey S, Jangra R, Ahlawat K, Mishra R, Mishra A, Jangra S and Prakash R. 2023. Selective generation of nitrate and nitrite in plasma activated water and its physicochemical parameters analysis, 474: 128832. <https://doi.org/10.1016/j.physleta.2023.128832>
- Park JS, Saquilayan GMO, Jallorina MA, and Culaba IB. 2013. Optical analysis of argon atmospheric plasma jet. *Proceedings of the Samahang Pisika ng Pilipinas*, 32: SPP2013-5A-7. <https://proceedings.spp-online.org/article/view/SPP2013-5A-7>
- Priatama RA, Pervitasari AN, Park S, Park SJ and Lee YK. 2022. Current advancements in the molecular mechanism of plasma treatment for seed germination and plant growth. *International Journal of Molecular Sciences*, 23(9): 4609. <https://doi.org/10.3390/ijms23094609>
- Raj A and Sheeja R. 2019. Seed priming: An approach towards agricultural sustainability. *Journal of Applied and Agricultural Science*, 11(1): 227-234. <http://dx.doi.org/10.31018/jans.v11i1.2010>
- Ranmeechai N, Lacap AT, Tac-an MIA and Bayogan ERV. 2022. Seed germination and vigor of Four Philippine Rice varieties as influenced by hydropriming and storage at various durations. *Philippine Journal of Science*. 151(2): 755-765. <https://doi.org/10.56899/151.02.18>
- Rao NK, Hanson J, Dulloo ME, Ghosh K, Nowell D and Larinde M. 2006. *Manual of seed handling in genebanks*. Handbooks for Genebanks No. 8. Bioversity International, Rome, Italy.
- Rathore V, Tiwari BS and Nema SK. 2022. Treatment of Pea Seeds with Plasma Activated Water to Enhance Germination, Plant Growth, and Plant Composition. *Plasma Chemistry and Plasma Processing*, 42: 109–129. <https://doi.org/10.1007/s11090-021-10211-5>
- Reed RC, Bradford KJ and Khanday I. 2022. Seed germination and vigor: ensuring crop sustainability in a changing climate. *Heredity*, 128: 450–459. <https://doi.org/10.1038/s41437-022-00497-2>
- Sajib SA, Billah M, Mahmud S, Miah M, Hossain F, Omar FB, Roy NC, Hoque KMF, Talukder MR, Kabir AH et al. 2020. Plasma activated water: the next generation eco-friendly stimulant for enhancing plant seed germination, vigor and increased enzyme activity, a study on black gram (*Vigna mungo* L.). *Plasma Chemistry and Plasma*

- Processing, 40: 119–143. <https://doi.org/10.1007/s11090-019-10028-3>
- Sivachandiran L and Khacef A. 2017. Enhanced seed germination and plant growth by atmospheric pressure cold air plasma: combined effect of seed and water treatment, *Royal Society of Chemistry Advances*, 7: 1822-1832. <https://doi.org/10.1039/C6RA24762H>
- Song I, Jeon H, Priatama RA, Gayathri S, Ko K and Lee YK. 2023. Effect of plasma-activated water on peanut seed germination and vegetative growth in a hydroponic system. *Plant Biotechnology reports*, 17(4): 573-583. <https://doi.org/10.1007/s11816-023-00847-4>
- Sukhani S, Punith N, Ekatpure A, Salunke G, Manjari M, Harsha R, Chanakya HN and Lakshminarayana R. 2021. Plasma-Activated Water as Nitrogen Source for Algal Growth: A Microcosm Study. *IEEE Transactions on Plasma Science*, 49(2): 551-556. <https://doi.org/10.1109/TPS.2021.3051728>
- Tadros MJ, Samarah NH and Alqudah AM 2011. Effect of different pre-sowing seed treatments on the germination of *Leucaena leucocephala* (Lam.) and *Acacia farnesiana* (L.). *New Forests*, 42(3): 397-407. <https://doi.org/10.1007/s11056-011-9260-1>
- The jamovi project. 2024. jamovi (Version 2.5) [Internet]. <https://www.jamovi.org>. Accessed on 12 July 2024.
- Tonmitr N and Yonesu A. 2023. Effect of plasma activated water treated by LF-microwave hybrid plasma on enhancement of seed germination and plant growth. *Japanese Journal of Applied Physics*, 62(SN): SN1017. <https://doi.org/10.35848/1347-4065/acfb8d>
- Wei T and Simko V. 2024. R package 'corrplot': Visualization of a Correlation Matrix. (Version 0.94) [Internet]. <https://github.com/taiyun/corrplot>. Accessed on 04 July 2024.
- Wong, KS, Chew NSL, Low M and Tan MK. 2023. Plasma-Activated water: physicochemical properties, generation techniques, and applications. *Processes*, 11(7): 2213. <https://doi.org/10.3390/pr11072213>
- Zulfiqar F. 2021. Effect of seed priming on horticultural crops. *Scientia Horticulturae*, 286: 110197. <https://doi.org/10.1016/j.scienta.2021.110197>

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## Insect-eating festivals as a tool for promoting entomophagy in Muslim Mindanao

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### ABSTRACT

Entomophagy, or the consumption of edible insects, offers a sustainable approach to addressing food insecurity and malnutrition, particularly in marginalized and post-conflict communities. Despite its benefits, cultural resistance and entomophobia hinder broader acceptance. This study aimed to assess the effectiveness of insect-eating festivals (IEFs) in promoting awareness and acceptance of entomophagy in the Bangsamoro Autonomous Region in Muslim Mindanao (BARMM), Philippines, a predominantly Muslim region. From 2012 to 2022, as part of an extension program at Mindanao State University, Marawi City, IEFs were organized involving 3,426 participants through superworm *Zophobas morio* (Fabricius, 1776)-based food tastings, educational lectures, quizzes, and eating challenges. A descriptive research design employing pre- (n = 111; 2017) and post-event (n = 102; 2020) surveys measured changes in knowledge, acceptability, and willingness to consume insects. Survey results showed marked improvements in participants' willingness to consume insects, with the proportion expressing interest increasing from 35% before the IEF to over 50% after participation. Negative reactions, such as disgust, decreased from 33% to less than 20%, while positive descriptors (e.g., "curious," "happy") became more frequent. Sensory exposure—particularly tasting fried *Z. morio*—combined with educational lectures significantly enhanced acceptability and reduced entomophobia ( $P < 0.05$ ). Beyond acceptance, insect-based foods offer practical nutritional and economic benefits, such as high-protein, resource-efficient alternatives that can help mitigate food insecurity in BARMM. Cultural contextualization—especially the integration of Islamic dietary norms—further enhanced acceptance, underscoring the potential for halal certification of insect-based products. These results demonstrate that culturally sensitive, community-driven interventions like IEFs can effectively normalize entomophagy and support alternative nutrition strategies in post-conflict settings.

**Keywords:** edible insects, food culture, sustainable food production, university extension programs



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## INTRODUCTION

The global rise in food insecurity alongside environmental degradation has driven interest in alternative food sources. Entomophagy, or the consumption of edible insects, offers a sustainable, efficient, and nutritionally rich option to complement conventional livestock, considering that insects constitute one of the Earth's most abundant biological resources, with approximately 1,900 edible species documented to date (Lisboa et al. 2024). Recognized by the United Nations Food and Agriculture Organization (FAO) for its role in food and feed security (van Huis et al. 2013), insects have been gaining global attention due to their high feed conversion efficiency, low environmental impact, minimal land and water use, and short production cycles (Yang et al. 2024). These characteristics make insect farming a viable option for addressing hunger, malnutrition, and rural livelihood challenges, especially in resource-constrained regions (Adundo and Mulungu 2024; Liceaga 2022).

Edible insects are also highly nutritious, often matching or even exceeding the nutritional value of traditional meat. This positions them as a strong solution to problems of protein deficiency and malnutrition. For example, species like *Locusta migratoria* (Linnaeus, 1758) and *Tenebrio molitor* (Linnaeus, 1758) are very protein-dense, with levels between 48% and 67% on a dry matter basis. Their amino acid profiles are also excellent, meeting or surpassing the daily requirements for essential amino acids such as lysine and threonine set by the World Health Organization (WHO) (Nachtigall et al. 2025). Moreover, other insects, including *Zonocerus variegatus* (Linnaeus, 1758) and *Periplaneta americana* (Linnaeus, 1758), have been found to possess even higher protein content—up to 74%—while also providing crucial iron, zinc, and dietary fiber, nutrients that are often less abundant in conventional meat (Omotoso and Adesola 2023). A unique benefit of insects is that they contain chitin-derived fiber and bioactive compounds not present in livestock, offering additional health advantages. Altogether, this combination of nutritional density and functional properties makes them a strategic food source for regions facing food insecurity, such as the BARMM, which has the Philippines' highest rate of food insecurity at 84% according to the DOST-Food and Nutrition Research Institute (Gutierrez 2023). Thus, our work underscores the urgency of exploring culturally relevant, resource-efficient solutions such as entomophagy in this region.

Even with these benefits, people in many cultures are still hesitant to eat insects. This resistance often stems from deep-seated cultural norms, food neophobia, and a sense of disgust (Aguilar-Toalá et al. 2025). A significant barrier is entomophobia, which is

a fear or aversion to insects that commonly manifests as disgust, avoidance, or anxiety. Research by Alhujaili et al. (2023) confirms that the main factors influencing willingness to eat insects include disgust, food neophobia, familiarity, visibility of insect in the dish, and taste. To mainstream insect-based foods, it is crucial to address these psychological and sociocultural hurdles. A growing body of research emphasizes that successfully changing perceptions requires communication and education strategies tailored to specific audiences (House 2016; Menozzi et al. 2017; Sogari et al. 2018; Verneau et al. 2016).

In the Philippines, the practice of eating insects, or entomophagy, is far from new. It has a long history within indigenous and rural communities, where insects have traditionally complemented staple diets as part of local food culture. For generations, Filipinos have incorporated locusts, beetles, bees, and crickets into various regional dishes—everything from simple fried snacks to the classic adobo—showcasing their dual role as seasonal treats and protein sources (Gibbs et al. 1912; Domoguen 1993). In fact, locusts (subfamily: Cyrtacanthacridinae) are the most well-documented edible insect in the country's history, with records of their consumption in places like the Batanes Islands (Starr 1991). However, despite this history, the broader Filipino public often perceives insect-eating as a primitive practice, which has prevented it from being integrated into the national food system.

This disconnect highlights the need for educational efforts that are sensitive to local culture and can help demystify the idea of eating insects. One promising approach is the use of insect-eating festivals (IEFs). Research indicates that these festivals are highly effective at reducing fear and normalizing insects as food. They achieve this by offering hands-on experiential learning, opportunities to taste the insects, and a fun, public setting for engagement (Feng et al. 2018; Hvenegaard 2016; Shin et al. 2018; Woolf et al. 2019). By combining food samples with educational activities, these events make edible insects more approachable and socially acceptable. A particularly compelling place to study this kind of intervention is the Bangsamoro Autonomous Region in Muslim Mindanao (BARMM). As a predominantly Muslim and post-conflict region, its cultural and religious play a crucial role. Notably, Islamic dietary laws classify specific insects—including locusts, grasshoppers, and in some views, bees—as halal, or permissible to eat. This religious acceptance creates a natural pathway for cultural acceptability (El-Mallakh and El-Mallakh 1994).

This study focuses on the Mindanao State University-Main Campus in Marawi City (MSU-Marawi), Lanao del Sur, BARMM, which has conducted IEFs as part of its agricultural extension program since 2012. These festivals aim to promote entomophagy not only as a nutritious food source but

also as a sustainable pest control strategy and alternative income stream for the community. Over the years, the IEFs have showcased a wide variety of insect-based dishes, including coconut pest larvae *Rhynchophorus ferrugineus* (A.G.Olivier, 1791), *Oryctes rhinoceros* (Linnaeus, 1758), rice moth *Corcyra cephalonica* (Stainton, 1866), Malaysian black bugs *Scotinophara coarctata* (Fabricius), green leafhoppers (*Nephotettix* spp.), rice weevils *Sitophilus oryzae* (Linnaeus, 1763) and farmed edible insects such as black soldier fly larvae *Hermetia illucens* (Linnaeus, 1758) and superworms *Zophobas morio* (Fabricius, 1776). While a previous report documented the outcomes of a 2014–2015 IEF involving 90 participants (Aguanta 2017), this paper expands the scope by analyzing the responses of 3,426 participants from 2012 to 2022.

The study aims to (1) evaluate the effectiveness of IEFs in improving awareness and acceptance of entomophagy in a culturally specific, post-conflict setting; (2) assess participants' knowledge, acceptability, and willingness to consume edible insects; and (3) explore how integrating Islamic food norms can enhance the acceptability of insect-based foods. By documenting this large-scale, community-based intervention, the study seeks to inform future programs targeting food security, cultural nutrition, and behavioral change in similar contexts.

## METHODS

### Study Design, Study Areas, and Organization of IEFs

This study employed a descriptive research design. The MSU-Marawi extension program partnered with various institutions and groups to organize a series of IEFs (Table 1; Figure 1). These events were held on campus on different dates from 2012 to 2022 and had a total of 3,426 registered participants. Specifically, the IEFs were conducted on 06 March 2012 (Botanical Garden; n = 285), 31 August 2012 (Botanical Garden; n = 470), 22 February 2013 (Botanical Garden; n = 522), 07 March 2014 (Botanical Garden; n = 298), 14 March 2015 (Academic Complex; n = 350), 27 April 2016 (Aggie Quadrangle; n = 253), 29 April 2017 (Aggie Quadrangle; n = 402), 06 April 2018 (CHARM Pavilion; n = 299), and 01 May 2019 (CHARM Pavilion; n = 337). The researchers prepared for a 10th IEF for 2020, but due to the COVID-19 pandemic, the activities were rescheduled to 23 November 2022, at the Aggie Audiovisual Hall, which was attended by 210 participants.

The insect-based menus were developed by students enrolled in crop protection, entomology, and integrated pest management courses at the College of

Agriculture, MSU-Marawi. The preparations utilized pest and non-pest insect species collected from the field and the university-based *Z. morio* mass rearing facility. Table 2 provides a representative snapshot of insect dishes exhibited and offered for free tasting; however, these were not formally subjected to sensory acceptability scoring. As MSU-Marawi has a predominantly Muslim population of students and faculty, the selection of insect species and menu design also considered participants' cultural and religious backgrounds. Insect species permissible under Islamic dietary norms (e.g. locusts, grasshoppers, and bees) were included in the menus to align with the predominantly Muslim context of BARMM. During the IEFs, the students attended entomophagy lectures, participated in quiz contests, and assisted in organizing competitions on live insect eating, insect menu preparation, and insect-themed face and body painting. The winners of various contests received special prizes, and there was a free tasting of the insect dishes and delicacies prepared by the students. The researchers noted the participants' reactions toward entomophagy during the events.

From 2017 to 2019, the Mr. and Ms. Entomophagist beauty pageant was held, with participation from entomology and agriculture students from MSU system external campuses, namely MSU-Naawan (Misamis Oriental) and MSU-Buug (Sibugay Province). The contestants paraded in insect-themed costumes and competed in the talent and question-and-answer rounds, with questions relevant to entomophagy and other insect-related issues. The pageant judges included top MSU administrators, professors, and agricultural officers from the Department of Agriculture.

### Preparation of Insects for the Live and Speed-eating Challenge

The IEFs included two types of entomophagy challenges, namely live and fried *Z. morio* eating. The first three IEFs utilized two common coconut insect pests, the Asiatic palm weevil (*R. ferrugineus*) and the rhinoceros beetle (*O. rhinoceros*), as well as the rice moth (*C. cephalonica*). These pests were collected from sawdust piles produced by a sawmill in Aurora, Zamboanga del Sur, while the larvae of the rice moth were obtained from the Regional Crop Protection Center (RCPC), Region IX, Molave, Zamboanga del Sur. Prior to the event, the insects were fasted for one day. In the succeeding IEFs, however, laboratory mass-reared *Z. morio* were used due to the unavailability of rice moth larvae, which were being used by the RCPC for the mass rearing of *Trichogramma* spp., a parasitic wasp used to control rice stemborers. For the speed-eating challenge of the Mr. and Ms. Entomophagist pageant, contestants consumed chocolate-coated *Z. morio*.

**Table 1.** Partner institutions and their roles in the IEFs. Abbreviations: NGA – national government agency; NGO – non-government agency; M – military; A – academe; LC – local community.

Partner	Sector	Role
Commission on Higher Education	NGA	Funder
Department of Agriculture, Region IX, Mahayag, Zamboanga del Sur	NGA	Funder
Department of Agriculture, Isabela City, Zamboanga Peninsula Region	NGA	Funder
Philippine Coconut Authority, Butuan City	NGA	Funder
Department of Education, Ramon Magsaysay, Zamboanga del Sur	NGA	Participant
Omega Team Philippines, Inc.	NGO	Participant
Philippine Army	M	Participant
MSU Integrated Laboratory School, MSU, Marawi City	A	Participant
MSU Institute of Science Education, MSU, Marawi City	A	Participant
MSU-Iligan, Iligan City	A	Participant
MSU-Naawan, Naawan, Misamis Oriental	A	Participant
MSU-Buug, Buug, Sibugay Province	A	Participant
Barrio Sikap, MSU, Marawi City	LC	Participant
Cabingan, Marawi, Lanao del Sur	LC	Participant
Palao, Ranaranao, Marantao, Lanao del Sur	LC	Participant



**Figure 1.** Activities in the insect-eating festivals. (A) Mr. and Ms. Entomophagist, 2019; (B) live speed insect-eating challenge, 2014; (C) insect cooking contest, 2012; (D) insect face and body painting, 2017; (E) insect quiz contest, 2014; (F) sale of edible insect-based food products, 2013; (G) insect-eating acceptability survey, 2019; (H) judging of best insect menu, 2019; (I) free tasting of insect recipes, 2012; (J) *Zophobas morio* (Fabricius, 1776) ice cream eating contest, 2019; (K) lecture series on entomophagy, 2018; (L) Pest Management Council of the Philippines Scientific Convention 2014.

**Pre-IEF and Post-IEF Online Survey**

To evaluate the effectiveness of the IEFs in promoting entomophagy and the acceptability of edible insects, two surveys were conducted. Participants were informed of the study's purpose, provided with informed consent, and assured of their anonymity and confidentiality.

The first survey took place on 23 November 2017, during the foundation day of the MSU-Marawi,

College of Agriculture. It involved 111 participants and served as a pre-IEF assessment. The instrument was a semi-structured questionnaire validated through pre-testing among fifteen students prior to administration. A five-point Likert scale (1 = strongly disagree; 5 = strongly agree) was used to measure willingness to taste insects, acceptability of entomophagy, and acceptability of insect dishes.

**Table 2.** Edible insects and menu in the insect-eating festivals.

Common Name	Scientific Name	Host Plant	Category	Food Preparations
Malaysian black bugs	<i>Scotinophara coarctata</i> (Fabricius)	Rice	Pest	Crispy fried insect; scrambled eggs; cookies, mango float
Green leafhoppers	<i>Nephotettix</i> spp.	Rice	Pest	Scrambled eggs
Grasshoppers	<i>Oxya chinensis</i> spp.	Rice	Pest	Gelatin; Noodles
Rice bugs	<i>Leptocorisa oratorius</i> (Fabricius, 1794)	Rice	Pest	Fried insects; scrambled eggs
Rice weevil	<i>Sitophilus oryzae</i> (A. Hustache, 1930)	Rice	Pest	Polvoron
Rice moth	<i>Crocyra cephalonica</i> (Stainton, 1866)	Rice	Pest	Sticky rice
Red flour beetle	<i>Tribolium castaneum</i> (Herbst, 1797)	Rice	Pest	Polvoron
Ladybird beetle	<i>Micraspis crocea</i> (Mulsant)	Rice	Non-pest	Fried peanut mix
Corn weevil	<i>Sitophilus zeamais</i> (Motschulsky & V.de, 1855)	Corn	Pest	Polvoron
Earwig	<i>Euborellia annulata</i> (Fabricius, 1793)	Corn	Non-pest	Banana rolls
Migratory locust	<i>Locusta migratoria</i> subsp. <i>manilensis</i> (Meyen, 1835)	Corn	Pest	Orange cake
Cabbage worm	<i>Crociodomia pavonana</i> (Fabricius, 1794)	Cabbage	Pest	Sticky rice cake
Curculionid beetle	<i>Metapocyrtus</i> spp.	Cabbage	Pest	Banana rolls
Banana pseudostem weevil	<i>Odoiporus longicollis</i> G.A.K.Marshall, 1930	Banana	Pest	Cookies
Coconut leaf beetle	<i>Brontispa longissima</i> (Gestro, 1885)	Coconut	Pest	Buttered cake
Asiatic palm weevil	<i>Rhynchophorus ferrugineus</i> (A.G.Olivier, 1791)	Coconut	Pest	Noodles; adobo
Rhinoceros beetle	<i>Oryctes rhinoceros</i> (Linnaeus, 1758)	Coconut	Pest	Vinegar-braised beetle
Cotton stainer	<i>Dysdercus cingulatus</i> (Fabricius, 1775)	Lady's fingers	Pest	Fried peanut mix
Leaf-footed bug	<i>Physomerus grossipes</i> (Fabricius, 1794)	Sweet potato	Pest	Fried peanuts mix
Lantana leaf-miner beetle	<i>Uroplata girardi</i> Pic, 1934	<i>Lantana camara</i> L.	Pest	Polvoron
Superworm	<i>Zophobas morio</i> (Fabricius, 1776)	Wheat bran	Non-pest	Spaghetti; stir-fried vegetables, pork rolls; deep fried insect; rice cake, macaroons, Polvoron, beef rendang, palapa, mango float, and superworm ice cream flavored with durian, mango and purple yam
Black soldier fly (larvae)	<i>Hermetia illucens</i> (Linnaeus, 1758)	Seaweed <i>Eucheuma cottonii</i> Weber Bosse, 1913	Non-pest	Fried maggots
Honeybee (larvae)	<i>Apis cerana</i> (Fabricius, 1793)	Coconut	Non-pest	Orange cake

The second survey was an online post-IEF assessment conducted from 26-30 May 2020. It aimed to evaluate the impact of the IEFs in promoting entomophagy and reducing entomophobia. The survey included 102 participants who responded to online

requests for feedback. Purposive sampling was employed to capture individuals who had attended at least one IEF, thereby ensuring that the sample represented those with direct exposure to insect-based foods. However, we acknowledge the potential for

self-selection bias, as only those willing to give feedback responded.

Part I of the instrument focused on respondents' general knowledge of entomophagy, their acceptability of edible insects, and the perceived usefulness of the lectures. Part II gathered information about the participants' experiences during the IEFs and their suggestions for future activities. While changes in dietary patterns or protein intake post-IEF were not formally measured, open-ended responses included remarks about the potential inclusion of insect dishes in participants' diets.

### Data Analysis

Survey data were analyzed using descriptive statistics (percentages, means) and chi-square tests to assess differences in reactions before and after the IEFs.

### Ethics

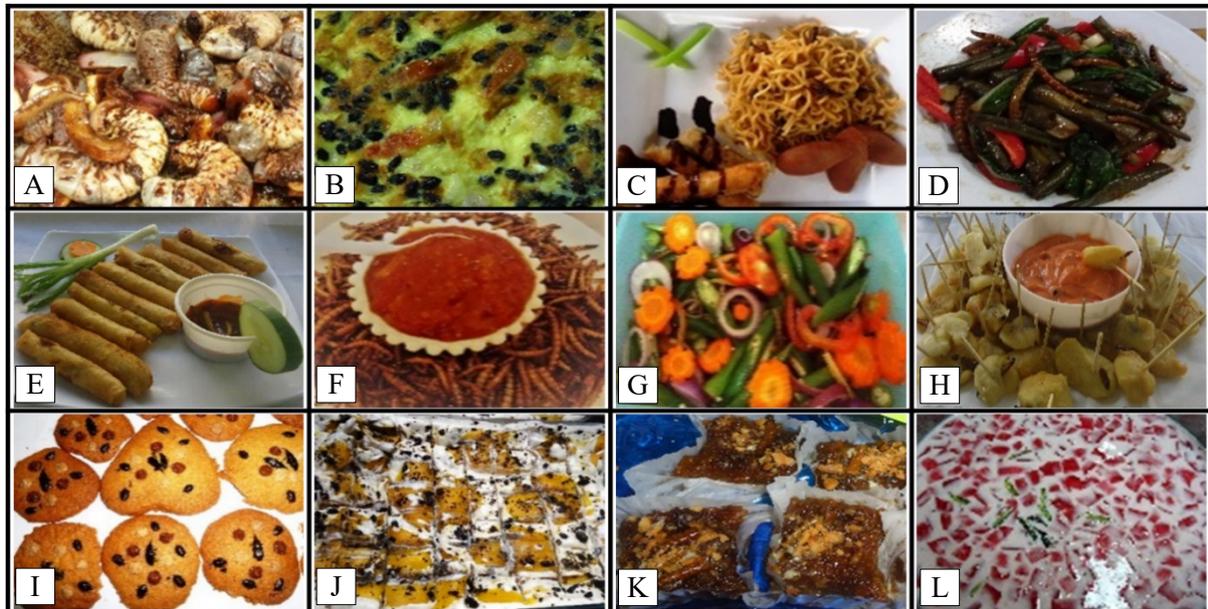
At the time the IEFs were conducted, MSU-Marawi did not yet have a formally established Institutional Review Board. Nevertheless, the research

adhered to the 2017 Philippine National Ethical Guidelines for Health and Health-Related Research developed by the Philippine Health Research Ethics Board (PHREB 2017). Participation was voluntary, informed consent was obtained, no minors (<18 years) were included, and anonymity and confidentiality were maintained. The study protocol, including survey design and participant recruitment, was reviewed by the College of Agriculture faculty panel to ensure compliance with ethical standards.

### RESULTS

The MSU-Marawi organized 10 IEFs as part of its extension program, providing students, employees, and local communities with direct experiential learning in a fiesta-like atmosphere. Entomophagy was promoted through brief lectures, contests (e.g. quizzes, cooking, face/body painting) and opportunities to taste insect-based menus (Figure 2).

**Figure 2.** Selected Filipino insect-based dishes developed by the students and served during the insect-eating festivals. (A) Adobong “batod” (*Rhynchophorus ferrugineus* A.G.Olivier, 1791); (B) scrambled eggs with Malaysian black bugs (*Scotinophara coarctata* (Fabricius)); (C) pansit (Filipino-style noodles) with grasshoppers *Oxya chinensis* (Thunberg, 1815); (D) stir-fried vegetables topped with crispy fried superworms *Zophobas morio* (Fabricius, 1776); (E) superworm *Z. morio* lumpia (fried insect rolls in rice wrapper); (F) deep-fried superworms (*Z. morio*) with spicy chili sauce; (G) steamed vegetable salad with fried superworms (*Z. morio*); (H) fried palapa (traditional crochets) with *Z. morio*; (I) buttered insect cookies (*S. coarctata*; and *Odoiporus longicollis* G.A.K.Marshall, 1930); (J) Graham mango float dessert with Malaysian black bugs (*S. coarctata*); (K) superworm (*Z. morio*); biko (steamed rice cake with caramelized coconut); and (L) cathedral window gelatin with grasshoppers (*Oxya chinensis* Thunberg, 1815).



**Attendee Demographics and Information Sources**

Most survey participants were aged 20-24 years (67.3%; n = 68), female (57.4%; n = 58), and Meranao (63.7%; n = 64)—the second-largest cultural group among Muslim Filipinos, rooted in Lanao. A majority first learned about entomophagy through the university's crop protection courses (55.4%; n = 55). Others were introduced through previous IEFs (32.6%; n = 33), by friends out of curiosity (35.6%; n = 36), or through IEC materials (4.9%; n = 5) and posters (9.9%; n = 10). Fifty-three (52.8%; n = 54) were first-time attendees, while 23 (22.8%; n = 23) had joined once before IEF, and the rest had participated several times. Among those who attended, most of the participants (72.8%; n = 74) engaged in at least one IEF activity.

The most popular event was the free fried *Z. morio* tasting, which was the common dish served across all IEFs (56.4%; n = 57). This activity was followed by the insect cooking contest (19.8%; n = 20), insect-themed face and body painting (11.9%; n = 12), insect quiz contest (9.9%; n = 10), live insect-eating challenge (8.9%; n = 9), and the Mr. and Ms. Entomophagist pageant (3.9%; n = 4).

**Knowledge and Awareness of Entomophagy**

Most participants learned about entomophagy through their crop protection courses (55.4%; n = 56). Additionally, a sizable portion became aware of entomophagy through the IEFs (32.7%; n = 33) and through word-of-mouth from friends (35.6%; n = 36).

Among the participants who had low awareness of entomophagy, the initial responses to tasting fried *Z. morio* and larvae of soldier flies (*H. illucens*) were primarily entomophobia (40.6%; n = 41) and disgust (67.2%; n = 68). It is worth noting that the

soldier fly maggots were reared on seaweed *Eucheuma cottonii* (Weber Bosse, 1913) organic fertilizer, while the *Z. morio* were reared on wheat bran under laboratory conditions.

**Edible Insect Acceptability**

Many participants found fried *Z. morio* and *H. illucens* maggots crispy (60.4%; n = 61), tasty (39.6%; n = 40), and even delicious (14.9%; n = 15). These acceptability ratings imply a reduction of entomophobia (31.7%; n = 32) and disgust (49.5%; n = 50). Once they discovered the taste and flavor of insects, they repeatedly consumed them. Lectures on entomophagy were also useful (83.2%; n = 84) in promoting entomophagy and in overcoming entomophobia and disgust. Most participants recommended entomophagy as part of the modules (72.3%; n = 73) in future Crop Protection classes. Only one out of those surveyed did not find the lectures useful (1.0%; n = 1), while the rest remained undecided (15.8%; n = 16) about the impact the lectures had on them in persuading them to attempt eating the insects. As individuals attend these events more frequently, their exposure to and familiarity with insect-based foods increase, contributing to a measurable reduction in entomophobia (Table 3).

While participants primarily engaged with the IEFs as an introduction to entomophagy, the surveys did not explicitly capture perceptions of edible insects as a sustainable protein source in the context of food scarcity or rising prices. Nonetheless, anecdotal observations during open discussions suggested that some participants informally associated entomophagy with alternative or future food options, indicating an interesting area for further investigation.

**Table 3.** Participants' reactions before and after participating in the insect-eating festivals. \*The *P*-value is less than the significance level ( $\alpha = 0.05$ ), indicating a statistically significant difference in participants' reactions, \*\* the participants were offered ten live superworms in the speed-eating challenge (see Methodology).

Question	Answer	Pre-IEF (n=111)	Post-IEF (n=102)	X <sup>2</sup> (P-value)
Are you interested in eating insects?	No	40.6	15.8	15.60* (p = 0.020)
	Yes	34.6	52.5	
	Undecided	26.7	34.6	
How do you feel about being challenged to taste an edible insect? **	Disgust	32.7	11.9	20.52* (p = 0.041)
	Positive	20.9	11.9	
	Surprised	16.8	20.8	
	Happy	5.9	1.9	
	Curious	34.7	58.4	

**DISCUSSION**

**Overcoming Entomophobia: Promoting Acceptance of Insect-based Foods**

The fear of eating insects prevails based on individual experiences and Filipino superstitious beliefs (DeFoliart 1999). Insects are seen as pests that

damage crops, invade kitchens, and feed on rotten foods, dung, dead bodies of animals, and even human corpses, and they are seen as vectors for malaria and dengue hemorrhagic fever. In addition, there is a traditional belief that “mambabarangs” (or sorcerers) use insects in their rituals to inflict injuries or torture their victims. Many Filipinos, especially in the

provinces, believe that hearing cricket songs and seeing a black butterfly fluttering augurs the spirit of a loved one relaying a message, which instills entomophobia and disgust (van Huis 2013).

Educational activities play a vital role in promoting entomophagy, as evidenced by our experiences organizing IEFs. By informing consumers about the numerous benefits of consuming insects, such as their potential as an alternative source of protein and their effectiveness in pest control, we take a significant step towards overcoming entomophobia and disgust among individuals (Looy and Wood 2006). Rather than simply providing knowledge, the combination of lectures, public demonstrations, and direct tasting helped participants replace negative associations with positive sensory experiences. The model used in these IEFs operates similarly to exposure therapy, wherein a person's fear is mitigated through gradual and repeated encounters with the source of anxiety (Pachana et al. 2007).

According to Sogari et al. (2018), curiosity is the main driver that leads people to sample insect-based dishes for the first time during such events. In their study, curiosity functioned as a psychological gateway. Once participants overcame their initial hesitation and actually tasted the insects, a positive experience with the flavor made them much more willing to eat them again. We can see a similar pattern in the work of Han et al. (2017) in South Korea. However, the situation in the BARMM was distinctive because curiosity was supported by a powerful framework of cultural and religious acceptance.

It is also critical to understand that the fear of insects is not solely psychological as it can also elicit observable physiological responses. These include facial distortion, eye closure, trembling, sweating, raised neck hairs, goosebumps, and even vomiting (Sikora and Rzymiski 2025). These findings indicate that role modeling by teachers and respected community members amplified the desensitization process, but unlike in European contexts where disgust dominates (Kröger et al. 2022), in BARMM, the embedding of insects in halal and culturally familiar dishes provided an additional layer of reassurance.

To further enhance consumer acceptance of insect-based foods, incorporating entomophagy-related topics in various courses such as entomology, integrated pest management, and crop protection could be an effective strategy. From 2012 to 2022, the annual IEFs helped dispel preconceived notions and fallacies about insects, leading to increased acceptance of entomophagy among participants.

It may be useful to consider the effects of exposure therapy, a common treatment for phobias (Pachana et al. 2007). Gradual desensitization through repeated exposure to insects in the form of food consumption can help individuals confront their fear of insects. However, it is important to note that not all

visitors to IEFs are willing to try insects, despite their curiosity. These non-insect eaters may benefit from alternative approaches such as processing insects into various food products, which may be more appealing and enticing to them. To further contextualize these findings, conventional and advanced processing methods (e.g. drying, milling, protein extraction) can transform insects into powders and analogs, making them less visually off-putting for hesitant consumers (Kozlu et al. 2024). Similarly, disgust and food neophobia have been documented as major psychological barriers to entomophagy within multiple cultural contexts (Kröger et al. 2022).

### **Factors Contributing to the Acceptance of Insect-based Foods**

One of the most persistent barriers to entomophagy is the widespread perception of insects as inherently disgusting and inedible, often triggering what is commonly referred to as the yuck factor (Volden 2024). This visceral reaction is deeply ingrained in many cultural contexts and contributes significantly to food neophobia—the reluctance to try unfamiliar foods. Taste functioned as the key counterpoint to this reaction: participants who moved past initial hesitation often expressed surprise that insect dishes were pleasant, demonstrating how flavor can reframe expectations and reduce disgust.

For many attendees, this sensory experience marked a turning point, with several expressing willingness to try insect-based foods again in future events. The observations from the IEFs are consistent with findings from previous studies, which highlight taste as a key determinant of consumer acceptance of edible insects (Hartmann et al. 2015; Schouteten et al. 2016). In BARMM, however, taste was seen to be reinforced by religious legitimacy and cultural resonance, showing that sensory appeal alone is not enough without alignment to values in a Muslim-majority, post-conflict setting.

Notably, a distinct pattern among younger participants was noticed, particularly children, who displayed a higher willingness to eat insects compared to their older counterparts. Although children were not part of the formal survey, informal observations suggested that taste mattered more than appearance to them, an insight worth looking into in future research. This suggests that positioning whole insect pieces—as also recommended by Castro and Chambers IV (2019)—may resonate with younger consumers in BARMM, where adventurousness and cultural curiosity intersect with communal eating practices. The more adventurous individuals, often described as brave participants, were usually the first to volunteer in tasting challenges. Once they discovered that the flavor of insects could be pleasant, they tended to consume them repeatedly, illustrating how curiosity

and initial sensory approval can create a reinforcing cycle of acceptance.

Curiosity emerged as a significant factor influencing participants' willingness to try insects for the first time during the IEFs. Unlike in Italy, where media promotion played the dominant role (Sogari et al. 2018), in BARMM peer encouragement, playful tasting challenges, and religious reassurance were equally critical in lowering barriers. For instance, food-tasting challenges and speed-eating of live insects created an atmosphere of laughter and exhilaration while simultaneously reducing disgust and entomophobia among the participants. Witnessing others accept the challenge of eating live insects, such as the Asiatic palm weevil (*R. ferrugineus*), rhinoceros beetles (*O. rhinoceros*), and mealworms (*Z. morio*), encouraged curious visitors to taste the insect-based menu themselves.

Popularizing insect-based foods through the media may have also played a significant role in promoting the consumption of insects in the region, particularly through the coverage of the IEFs held at MSU Main Campus. The IEFs gained visibility and reached a wider audience through their features on the prominent and multi-awarded television programs “Kapuso Mo, Jessica Soho” and “I-Witness” by Howie Severino both on GMA 7 (GMA Network, Inc., Quezon City), as well as national radio programs, such as “Pinoy Scientist” on DZEC 1062-Radyo Agila (Eagle Broadcasting Corporation, Quezon City) and “Sa Kabukiran” on DZMM (ABS-CBN Broadcasting Center, Quezon City, Metro Manila). These are examples of media appearances are effective in increasing consumers' attention and awareness of entomophagy in Italy (Sogari et al. 2018). The coverage provided an opportunity to highlight the IEFs, educate the public about the benefits of consuming insects, and challenge prevalent misconceptions.

### University-led Research and Agricultural Extension

Through its agricultural extension program, MSU-Marawi campus is actively contributing to the development and promotion of entomophagy as a sustainable and innovative food solution. The university provides valuable educational opportunities and immersive experiences through the IEFs, which serve as a platform for promoting entomophagy. Since 2012, the university has been actively integrating entomophagy-related topics into class lectures, ensuring that students have access to comprehensive knowledge on this subject.

Beyond education, these efforts illustrate how MSU-Marawi has helped bridge the gap between scientific nutrition and cultural acceptability in a Muslim-majority region. By contextualizing entomophagy within halal principles and presenting it through culturally familiar dishes, the IEFs moved

insect consumption beyond a laboratory experiment and into a socially and religiously meaningful practice. Such convergence of scientific education and cultural legitimacy not only reduced entomophobia but also fostered trust, highlighting the capacity of universities to mediate between modern nutritional science and deeply rooted community values.

Recognizing the importance of research and development, the university has secured both in-house funding and external research grants from the Commission on Higher Education. These resources supported the mass-rearing of edible insects, contributing to the growth and sustainability of this emerging industry. Furthermore, the university has fostered collaborative partnerships to further advance the field of entomophagy in allied fields. By collaborating with the DOST-Philippine Nuclear Research Institute and receiving assistance from the International Atomic Energy Agency (IAEA), the university is conducting research on the functionality of insect-based foods, given the recent discoveries that insect-based peptides have antioxidant, anti-angiotensin-converting enzyme, anti-dipeptidyl peptidase-IV, anti-glucosidase, anti-lipase, anti-lipoxygenase, anti-cyclooxygenase, anti-obesity, and hepatoprotective activities (Quah et al. 2023). This collaboration aims to explore the potential of these innovative food sources and promote their sustainable utilization. This commitment to research and innovation has positioned the university as a leader in exploring possibilities for insect-based food production in the country.

### Cultural Nuances and Post-conflict Recovery

Insects deemed edible in early Christian, Jewish, and Islamic sacred texts align with Islamic tradition, permitting the consumption of specific insects like locusts, bees, ants, and termites (El-Mallakh and El-Mallakh 1994). During the IEFs, Muslim students adhered to Islamic principles, preparing regional cuisines, such as palapa and beef rendang infused with locusts, exemplifying a harmonious blend of tradition and innovation. This culinary pathway—where halal framing and cultural familiarity intersect—differs from European interventions, where entomophagy often enters as an exotic novelty rather than a recovery and resilience tool.

The university's initiatives to promote entomophagy received reinforcement from influential Muslim women academic leaders who recounted their childhood experiences of consuming locusts during the IEFs. Such leaders function as “embodied advocates,” whose credibility is rooted in both scholarly expertise and cultural authenticity. Their endorsement of insect-based dishes—especially when prepared in culturally resonant formats like palapa or rendang—helped reduce entomophobia and fostered

openness among Meranao participants. In post-conflict settings like BARMM, where identity and trust are fragile, the presence of respected female figures helped bridge the gap between scientific nutrition and cultural acceptability.

This interpretation aligns with the FAO's Gender Transformative Approaches framework, which emphasizes the need to challenge structural barriers and power imbalances by empowering women as agents of change in food systems. The FAO highlights that mentorship, peer networks, and leadership grounded in lived experience are essential for catalyzing sustainable and equitable food innovations. By fostering collective agency and promoting courageous leadership practices, women not only influence dietary norms but also reshape governance structures to be more inclusive and resilient (FAO 2023).

Finally, the advocacy of entomophagy in the region has also played a role in Marawi's post-conflict recovery. The Marawi Siege, a five-month-long armed conflict, left a lasting impact on the community. Insect-based food has emerged as a sustainable and innovative solution, not only addressing nutritional challenges but also providing economic opportunities. The university's engagement in entomophagy, especially in the aftermath of the conflict, has contributed to rebuilding livelihoods and fostering resilience in Marawi.

This study demonstrates that IEFs in post-conflict BARMM can effectively promote entomophagy by improving awareness, reducing entomophobia, and fostering cultural acceptance through community-based engagement. Beyond enhancing knowledge and attitudes, the integration of Islamic dietary principles reinforced legitimacy, supporting the potential development of halal-certified insect products.

From a policy and development perspective, the IEF model provides a scalable approach for local governments, educators, and extension programs to integrate insect-based foods into nutrition, food security, and livelihood initiatives. Such culturally grounded interventions are particularly relevant in disaster-prone and resource-limited areas, where resilience and affordable protein alternatives are urgently needed.

While the IEFs demonstrated clear potential in improving awareness and acceptability of entomophagy, the study is limited by its descriptive, cross-sectional design and the use of different participant groups and insect recipes across years. These constraints preclude direct longitudinal comparisons, but the findings nonetheless highlight the potential for culturally grounded interventions in normalizing edible insects in post-conflict and food-insecure settings. Future research should examine sustained adoption, economic and health outcomes,

and the role of entomophagy in broader resilience strategies in both local and regional food systems.

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## **DECLARATION OF COMPETING INTEREST**

The authors declare that they have no competing interests.

## **GENERATIVE AI STATEMENT**

During the preparation of this work the authors utilized ChatGPT-5 to enhance grammar and improve the clarity of the text. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

## **ETHICAL CONSIDERATIONS**

We adhered to the Philippine National Ethical Guidelines for Health and Health-Related Research developed by the Philippine Health Research Ethics Board (PHREB 2017).

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## REFERENCES

- Adundo LA and Mulungu K. 2024. Nature's food—the contribution of edible insects to household nutrition and income in Western Kenya. *Journal of Insects as Food and Feed*, 10(12): 1991-2002. <https://doi.org/10.1163/23524588-00001016>
- Aguanta LM. 2017. Consumer awareness and acceptance of edible insects in Lanao del Sur, ARMM: potentials for food security. *The Philippine Entomologist*, 31(2): 157.
- Aguilar-Toalá JE, Vidal-Limón AM and Liceaga AM. 2025. Advancing food security with farmed edible insects: economic, social, and environmental aspects. *Insects*, 16(1): 67. <https://doi.org/10.3390/insects16010067>
- Alhujaili A, Nocella G and Macready U. 2023. Insects as food: consumers acceptance and marketing. *Foods*, 12(4): 886-907. <https://doi.org/10.3390/foods12040886>
- Castro M and Chambers IV E. 2019. Willingness to eat an insect-based product and impact on brand equity: A global perspective. *Journal of Sensory Studies*, 34(2): e12486. <https://doi.org/10.1111/joss.12486>
- DeFoliart GR. 1999. Insects as Food: Why the western attitude is important. *Annual Review of Entomology*, 44(1): 21-50. <https://doi.org/10.1146/annurev.ento.44.1.21>
- Domoguen RL. 1993. Hard times in the Cordilleras: back to insect-eating. *Food Insects Newsletter*, 6(1).
- El-Mallakh OS and El-Mallakh RS. 1994. Insects of the Quran. *American Entomologist*, 40(2): 82-84. <https://doi.org/10.1093/ae/40.2.82>
- FAO (Food and Agriculture Organization). 2023. Gender transformative approaches for food security, improved nutrition and sustainable agriculture. <https://openknowledge.fao.org/server/api/core/bitstreams/6990109c-60d5-4d36-b9b0-5d456587bb16/content>. Accessed on 15 March 2021.
- Feng Y, Chen XM, Zhao M, He Z, Sun L, Wang C and Ding W. 2018. Edible insects in China: Utilization and prospects. *Insect Science*, 25(2): 184-198. <https://doi.org/10.1111/1744-7917.12449>
- Gibbs HD, Agcaoili F and Shilling GR. 1912. Some Filipino foods. *Philippine Journal of Science*, 7: 383-401.
- Gutierrez P. 2023. BARMM has highest incidence of food insecurity: DOST-FNRI. ABS-CBN News. <https://www.abs-cbn.com/news/12/21/23/barmm-has-highest-incidence-of-food-insecurity-dost-fnri>. Accessed on 10 October 2024.
- Han R, Shin JT, Kim J, Choi YS and Kim YW. 2017. An overview of the South Korean edible insect food industry: challenges and future pricing/promotion strategies. *Entomological Research*, 47(3): 141-151. <https://doi.org/10.1111/1748-5967.12230>
- Hartmann C, Shi J, Giusto A and Siegrist M. 2015. The psychology of eating insects: A cross-cultural comparison between Germany and China. *Food Quality and Preference*, 44: 148-156. <https://doi.org/10.1016/j.foodqual.2015.04.013>
- House J. 2016. Consumer acceptance of insect-based foods in the Netherlands: Academic and commercial implications. *Appetite*, 107: 47-58. <https://doi.org/10.1016/j.appet.2016.07.023>
- Hvenegaard G. 2016. Insect festivals in North America: Patterns and purposes. *American Entomologist*, 62(4): 235-240. <https://doi.org/10.1093/ae/tmw078>
- Kozlu A, Ngasakul N, Klojdrová I and Baigts-Allende DK. 2024. Edible insect-processing techniques: a strategy to develop nutritional food products and novelty food analogs. *European Food Research and Technology*, 250: 1253-1267. <https://doi.org/10.1007/s00217-024-04474-3>
- Kröger T, Dupont J, Büsing L and Fiebelkorn F. 2022. Acceptance of insect-based food products in Western Societies: Systematic Review. *Frontiers in Nutrition*, 8: 759885. <https://doi.org/10.3389/fnut.2021.759885>
- Liceaga AM. 2022. Edible insects, a valuable protein source from ancient to modern times. *Advances in Food and Nutrition Research*, 101: 129-152. <https://doi.org/10.1016/bs.afnr.2022.04.002>
- Lisboa HM, Nascimento A, Arruda A, Sarinho A, Lima J, Batista L, Dantas MF and Andrade R. 2024. Unlocking the potential of insect-based proteins: Sustainable solutions for global food security and nutrition. *Foods*, 13(12): 1846. <https://doi.org/10.3390/foods13121846>
- Looy H and Wood JR. 2006. Attitudes toward invertebrates: are educational "bug banquets" effective? *Journal of Environmental Education*, 37(2): 37-48. <https://doi.org/10.3200/JOEE.37.2.37-48>
- Menozi D, Sogari G, Veneziani M, Simoni E and Mora C. 2017. Eating novel foods: An application of the Theory of Planned Behaviour to predict the consumption of an insect-based product. *Food Quality and Preference*, 59: 27-34. <https://doi.org/10.1016/j.foodqual.2017.02.001>
- Nachtigall L, Grune T and Weber D. 2025. Proteins and amino acids from edible insects for the human diet—A narrative review considering environmental sustainability and regulatory challenges. *Nutrients*, 17(7): 1245. <https://doi.org/10.3390/nu17071245>
- Omotoso OT and Adesola AA. 2023. Comparative studies of the nutritional composition of some insect orders. *International Journal of Entomology and Nematology Research*, 2(1): 45-58.
- Pachana NA, Woodward RM and Byrne GJ. 2007. Treatment of specific phobia in older adults. *Clinical Interventions in Aging*, 2(3): 469-476. <https://doi.org/10.2147/cia.S12159926>
- PHREB (Philippine Health Research Ethics Board). 2017. National Ethical Guidelines for Health and Health-Related Research. Department of Science and Technology – Philippine Council for Health Research and Development. <https://www.pchrd.dost.gov.ph/wp-content/uploads/2022/03/Annex-5.-National-Ethical-Guidelines-for-Health-and-Health-Related-Research-2017-1.pdf>. Accessed on 05 April 2021.
- Quah Y, Tong SR, Bojarska J, Giller K, Tan SA, Ziora ZM, Esatbeyoglu T and Chai TT. 2023. Bioactive peptide discovery from edible insects for potential applications in human health and agriculture. *Molecules*, 28(3): 1233-1256. <https://doi.org/10.3390/molecules28031233>
- Schouteten JJ, De Steur H, De Pelsmaecker S, Lagast S, Juvinal JG, De Bourdeaudhuij I, Verbeke W and Gellynck X. 2016. Emotional and sensory profiling of insect-, plant-, and meat-based burgers under blind, expected and informed conditions. *Food Quality and Preference*, 52: 27-31. <https://doi.org/10.1016/j.foodqual.2016.03.011>
- Shin JT, Baker MA and Kim YW. 2018. Edible Insects Insect in South Korean Gastronomy: "Korean Edible Insect Laboratory" Case Study. In: Halloran A, Flore R, Vantomme P and Roos N (eds). *Edible Insects in Sustainable Food Systems*. Cham: Springer International Publishing. pp. 147-159. [https://doi.org/10.1007/978-3-319-74011-9\\_10](https://doi.org/10.1007/978-3-319-74011-9_10)
- Sikora D and Rzymiski P. 2025. Disgust or curiosity? Acceptance of edible insects as food alternative in Poland. *European Food Research and Technology*, 251(6): 943-954. <https://doi.org/10.1007/s00217-025-04673-6>
- Sogari G, Menozzi D and Mora C. 2018. Sensory-liking expectations and perceptions of processed and unprocessed insect products. *International Journal of Food System Dynamics*, 9(4): 314-320. <https://doi.org/10.18461/ijfsd.v9i4.942>
- Starr C. 1991. Notes on entomophagy in the Philippines. *Food Insects Newsletter*, 4(3).
- van Huis A, Van Itterbeeck J, Klunder H, Mertens E, Halloran A, Muir G and Vantomme P. 2013. *Edible Insects: Future*

- Prospects for Food and Feed security. Food and Agriculture Organization of the United Nations.
- Verneau F, La Barbera F, Kelle S, Amato M, Del Giudice T and Grunert K. 2016. The effect of communication and implicit associations on consuming insects: An experiment in Denmark and Italy. *Appetite*, 106: 30-36. <https://doi.org/10.1016/j.appet.2016.02.006>
- Volden J. 2024. 'A wing in the throat': Negotiating edibility in everyday insect consumption. *Geoforum*, 157: 104132. <https://doi.org/10.1016/j.geoforum.2024.104132>
- Woolf E, Zhu Y, Emory K, Zhao J and Liu C. 2019. Willingness to consume insect-containing foods: A survey in the United States. *LWT - Food Science and Technology*, 102: 100-105. <https://doi.org/10.1016/j.lwt.2018.12.010>
- Yang J, Zhou S, Kuang H, Tang C and Song J. 2024. Edible insects as ingredients in food products: nutrition, functional properties, allergenicity of insect proteins, and processing modifications. *Critical reviews in Food Science and Nutrition*, 64(28): 10361-10383. <https://doi.org/10.1080/10408398.2023.2223644>

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## DNA barcoding of “Sibujing” (*Allium chinense* G.Don) accessions from Mindanao, Philippines

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### ABSTRACT

Through an integrative taxonomic approach encompassing morphological, phytochemical, and molecular analyses, Mindanao’s famous “Sibujing” was conclusively identified as *Allium chinense* G.Don in 2023. The initial molecular analyses of “Sibujing” were predominantly based on nucleotide sequences from internal transcribed spacer (ITS) region, with sampling limited to few localities. To broaden the scope of the molecular assessment, maturase K (*matK*) region was subsequently utilized to validate the identity of “Sibujing” samples from three major cultivation sites in Mindanao. The *matK* gene was utilized because of its effective species discrimination power, high quality sequence recovery and simple laboratory procedures. The “Sibujing” accessions from (1) Amai Manabilang, Lanao Del Sur (2) Parang, Maguindanao del Norte, and (3) Rangayen, Alamada, Cotabato were molecularly identified as *A. chinense* based on their *matK* partial gene sequences. The result coincides with the previous molecular identification report of this *Allium* species in the Philippines. The findings of this research contribute to the ongoing efforts to resolve the taxonomic ambiguities involving “Sibujing” in the country.

**Keywords:** *Allium chinense*, ITS, *matK*, partial gene sequences, “Sibujing”, taxonomic ambiguities

*Allium chinense* G.Don or commonly known as “Sibujing” is an integral component of Maranao’s dishes. It is a species related to leek (*Allium porrum* L.) and is traditionally considered as its wild progenitor (Garcia-Herrera et al. 2014). The classification of *Allium* plants indicated that this particular species is a member of the *Amaryllidaceae* family, known for its perennial herbaceous geophytes (Wheeler et al. 2013). A popular product generated from this harvest is called “palapa,” a seasoned blend or condiment commonly used in Muslim Regions.

For the past 25 years, “Sibujing” has always been regarded as *Allium sativum* L. (Madulid 2001), *Allium ampeloprasum* L. (Añides et al. 2019; Dapanas and Duero 2020), *Allium fistulosum* L. (Casim 2021) and *Allium ascalonicum* L. (Nuñez et al. 2021). In 2023, Vera Cruz et al. reported that the said *Allium* species is *A. chinense*. For further confirmation of this finding, “Sibujing” accessions were obtained from selected major production sites in Mindanao and subjected to deoxyribonucleic acid (DNA) barcoding.



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The DNA barcodes have been developed and utilized to overcome the problems faced by conventional characterization techniques (Saddhe and Kumar 2018). Since it is proven to be unaffected by external or other environmental factors, the markers are considered as a reliable, stable and rapid method for the identification of various organisms (Tnah et al. 2019). To identify “Sibujing” at the molecular level, Vera Cruz et al. (2023) employed internal transcribed spacer gene as their nuclear barcode. Recognizing the value of complementary genetic markers in plant barcoding, the maturase K (*matK*) gene was utilized in the present study to enhance taxonomic resolution. This is to augment the current molecular dataset and explore additional discriminatory power. The *matK* is one of the most rapidly evolving coding sections of the plastid genome (Ford et al. 2009) and has been commonly utilized for identification of plants at the family, genus and even at the species level (Dong et al. 2012). Hence, the Consortium for the Barcode of Life (CBOL) Plant Working Group has recognized its discrimination power as a single barcode region or in combination with other proposed plant barcode sequences (CBOL Working Group et al. 2009).

This research endeavor aimed to validate the genetic identity of “Sibujing” in Mindanao, Philippines using *matK* gene. This gene has vast substitution rates compared to other chloroplast gene sequences and has been proven to be effective in solving phylogenetic problems (Fazekas et al. 2008). The utility of the *matK* gene as a molecular marker for *Allium* taxonomy and genetic diversity has been well-established in various geographical regions. For instance, it has been successfully applied in Iran (Zarei et al. 2020), Kazakhstan (Abugalieva et al. 2017), and Turkey (İpek et al. 2014) to characterize different *Allium* species.

Leaf samples of different “Sibujing” accessions were collected from three production sites in Mindanao: Amai Manabilang, Lanao Del Sur; Parang, Maguindanao; and Rangayen, Alamada (Figure 1). These leaf samples were then submitted to the Philippine Genome Center (PGC) Mindanao for genomic DNA isolation; gene amplification using *matK* gene (Fazekas et al. 2008); agarose gel electrophoresis; gene sequencing; and sequence analyses. Electrophoretograms, generated sequences, and Basic Local Alignment Search Tool (BLAST)n analysis results were sent as data sets by PGC Mindanao via email.

In the advent of modern biotechnology, DNA barcoding techniques have been proven as an efficient procedure for assessing plant phylogenies and presented successful applications in the molecular classification of *Allium* species (Zarei et al. 2020). The DNA barcoding of *matK* nucleotide sequences revealed that “Sibujing” accessions from Amai Manabilang, Lanao Del Sur; Parang, Maguindanao;

and Rangayen, Alamada were 100% homologous to *A. chinense*'s chloroplast genome (Table 1).



**Figure 1.** “Sibujing” (*Allium chinense* G. Don) samples from (a) Amai Manabilang, Lanao Del Sur (b) Parang, Maguindanao del Norte and (c) Rangayen, Alamada, Cotabato.

This 100% match suggests that the *matK* region, at least for this particular species, is highly conserved. The *matK* gene, while a good barcode due to its variability in some groups, can be highly conserved within a species, especially in cultivated plants (CBOL Working Group et al. 2009). Furthermore, the result also strongly suggests very low genetic variation among the studied samples for this specific gene region.

The primary mode of propagation for “Sibujing” in Mindanao is asexual, utilizing bulbs for vegetative reproduction. Sexual reproduction through seeds is also a common practice. The widespread reliance on bulb propagation leads to the establishment of genetically uniform clones, consequently resulting in minimal intraspecific genetic diversity.

The DNA barcoding results obtained are consistent with the research of Vera Cruz et al. (2023), who initially elucidated the identity of “Sibujing” accessions from Magpet, Cotabato, and Zamboanga del Norte. These findings corroborate the earlier reports, confirming “Sibujing”, a traditional condiment crop of the Maranaos in Mindanao, Philippines, as *A. chinense* G. Don, a species native to China.

Significantly, this study provides the second molecular-based confirmation of “Sibujings” identity in the Philippines, following the initial report of Vera Cruz et al. in 2023.

**Table 1.** Basic Local Alignment Search Tool (BLAST)n result of *matK* conserved sequences from three “Sibujing” (*Allium chinense* G.Don) samples.

Sample	Basic Local Alignment Search Tool (BLAST)n Best Match (top three hits)	Query cover (%)	E-value	Percent Identity (%)
“Sibujing” from Amai Manabilang, Lanao del Sur	1. <i>Allium chinense</i> chloroplast, complete genome NC 043922.1	100	0.0	100
	2. <i>Allium thunbergia</i> var. <i>deltoides</i> chloroplast, complete genome OQ701006.1	99	0.0	99.87
	3. <i>Allium sacculiferum</i> chloroplast, complete genome NC 070336.1	99	0.0	99.87
“Sibujing” from Parang Maguindanao del Norte	1. <i>Allium chinense</i> chloroplast, complete genome NC 043922.1	100	0.0	100
	2. <i>Allium longistylum</i> chloroplast, complete genome NC 080285.1	99	0.0	99.87
	3. <i>Allium thunbergia</i> var. <i>deltoides</i> chloroplast, complete genome OQ701006.1	99	0.0	99.83
“Sibujing” from Rangayen, Alamada, Cotabato	1. <i>Allium chinense</i> chloroplast, complete genome NC 043922.1	100	0.0	100
	2. <i>Allium longistylum</i> chloroplast, complete genome NC 080285.1	99	0.0	99.92
	3. <i>Allium microdictyon</i> chloroplast, complete genome JF972927.1	99	0.0	99.87

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## GENERATIVE AI STATEMENT

The concepts, results, and discussion within this paper are the authors' original work. An AI tool (Gemini 1.5 Pro) was used only for editing and improving the manuscript's readability. It was not involved in the generation of data and interpretation of results.

## ETHICAL CONSIDERATIONS

All necessary permits for specimen collection were secured before sampling.

## DECLARATION OF COMPETING INTEREST

The authors declare no competing interests.

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## REFERENCES

- Abugalieva S, Volkova L, Genievskaia Y, Ivaschenko A, Kotukhov Y, Sakauova G and Turuspekov Y. 2017. Taxonomic assessment of *Allium* species from Kazakhstan based on ITS and *matK* markers. *BMC Plant Biology*, 17(Suppl 2): 258. <https://doi.org/10.1186/s12870-017-1194-0>
- Añides JA, Dapar MLG, Aranas AT, Mindo RA, Manting MM, Torres MA and Demayo CG. 2019. Phytochemical, antioxidant and antimicrobial properties of the white variety of ‘Sibujing’ (*Allium ampeloprasum*). *Pharmacophore*, 10(1): 1-12.
- Casim R. 2021. Growth and yield performance of “Sakurab scallion green onion (*Allium fistulosum* L.) under different rates of chicken dung and sources of soil amendment. *IAR Journal of Agriculture Research and Life Sciences*, 2(1):50-58.

- CBOL Working Group, Hollingsworth PM, Forrest LL, Spouge JL, Hajibabaei M, Ratnasingham S, van der Bank M, Chase MW, Cowan RS, Erickson DL, et al. 2009. A DNA barcode for land plants. *Proceedings of the National Academy of Sciences*, 106(31): 12794–12797. <https://doi.org/10.1073/pnas.0905845106>
- Dapanas MM and Duero JG. 2020. Product innovation, acceptability and cost analysis of the white variety of ‘sibujing’ (*Allium ampeloprasum*) for potential beverage. *Pakistan Journal of Nutrition*, 19(6): 271-278.
- Dong W, Liu J, Yu J, Wang L and Zhou S. 2012. Highly variable chloroplast markers for evaluating plant phylogeny at low taxonomic levels and for DNA barcoding. *PLoS ONE*, 7(4): e35071. <https://doi.org/10.1371/journal.pone.0035071>
- Fazekas AJ, Burgess KS, Kesanakurti PR, Graham SW, Newmaster SG, Husband BC, Percy, DM, Hajibabaei M and Barrett SCH. 2008. Multiple multilocus DNA barcodes from the plastid genome discriminate plant species equally well. *PLoS ONE*, 3(7): e2802. <https://doi.org/10.1371/journal.pone.0002802>
- Ford CS, Ayres KL, Toomey N, Haider N, Stahl JVA, Kelly LJ, Wikström N, Hollingsworth PM, Duff RJ, Hoot SB, Cowan RS, Chase MS and Wilkinson MJ. 2009. Selection of candidate coding DNA barcoding regions for use on land plants. *Botanical Journal of the Linnean Society*, 159(1): 1-11. <https://doi.org/10.1111/j.1095-8339.2008.00938.x>
- García-Herrera P, Morales P, Fernández-Ruiz V, Sánchez-Mata MC, Cámara M, Carvalho AM, Ferreira ICFR, Pardo-de-Santayana M, Molina M and Tardío J. 2014. Nutrients, phytochemicals and antioxidant activity in wild populations of *Allium ampeloprasum* L., a valuable underutilized vegetable. *Food Research International*, 62: 272-279. <https://doi.org/10.1016/j.foodres.2014.03.004>
- İpek M, İpek A and Simon PW. 2014. Testing the utility of *matK* and ITS DNA regions for discrimination of *Allium* species. *Turkish Journal of Botany*, 38(2): 203–212.
- Madulid D. 2001. A dictionary of Philippine plant names. The Bookmark Inc., Makati City, Philippines.
- Nuñeza O, Rodriguez B and Naisad JG. 2021. Ethnobotanical survey of medicinal plants used by the Mamanwa tribe of Surigao Del Norte and Agusan Del Norte, Mindanao, Philippines. *Biodiversitas Journal of Biological Diversity*, 22(6): 3284-3296. <https://doi.org/10.13057/biodiv/d220634>
- Saddhe AA and Kumar K. 2018. DNA barcoding of plants: selection of core markers for taxonomic groups. *Plant Science Today*, 5(1): 9-13. <https://doi.org/10.14719/pst.2018.5.1.356>
- Tnah, LH, Lee SL, Tan AL, Lee CT, Ng KK, Ng CH and Farhana ZN. 2019. DNA barcode database of common herbal plants in the tropics: a resource for herbal product authentication. *Food Control*, 95: 318-326. <https://doi.org/10.1016/j.foodcont.2018.08.022>
- Vera Cruz JRA, Gentallan Jr. RP, Bartolome MCB, Calimpang IA, Quiñones KJO, Manting MM, Madayag RE, Lalusin AG, Hernandez JE, Manuel CCM, Endonela LE and Borromeo TH. 2023. Taxonomic evidences supporting the establishment of “Sibujing”, a traditional condiment crop from the Philippines, as *Allium chinense* G.Don. *Nordic Journal of Botany*, 2023(11): e04054. <https://doi.org/10.1111/njb.04054>
- Wheeler EJ, Mashayekhi S, McNeal DW, Columbus JT and Pires JC. 2013. Molecular systematics of *Allium* subgenus *Amerallium* (*Amaryllidaceae*) in North America. *American Journal of Botany*, 100(4): 701-711.
- Zarei H, Fakheri, BA, Naghavi MR and Mahdinezhad N. 2020. Phylogenetic relationships of Iranian *Allium* species using the *matK* (cpDNA gene) region. *Journal of Plant Biotechnology*, 47(1): 15-22. <https://doi.org/10.5010/JPB.2020.47.1.015>

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# Botanical data for the conservation of the Victoria-Anepahan Mountain Range, Palawan, Philippines

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## ABSTRACT

The Victoria-Anepahan Mountain Range (VAMR) in Palawan, Philippines, is a botanically significant yet threatened mountain range that harbors a rich diversity of plant species. This paper reviewed the botanical studies conducted in VAMR, collated published species data and produced a consolidated and updated species list of plants in VAMR. Analysis of temporal and spatial distribution of botanical studies showed that research activities have been sporadic, with periods of high activity followed by gaps. The dominant focus has been on new species discovery, accounting for over 70% of the studies. In contrast, floristic surveys and ethnobotanical studies have been relatively limited. Consolidation of data from 18 studies resulted in a list comprised of 255 species representing 187 genera and 93 families. The findings revealed a significant number of endemic plant species (91), many of which are assessed as threatened (51). Critical knowledge gaps were identified, such as the lack of comprehensive floristic surveys in specific areas in VAMR, and insufficient species threat assessments. The geographical coverage of plant species discovery efforts has been limited in the municipality of Narra, while other areas within the larger VAMR, such as Aborlan, Quezon, and Puerto Princesa City, remain underexplored. Additionally, ethnobotanical research has been confined to only two Tagbanua communities, leaving out valuable traditional ecological knowledge from other areas in the region. Addressing these gaps by expanding the geographical and taxonomic scope of botanical and ethnobotanical studies could deepen the understanding of the mountain range's unique plant biodiversity and its traditional uses.

**Keywords:** biodiversity, conservation, ethnobotany, floristic survey, Tagbanua

## INTRODUCTION

The Philippines is widely recognized as a global biodiversity hotspot, known for its exceptional levels of diversity. However, despite this overall biodiversity richness, the country also contains distinct regions that can be considered global plant diversity

darkspots. An area is considered a biodiversity darkspot when it possesses high levels of endemism and significant biodiversity, yet remains poorly studied or neglected by conservation efforts, leading to potential unknown losses of life. Palawan is an actual "darkspot" in the biodiversity landscape of the country. Palawan is the fifth-largest island (>11,000 square



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kilometers land area) in the Philippine archipelago (Fidenci and Castillo 2009), and the largest island in the Philippine province of the same name, situated between 7°47' and 12°22' north latitude and 117°00' and 119°51' east longitude. Palawan is renowned as the Philippines' last ecological frontier because it still has vast forests, rich marine life, and high levels of biodiversity compared to more developed and exploited regions. It is also acknowledged as one of the country's most diverse and abundant island (Sandalo and Baltazar 1997), home to a remarkable estimated tally of 3,000-3,500 flowering species, many of which are exclusive to it (Madulid 2002). Recognizing its immense ecological value, Palawan was designated as a biosphere reserve within the Philippines through United Nations Educational, Scientific and Cultural Organization's Man and Biosphere Reserve Program in 1990, underscoring its significant role in global biodiversity preservation (Diesmos and Palomar 2004).

Palawan holds a unique phytogeographical significance, occupying a distinct and secluded position, and acting as a connecting bridge between Luzon and Mindoro in the north and Borneo in the south. Its isolation from the main island chain of the Philippine archipelago presents compelling opportunities for conducting comprehensive

biodiversity studies. However, in spite of the reported high endemism, many areas are not yet explored, making it a biodiversity darkspot as mentioned earlier. Additionally, despite the popularity of Palawan's natural attractions, its biodiversity is currently experiencing threats such as illegal and unregulated logging, conversion of land for different purposes, and mining operations (Arriola et al. 2017; Sopsop and Buot 2009; Cruz et al. 2007).

The Victoria-Anepahan Mountain Range (VAMR) rises as one of the prominent ecologically significant areas in Palawan (Reyes 2019) (Figure 1). It measures about 210,000 ha and extends to Aborlan, Narra, and Quezon municipalities, and Puerto Princesa City. It is known to be a biodiversity hotspot exhibiting 41.18% endemism rate in its plant and animal species. Moreover, 31% of the species of the mountain range need conservation and protections efforts for these critical species are identified as high priority (Maclang 2023). This mountain range is also known to have the largest ultramafic forest in Palawan, hosting unique plant communities as well as rich wildlife diversity (Magdayao 2021). The VAMR is truly an important habitat for a great number of species maintaining the rich biodiversity of the greater Palawan biogeographic region (Tandang et al. 2020).

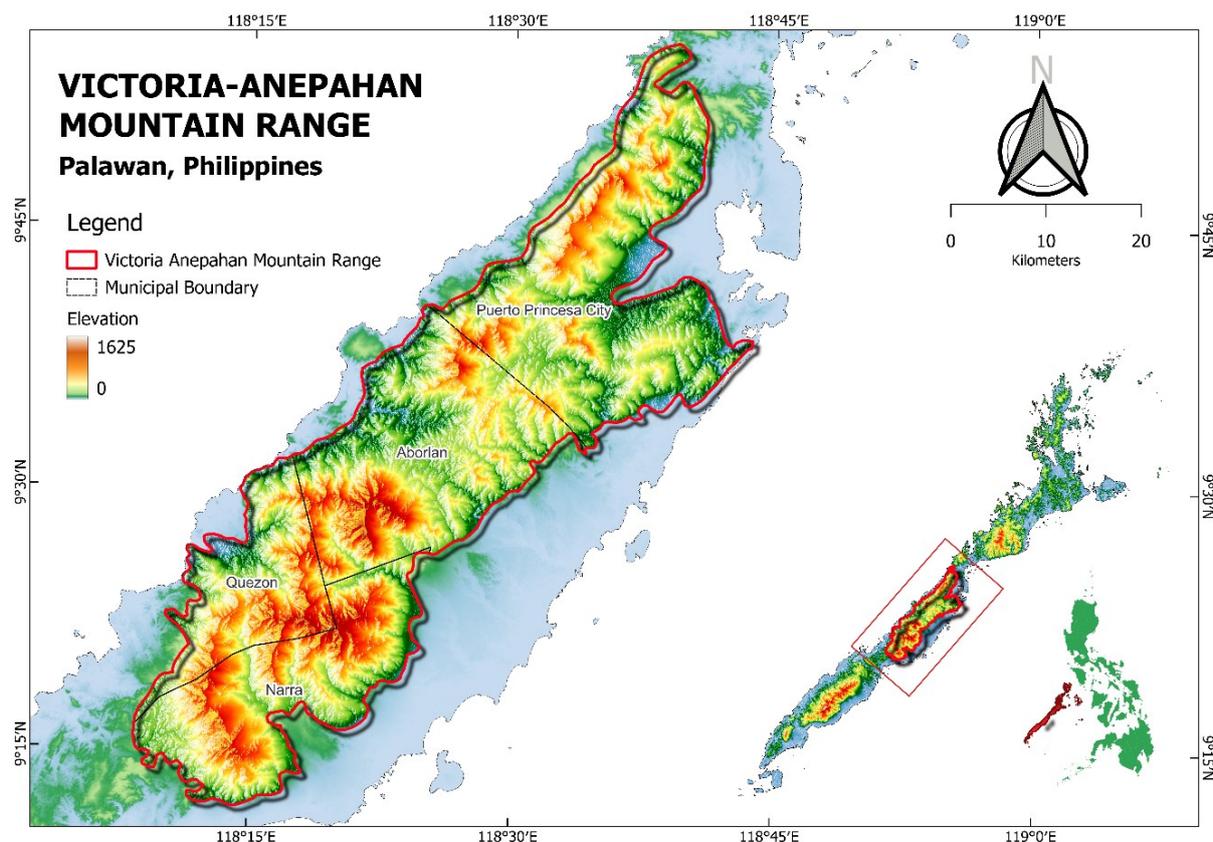


Figure 1. Map of the Victoria-Anepahan Mountain Range (VAMR).

Currently, the majority of the natural areas covered by VAMR is not yet a protected area (PA). It is very alarming given the fact that this mountain range is home to a wide range of flora and fauna that are endemic and threatened (Maclang 2023). In addition to that, it also serves as an essential watershed that gives water supply to the communities surrounding it (Magdayao 2021). Given its remarkable biodiversity and vulnerability to surrounding threats, VAMR is in dire need of a PA status. Being a PA would give it legal recognition that would enforce regulations leading to a decreased degree of unsustainable exploitation of VAMR, safeguarding its natural resources (Maclang 2023). A PA status would support sustainable practices in resources extraction, encourage responsible ecotourism, and push more efforts for conservation (Magdayao 2021).

The Palawan Council for Sustainable Development (PCSD) is now initiating an inter-local government unit agreement that involves the municipalities of Aborlan, Narra, and Quezon, and the city of Puerto Princesa City to jointly manage VAMR (Maclang 2023). The information about the diversity of plants and other biodiversity aspects are important in supporting this initiative of PCSD. Conducted comprehensive floristic studies in VAMR can give details about the diversity and distribution of plant species which can help in identifying the presence of endemic and endangered species that need measures for conservation. Biodiversity data from existing studies can also show the unique vegetation profile and ecological significance of VAMR. This supports the call for coordinated management initiatives.

This paper aimed to assess the current state of plant biodiversity and research in VAMR based on existing literature. The research objectives were to: 1) document and review the botanical studies conducted in VAMR; 2) synthesize published species data and produce a consolidated list of plant species with their updated information, and 3) identify botanical research gaps that need to be addressed for the effective protection and conservation of plant biodiversity in VAMR.

## THE REVIEW PROCESS

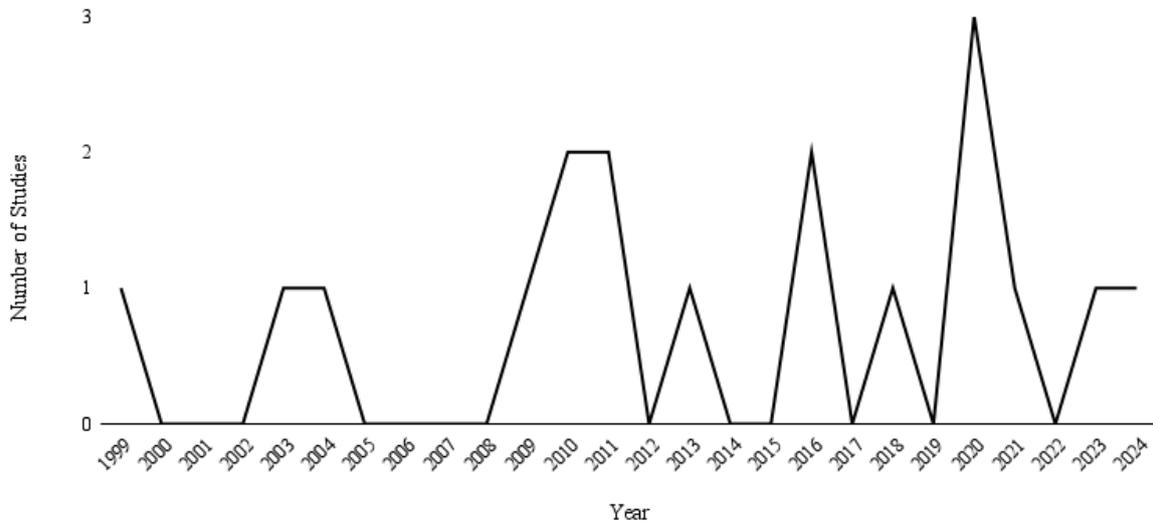
A systematic review of published literature was performed in this study. Google Scholar and Science Direct were utilized in conducting a comprehensive literature search to investigate the floristic studies conducted at VAMR. Keywords “Victoria-Anepahan Mountain Range” and “Flora” were used, and the search results were critically reviewed. There were 54 searched articles of which 18 were given preference for they specifically addressed the desired aspects of the study.

The databases used in checking the updated and accepted names of species and their families were the Royal Botanic Gardens, Kew’s Plants of the World Online (<https://powo.science.kew.org>) and Co’s Digital Flora of the Philippines (CDFP) (Pelsner et al. 2011 onwards). The Global Biodiversity Information Facility (<https://www.gbif.org>) was used to obtain species distribution data. Co’s Digital Flora of the Philippines (CDFP) (Pelsner et al. 2011 onwards) was also employed to further confirm the distribution of species at the country level. The conservation status of species was assessed using the International Union for Conservation of Nature (IUCN) Red List for global status and the Department of Environment and Natural Resources (DAO-2017-11) for national classification. Furthermore, the Botanic Gardens Conservation International (BGCI 2024) ThreatSearch tool was employed to gather supplementary data on species conservation.

## EXPLORATIONS IN VAMR

The rich plant life and hidden botanical wonders of the VAMR in Palawan, Philippines, are brought to light through the indispensable work of botanical explorations. The work of researchers and botanists has greatly advanced knowledge of the mountain range’s plant life and highlighted its ecological value. Botanical studies conducted in the VAMR covers three primary areas: vegetation surveys and analyses, ethnobotany, and new species discovery.

As shown in Figure 2, botanical studies in VAMR have occurred sporadically over the 25-year period from 1999 to 2024. The earliest study was recorded in 1999, followed by a single study in 2003, 2004, and 2009. Research activity slightly increased in 2010 and 2011, with two studies conducted in each of these years. A similar level of activity was observed in 2016. The highest number of studies was recorded in 2020, with three published works. In the remaining years, most activity was limited to one study per year, including 2013, 2018, 2021, 2023, and 2024. There were also several years when no botanical studies were reported, specifically from 2000 to 2002, 2005 to 2008, 2012, 2014 to 2015, 2017, 2019, and 2022. It can be gleaned that the occurrence of research activities at VAMR have been inconsistent, with periods of high activity followed by gaps where no studies were conducted. The reasons for this fluctuation could be due to factors such as funding availability, changes in research priorities, or other institutional or external influences.



**Figure 2.** Number of studies conducted at Victoria-Anepahan Mountain Range (VAMR).

There were 18 botanical studies conducted at VAMR across the past 25 years. The majority of these studies (72.20%) were focused on new species discovery (Figure 3). It means that a significant portion of VAMR's research efforts has been directed towards the identification, documentation, and taxonomic analysis of previously unknown plant species. In contrast, 16.7% are floristic surveys. These types of surveys aimed to comprehensively document and analyze the plant species found in a specific area or ecosystem. The third category of studies was ethnobotany, which examined the traditional knowledge and uses of plants by indigenous or local communities, had the lowest proportion at 11.1% (Figure 3). While discovery and documentation of novel species significantly enrich biodiversity data and reinforce the ecological importance of VAMR as an area of high endemism, the other aspects of botanical research should also be tended to. Conducting more floristic surveys and ethnobotanical studies could provide a more holistic understanding of plant life and its interactions with human communities at VAMR.

The distribution of studies conducted within the Victoria-Anepahan Mountain Range indicates that the majority of the research activities have been concentrated in the municipality of Narra (Figure 4). Nine studies focusing on new species discovery and ethnobotany have been conducted in this area. This municipality, occupying a large portion of the mountain range, has been a prime location for researchers to focus their efforts. Additionally, the municipality of Aborlan has been the site of notable research activities, with five studies conducted focusing on discovery of new species, floristic surveys and ethnobotanical investigations. Its position within the central zone of the VAMR renders Aborlan a suitable and accessible area for scientific studies.

Similarly, Puerto Princesa City, the capital and only city in the province, had four documented studies for a floristic survey and new species discovery. However, there is a complete absence of research activity in the municipality of Quezon. The absence of research in the Quezon area of the VAMR indicates that it may not have been prioritized by researchers. A possible reason for this is that only a limited portion of Quezon lies within the VAMR boundary. This municipality is, in fact, also situated within the bounds of Mt. Mantalingahan Protected Landscape, a separate key biodiversity area in Palawan. Consequently, researchers may have focused on core VAMR areas located in Narra, Aborlan and Puerto Princesa City where the mountain range occupies a larger portion.

The first type of botanical exploration—vegetation surveys and analyses—was carried out by Sopsop and Buot (2013) and Beijnen and Jose (2020), who documented patterns in plant species richness, diversity, and distribution within particular sites of the VAMR. The study by Sopsop and Buot (2013) was focused on evaluating forest types and plant species across the Aborlan Guba System, within an elevation range of 150–760 meters above sea level (masl). In total, 324 plant species were observed, and four distinct vegetation zones were delineated based on species dominance. Each zone was named according to the dominant tree species present within its specific elevation range. The forest types identified were *Artocarpus-Ganophyllum* forest (Zone I), *Koordersiodendron-Litsea* forest (Zone IIA), *Pouteria-Ficus-Neonauclea-Quercus-Syzygium-Tristaniopsis* forest (Zone IIB), *Swintonia-Agathis-Magnolia* forest (Zone III), and *Pinus* forest (Zone IV) (Sopsop and Buot 2013). The dissertation conducted by Sopsop (2010) reported that her vegetation survey conducted in Aborlan Guba System had identified 324

species across 89 families and 192 known genera, of which some were undetermined. Meanwhile, Beijnen and Jose (2020) conducted a vegetation survey at the threatened riverine lowland forest ecosystem in the Talakaigan Watershed, which is also situated in Aborlan, Palawan. They identified a total of 22 plant species from 17 genera and 11 families. The small

number of plant species recorded in their study may not fully reflect the complete picture of the biodiversity of the riverine lowland forests of the study site. One possible reason is the limited sections of the area they investigated due to accessibility challenges, such as the remote and inaccessible nature of some areas.

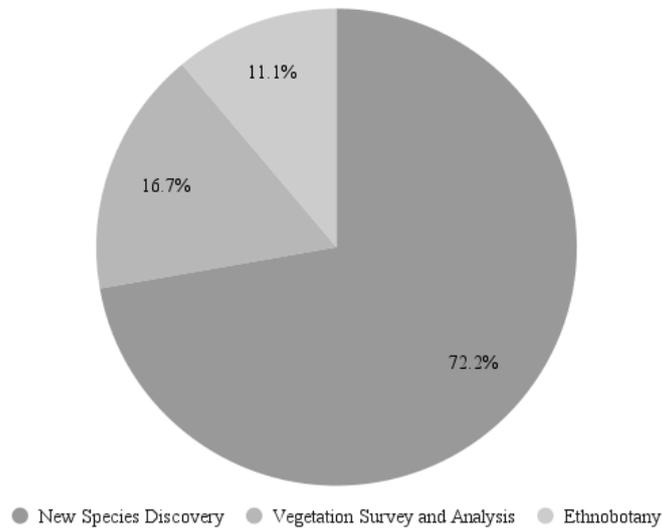


Figure 3. Types of studies in Victoria-Anepahan Mountain Range (VAMR).

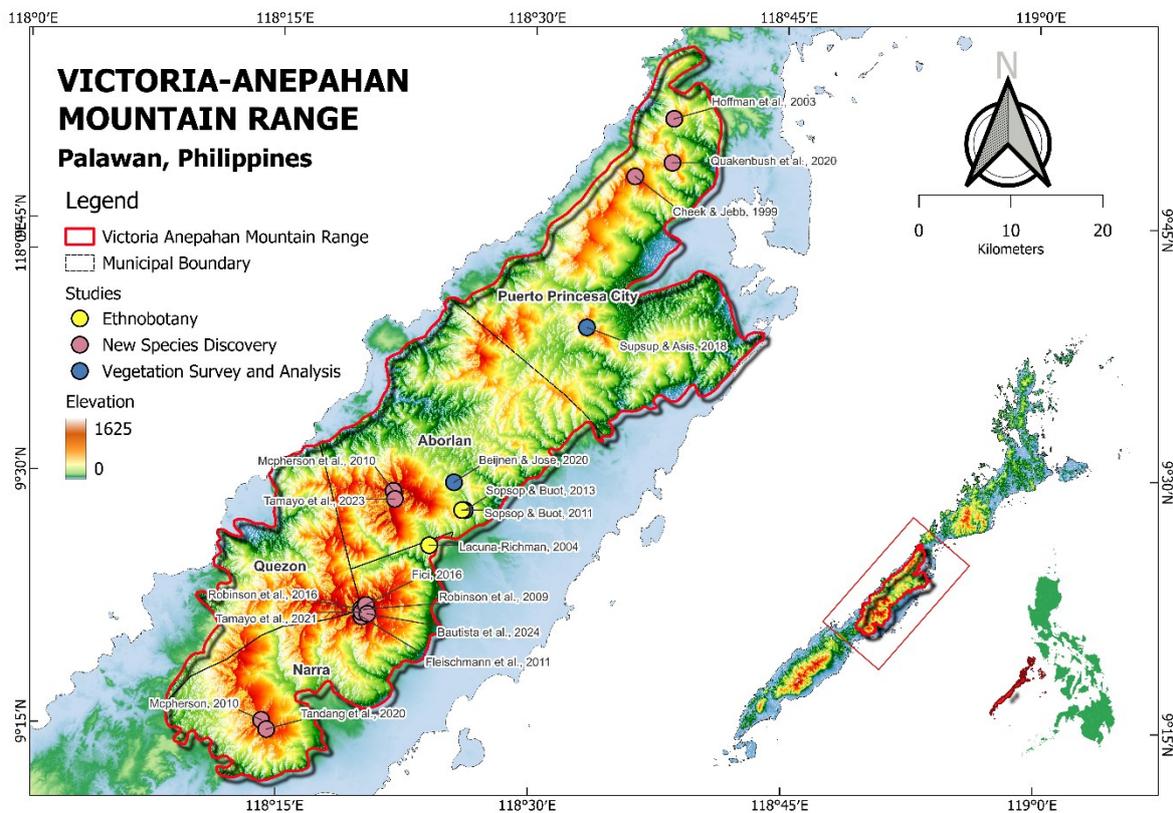


Figure 4. Distribution map of Victoria-Anepahan Mountain Range (VAMR) botanical studies (Blue: Vegetation survey and analyses; Yellow: Ethnobotany; Violet: New Species Discovery).

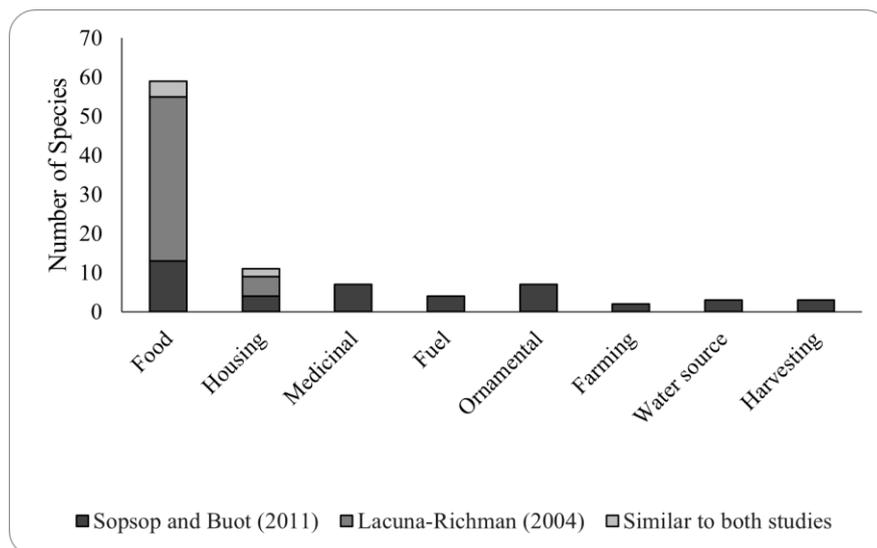
Aside from the vegetation surveys and analyses conducted by Sopsop and Buot (2013) and Beijnen and Jose (2020), Supsup and Asis (2018) also identified plant species and the important habitats for endemic and threatened species in the northern part of the Victoria-Anepahan Mountain Range, Puerto Princesa City, Palawan. They conducted a rapid assessment of vegetation covering the barangays of Bacungan, Simpocan, Bagong Bayan, Napsan, Montible, Iwahig, and Irawan. During the assessment, they collected geographic coordinates of different habitat types, such as cultivated areas, mangroves, secondary growth, and pristine forests, to be used as reference points for supervised vegetation analysis. Through this, they identified 12 plant species, and provided information on their conservation status, distribution, habitat, and number of records.

Since the aforementioned studies focused on a few specific locations, their findings may not be directly applicable to other areas in VAMR with different environmental conditions and forest types. Figure 4 reveals that the mentioned vegetation studies are limited to the specific areas in Aborlan and Puerto Princesa. While these botanical surveys have contributed to a better understanding of the local flora, there is still a need to further examine the other unexplored areas in VAMR. A more complete assessment of the VAMR's botanical richness and diversity can be achieved by extending surveys to cover additional areas like in Narra and Quezon.

The second type of botanical explorations in VAMR involves ethnobotanical studies. Various indigenous ethnolinguistic groups reside in the province of Palawan. Among these groups are the Pala'wan, Tau't Bato, Molbog, Batak, Agutaynen, Cuyonon, and Tagbanua (Acero 2020). The Tagbanua, often referred to as the intermediate indigenous group

of Palawan, apply this label to themselves and are likewise recognized as such by other local communities. The Tagbanua have traditionally sustained their livelihood through the local practice of subsistence slash-and-burn agriculture, known in Filipino as “kaingin”, cultivating rice and other crops (Dressler 2005). However, modifications to this traditional farming practice have occurred in response to changing land-use policies, including restrictions on logging and forest conversion, alongside growing population demands (Acero 2020). Harvesting forest products remains the chief livelihood activity providing cash income for the Tagbanua.

The utilization of plants by the Tagbanua people in the VAMR has been documented in two ethnobotanical studies. Lacuna-Richman (2004) conducted her study in Sitio Boong, Barangay Dumanguena, Narra, Palawan, while Sopsop and Buot (2011) focused their research on the Aborlan Guba System in Aborlan, Palawan. Figure 5 compares the number of plant species utilized for different purposes, drawing on data from Lacuna-Richman (2004) and Sopsop and Buot (2011). It can be discerned that Lacuna-Richman (2004) mainly reported food plants and species used in house construction while Sopsop and Buot (2011) were able to document other purposes such as medicinal, ornamental, as sources of water and fuel, and for farming and harvesting. It should be noted that in the study Lacuna-Richman (2004), participants did not disclose their medicinal plants due to concerns about biopiracy and the loss of intellectual rights over traditional knowledge. The threat of bioprospectors has led to a reluctance to share information about medicinal plants with non-tribal members (Lacuna-Richman 2004). This connotes the importance of respecting indigenous communities' rights and knowledge when engaging with traditional plant resources.



**Figure 5.** Comparison of plant use data of Tagbanua communities obtained from the ethnobotanical studies of Lacuna-Richman (2004) and Sopsop and Buot (2011).

Interestingly, the two studies shared four common edible plant species and two common plant species used for traditional house-building, highlighting some consistencies in the Tagbanua's reliance on locally available resources. Nevertheless, there are more species that are unique to each study. The differences in the findings between the two studies reflect the localized variation in Tagbanua traditional ecological knowledge and plant resource utilization across the VAMR. These studies show the Tagbanua's deep understanding and sustainable use of the diverse plant life in their forest environment, which is crucial to preserve and promote for the continuation of their cultural practices.

The studies conducted by Lacuna-Richman (2004) and Sopsop and Buot (2011) demonstrate the abundant plant diversity and indigenous knowledge possessed by the Tagbanua communities residing in VAMR. The Tagbanua people have deep knowledge of and reliance on the local environment for food, housing, medicine, fuel, ornamentation, farm implements, and alternative water sources. Although the aforementioned studies offer ideas about the utilization of plant resources by the Tagbanua community within certain parts of the mountain range, there is a necessity for additional research to thoroughly document the entirety of plant diversity, traditional knowledge, and cultural practices among Tagbanua communities in various other areas of VAMR. Moreover, considering the vital role forest resources play in the lives of the Tagbanua people, prioritizing sustainable forest management is essential. Key actions include supporting sustainable resource use, protecting indigenous knowledge on plant utilization, and ensuring community participation in management decisions. Protecting and conserving plant species with cultural, ecological, and economic value to the Tagbanua should be a key priority in conservation strategies for VAMR.

The third type of botanical exploration in VAMR is the discovery of novel species and records. The identification of new species and an additional distribution record in VAMR reveals the mountain range's ecological richness and botanical uniqueness. A total of 15 new species and record from 11 different genera and eight families have been discovered and officially documented over the past 25 years (Cheek and Jebb 1999; Hoffmann et al. 2003; Robinson et al. 2009; McPherson 2010; McPherson et al. 2010; Fleischmann et al. 2011; Fici 2016; Robinson et al. 2016; Quakenbush et al. 2020; Tandang et al. 2020; Tamayo et al. 2021; Tamayo et al. 2023; Bautista et al. 2024).

Table 1 provides a list of new plant species and records belonging to several botanical families, including Capparaceae, Droseraceae, Ericaceae, Euphorbiaceae, Melastomataceae, Nepenthaceae, Orchidaceae, and Rubiaceae. The families with the

largest number of species represented are Orchidaceae and Nepenthaceae, with four (4) species each. Most of these species were observed in the municipality of Narra, Palawan, as indicated by the violet points in Figure 4. It can be gleaned that the municipality of Narra has been a focus of new species discoveries. Most of the newly discovered plant species were found in the ultramafic peak of Mt. Victoria in Narra.

There were three new species and one new distribution record under the family Orchidaceae. The terrestrial orchids *Stigmatodactylus dalagangpalawanicum* A.S.Rob. and *Stigmatodactylus aquamarinus* A.S.Rob. & Gironella were discovered growing in the upper montane forest of Mt. Victoria in Narra (Robinson et al. 2016) as well as *Corybas circinatus* Tandang & R.Bustam. which was found in the mid-montane forest Mt. Victoria and Sultan Peak in Narra at 331 masl (Tandang et al. 2020). A new distribution record was reported for *Cryptostylis carinata* J.J.Sm., found in an upland swamp overlying ultramafic rock in Mt. Victoria, Narra (Robinson et al. 2016).

The two new species of Nepenthaceae found in Narra were *Nepenthes attenboroughii* A.S.Rob., S.McPherson & V.B.Heinrich found in the summit region of Mt. Victoria and *Nepenthes palawanensis* S.McPherson, Cervancia, Chi.C.Lee, Jaunzems, Mey & A.S.Rob. found in the summit ridge of Sultan Peak, each with its specific habitat preferences. The *N. attenboroughii* flourishes in open, exposed habitats characterized by low-growing upper montane scrub. It was found by Robinsons et al. (2009) at an elevation of 1,650 masl. The *N. palawanensis* is another species from the Nepenthaceae family which is found in a low-growing upper montane scrub and has not been documented in forested or shaded areas, just like *N. attenboroughii*. Its elevation ranges from 1,100 to 1,236 masl (McPherson 2010). Meanwhile, *Nepenthes leonardoii* S.McPherson, Bourke, Cervancia, Jaunzems & A.S.Rob. was documented in Mt. Shumkat. According to the literature, Mt. Shumkat is also located in Narra, Palawan; however, based on the National Mapping and Resource Information Authority data used in creating Figure 4, the mountain falls within the jurisdiction of Aborlan. This discrepancy may be attributed to the authors' jump off point in Narra, while the actual collection site of the species was already within Aborlan. This species has a different habitat preference as it occurs in upper montane forests but in shaded areas and thrives in open scrub on ridges and summit zones. It was recorded at an elevation of 1,490 masl (McPherson et al. 2010). *Nepenthes philippinensis* Macfarl. found on Mt. Pulgar in Puerto Princesa City thrived on ultramafic soils in lower elevations of 25-250 masl (Cheek and Jebb 1999).

These literatures about new plant species discoveries show the richness and endemic nature of plants that thrive in the montane and ultramafic

ecosystems. The distribution elevation of the discovered species ranges from as low as 25-250 masl like the *N. philippinensis* to as high as 1,700 masl like the *Vaccinium exiguum* M.N.Tamayo, R.Bustam. & P.W.Fritsch, reflecting the plants' adaptations to diverse montane environments. There were restricted occurrences of species like the *Drosera ultramafica* A.Fleischm., A.S.Rob. & S.McPherson, *Rhododendron astrophorum* M.N.Tamayo, Y.P.Ang & A.S.Rob., and *Nepenthes* species that were found in ultramafic and montane forest habits, which shows that these areas are critical centers of endemism shaped by the distinctive soil composition and microclimates that support rapid diversification (De Castro et al. 2020). There were also several species that were restricted in particular peaks of VAMR in Narra, Palawan, such as the *Nepenthes* and *Stigmatodactylus* orchids. It

emphasizes the high level of localized endemism and the likelihood that these species are adapted to narrowly defined environmental conditions. According to Galey et al. (2017), montane and ultramafic environments can serve as engines of evolution where the combination of unique physical and biological conditions promotes the rise of narrowly adapted and endemic plant species. The adaptability and the diversity of montane ecosystems are supported by the wide altitudinal ranges occupied by some of the newly discovered plant organisms. It supports the idea that there should be a high conservation priority to be given to the montane and ultramafic habitats of VAMR. These environments act as biodiversity hotspots and give an opportunity to study the ecological and evolutionary forces behind species diversification.

**Table 1.** Newly described species and record from the Victoria-Anepahan Mountain Range, with details on family, species, habitat and ecology, elevation, and source.

Family	Species	Habitat and Ecology	Elevation (masl)	Source
Capparaceae	<i>Capparis tagbanuorum</i> Fici	known from a single locality in Mt. Victoria, in forest habitats along a stream valley; ultramafic soils	490-590	Fici 2016
Droseraceae	<i>Drosera ultramafica</i> A.Fleischm., A.S.Rob. & S.McPherson	growing on high montane forest, on ultramafic soils	1680	Fleischmann et al. 2011
Ericaceae	<i>Rhododendron astrophorum</i> M.N.Tamayo, Y.P.Ang & A.S.Rob.	found as an epiphyte on the larger limbs of shrubs and small trees from mid-montane ultramafic forest	1100-1477	Tamayo et al. 2023
	<i>Vaccinium exiguum</i> M.N.Tamayo, R.Bustam. & P.W.Fritsch	known from a single individual from a single location at the exposed ultramafic forest summit of Mt. Victoria	1700	Tamayo et al. 2021
Euphorbiaceae	<i>Phyllanthus balgooyi</i> Petra Hoffm. & A.J.M.Baker	growing as isolated individuals, restricted to ultramafic substrates in scrub or very stunted open forest	150-200	Hoffmann et al. 2003
Melastomataceae	<i>Medinilla ultramaficola</i> Quakenbush, Y.P.Ang & R.Bustam.	found on exposed ultramafic ridges	150-1010	Quakenbush et al. 2020
Nepenthaceae	<i>Nepenthes attenboroughii</i> A.S.Rob., S.Mc Pherson & V.B.Heinrich	exposed, open areas of low growing upper montane scrub; not known from forest or shaded habitat	1650	Robinson et al. 2009
	<i>Nepenthes leonardoi</i> S.McPherson, Bourke, Cervancia, Jaunzems & A.S.Rob.	growing in upper mountain forest, often in shade, as well as in open scrub in ridge top and summit areas	1490	McPherson et al. 2010

Family	Species	Habitat and Ecology	Elevation (masl)	Source
	<i>Nepenthes palawanensis</i> S.McPherson, Cervancia, Chi.C.Lee, Jaunzems, Mey & A.S.Rob.	areas of low growing upper montane scrub; not known from forest or shaded habitat.	1100-1236	McPherson 2010
	<i>Nepenthes philippinensis</i> Macfarl.	scrub on ultramafic soils	25-520	Cheek and Jebb 1999
Orchidaceae	<i>Corybas circinatus</i> Tandang & R.Bustam.	skeletal, humic soils overlying mafic rocks; stunted, mid-montane trees close to the waterfall camp	331	Tandang et al. 2020
	<i>Cryptostylis carinata</i> J.J.Sm. (new record)	upland swamp overlying ultramafic rock	1459	Robinson et al. 2016
	<i>Stigmatodactylus aquamarinus</i> A.S.Rob. & Gironella	growing terrestrially in moss pads overlying matted tree roots or steeply inclined ultramafic rock, generally occurring singly beneath closed canopy forest of upper montane trees 4–6 m tall or climbing bamboo; or in ultramafic rubble in open summit scrub, sheltered by large boulders	1460	Robinson et al. 2016
	<i>Stigmatodactylus dalagangpalawanicum</i> A.S.Rob.	growing terrestrially in humus layer overlying ultramafic rock in upper montane, closed canopy forest below summit scrub zone	1600	Robinson et al. 2016
Rubiaceae	<i>Psychotria pendens</i> M.Bautista & R.Bustam.	growing in moist, shady lowland and mid-elevation forest areas	500	Bautista et al. 2024
<b>Total Number of Species</b>	15			

### UPDATED SPECIES LIST

The comprehensive updated species list compiled from 18 botanical studies in the VAMR gives a snapshot of the rich plant diversity in the region. Spanning 93 families, 187 genera, and a total of 255 species, the list offers important insights into the taxonomic breadth and composition of the local flora. There were 56 species that were previously documented under outdated taxonomic names. These 56 species have been cross-checked and updated to their current nomenclature. Geographically, the list includes 196 species that are already known to occur in Palawan, while 58 species have not yet been recorded in the province (as per Pelsner et al. 2011 onwards) but are on the list.

The comprehensive species list provides the geographic distribution and endemism levels of the documented plant diversity (Figure 6). Over 164 (64.3%) of the 255 species were categorized as non-endemic, indicating they have a wider distribution

beyond the Philippine archipelago. However, a substantial proportion, accounting for over 35.7% (91 species) of the total, are Endemic to the Philippines, emphasizing the unique and irreplaceable nature of the plant life in this region. Of the 91 Philippine endemic species, 24 were restricted to Palawan, underscoring the exceptional level of localized endemism within VAMR. These 24 Palawan-endemic species represent a distinct and highly specialized component of the regional flora, warranting targeted conservation efforts to protect them from any threats. While the majority of the species have a broader area distribution, the substantial proportion of endemic Philippine species, and the even more specialized Palawan-endemic subset, emphasizes the unique and irreplaceable nature of this flora.

The species list includes data on the conservation status of the documented plant species, as assessed under both the global International Union for Conservation of Nature and the national DAO 2017-11 framework in the Philippines. From the IUCN

assessment, 28 species were classified as critically endangered, endangered or vulnerable, indicating they face a risk of extinction worldwide (Figure 7). An additional 12 species were categorized as Near Threatened, meaning they are close to qualifying for a threatened status. However, a significant portion (98 species) has not yet been evaluated by IUCN, leaving their global conservation status unknown. Conversely, the national classification under DAO 2017-11 presents a more alarming scenario for plant conservation in the Philippines. There were 51 species identified as critically endangered, endangered, and vulnerable. It indicates that these species are under alarming threats and are at risk of extinction in the Philippines. There were also 14 species that fall under the classification other threatened species in DAO 2017-11 which means that there is a pressing need for conservation actions. The results of conservation status evaluation of plant species in the IUCN and DAO 2017-11 point out a need for critical and focused protection strategies for the threatened plant species of VAMR. Prioritizing both the protection of these threatened species and the conservation of the VAMR's unique natural heritage is essential. The conservation status of the 98 unevaluated species must also be assessed to give a more accurate estimate of the extent of threatened species.

The BGCI ThreatSearch database (BGCI 2024) was used to evaluate the conservation status of species lacking IUCN or DAO 2017-11 assessments. Among the 24 species with available data, 15 were identified as threatened, one as possibly threatened, seven as not threatened, and one as data deficient (Figure 8). These threatened species should also be assessed by IUCN and DAO 2017-11 to confirm their

status and attract attention from conservation professionals and authorities. The BGCI database serves as a valuable alternative tool for assessing species not classified under IUCN or DAO 2017-11, enhancing our understanding of biodiversity and the risks faced by these species.

#### DATA GAPS AND OTHER OPPORTUNITIES

Based on the existing body of knowledge about the flora of VAMR, there are several significant research gaps identified. A major research gap pertains to the narrow geographic and taxonomic scope of previous botanical research efforts. While new plant species have been found across two peaks in the municipality of Narra, there is significant potential for discovering additional undescribed species in other areas and peaks within the larger VAMR, such as in the municipalities of Aborlan and Quezon, and the city of Puerto Princesa. Even in the case of the Puerto Princesa City area, where a rapid assessment has been done by Supsup and Asis (2018), a more comprehensive botanical survey of the mountain regions could uncover additional plant diversity and distribution patterns. Expanding the geographical scope of plant species surveys and exploration to these other uncharted regions will reveal the actual plant diversity in VAMR. Moreover, the existing data points out that the available literature has primarily focused on the discovery of new plant species. It is essential to broaden the research scope to encompass more extensive botanical and ethnobotanical studies in order to better understand the plant biodiversity, traditional knowledge, and resource use within the VAMR.

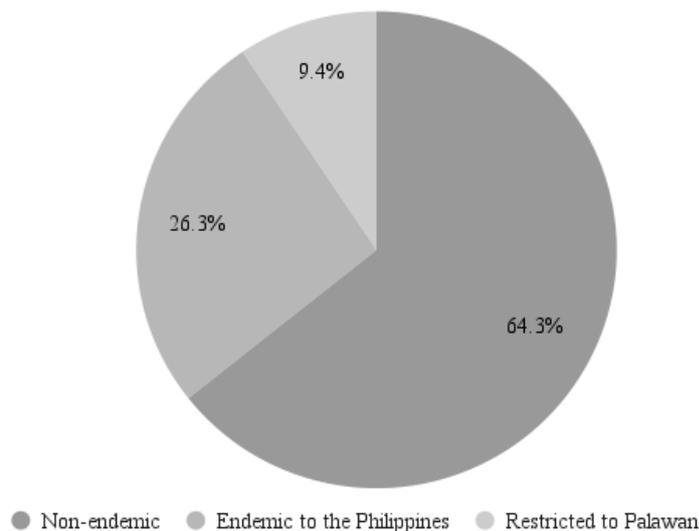
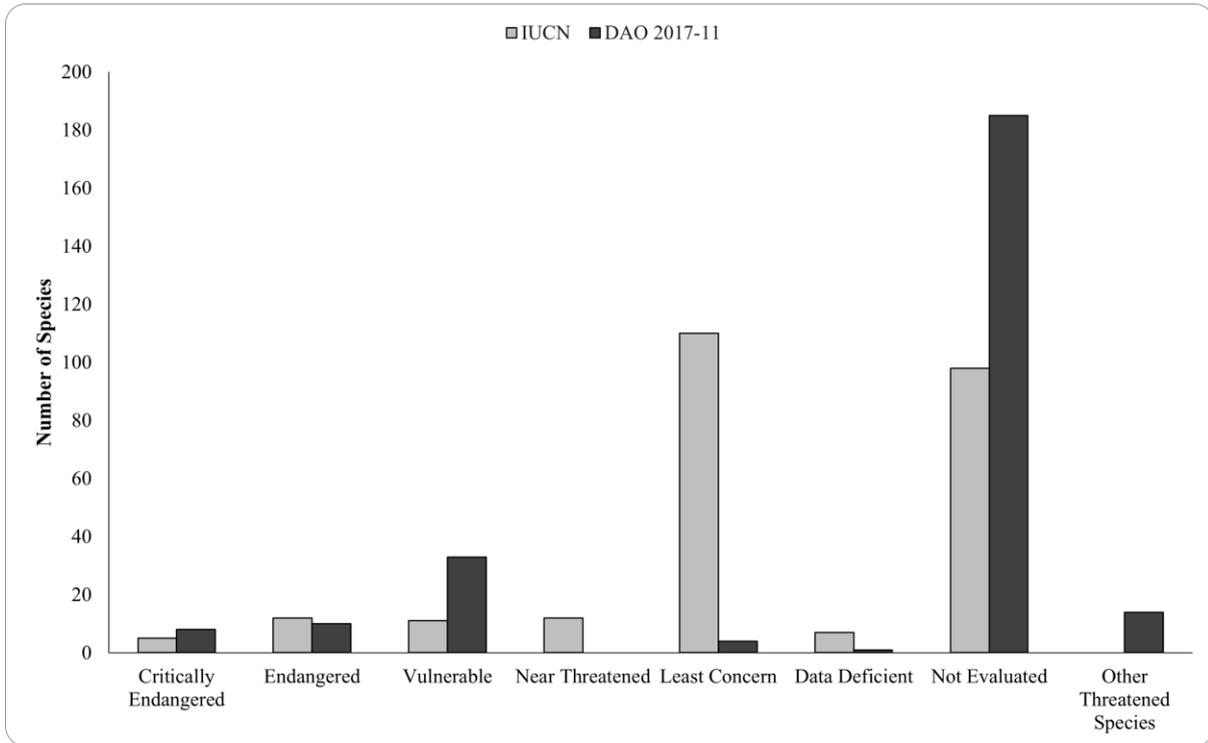
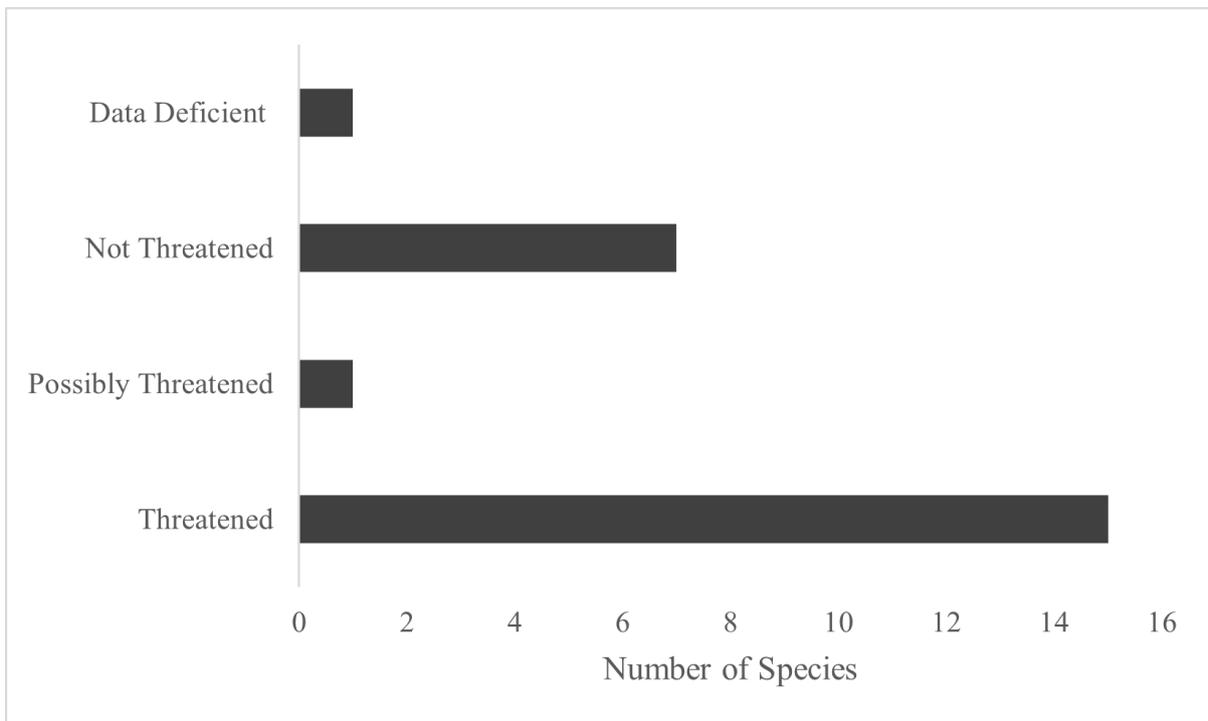


Figure 6. Endemism of Victoria-Anepahan Mountain Range's Flora.



**Figure 7.** International Union for Conservation of Nature and Department of Environment and Natural Resources Administrative Order 2017-11 conservation status of Victoria-Anepahan Mountain Range’s Flora.



**Figure 8.** Conservation status of plant species in the Victoria-Anepahan Mountain Range according to Botanic Gardens and Plant Conservation ThreatSearch.

Botanical surveys in VAMR have largely concentrated on the angiosperms. It is also noteworthy that the recently discovered plant species in VAMR are mainly shrubs and herbs. These studies have not thoroughly included bryophytes and other understory plants. Understory vegetation should also be studied as they are key components of forest ecosystems contributing to ecological balance and function. They can reflect the condition of their environment and can also support the wildlife by giving them nourishment and habitat. More comprehensive floristic surveys should be conducted and should include understory vegetation within the different forest types in VAMR. Understory vegetations are often underrepresented in botanical studies even though they hold valuable information about the environmental state and conservation needs of a particular site. They are highly responsive to environmental shifts and can function as bioindicators of habitat quality, microclimate, and levels of disturbance (Deng et al. 2023).

The limited ethnobotanical research conducted in the mountain range is another identified data gap. There are only two Tagbanua communities that were investigated, one located in Narra and the other in Aborlan. There are other Tagbanua communities that remain unexplored and hold potential for investigating ethnobotanical knowledge and traditional plant use. Other communities situated in other sitios and barangays within VAMR, such as those in Puerto Princesa City (Barangays Napsan, Simpocan, and Bagong Bayan), can also provide ethnobotanical data and reveal a fuller picture of traditional knowledge and plant resource utilization in the VAMR. Conducting ethnobotanical studies in VAMR beyond the Tagbanua communities that have already been the focus of previous studies is truly important. Each community may have distinct traditional ecological knowledge and practices related to plant utilization. Other Tagbanua settlements may reveal new information that can possibly lead to the identification of medicinal and culturally important species.

The analysis of the phytochemical properties of medicinal plants utilized by the Tagbanua people should be conducted, especially for those species that have not yet been subjected to scientific study. According to Recuenco et al. (2020), scientific validation through phytochemical studies can reinforce claims of traditional plant use. The exploration of the active compounds, modes of action, and therapeutic potential of medicinal plants allows researchers to deepen scientific understanding of their efficacy while promoting the preservation and continued practice of traditional knowledge. Furthermore, there are occurrences of the migration of tribe members to lowland areas or urban centers. This poses a risk of eroding the traditional ecological knowledge of the Tagbanua communities in VAMR. According to Abdullah and Khan (2023), migration and

displacement from their traditional lands and practices can result in the erosion of important information about the mountain's plant diversity, medicinal uses, and sustainable resource management practices. Hence, there is a greater need to conduct more ethnobotanical studies. There should be collaboration with the Tagbanua people to document and preserve their traditional knowledge. This is vital to the preservation of valuable traditional ecological knowledge.

The absence of extensive floral explorations in ultramafic forests presents an opportunity for future botanical studies. Results of this study show that ultramafic forests are home to many newly discovered species. Ultramafic forests are those that grow on soils derived from ultramafic rocks, such as serpentinite, and are known to harbor unique and specialized plant communities adapted to the challenging environmental conditions associated with these substrates (Galey et al. 2017). According to De Castro et al. (2020), ultramafic environments are often characterized by high levels of endemism, as plants evolve unique adaptations to cope with the harsh soil conditions. The ultramafic forests of VAMR could be a home to more endemic flora. Investigating the VAMR's ultramafic habitats may reveal significant biodiversity. It can contribute to both species' discovery and a more profound and comprehensive understanding of plant community structure. There are still many ultramafic peaks in VAMR that are unexplored. These habitats tend to be patchy and geographically limited, where each mountain or peak may host unique assemblages of plant species. Conducting more botanical studies to other ultramafic zones of VAMR may offer deeper understanding of the plant diversity associated with these specialized habitats. Ultramafic forests are often seen as delicate and ecologically vulnerable habitats due to their narrow distribution and the unique adaptations of their flora. There should be more efforts to protect the ultramafic habitats of the VAMR to maintain the rare and unique plant biodiversity they harbor.

Expand geographic and taxonomic coverage in future botanical studies, conduct more ethnobotanical studies, and focus more on the ultramafic habitats can contribute to a deeper understanding of plant diversity, traditional ecological knowledge, and conservation needs within VAMR. This will ultimately inform a more effective management, protection, and conservation of the Victoria-Anepahan Mountain Range in the island of Palawan, Philippines.

The VAMR harbors exceptional plant diversity and endemism, emphasizing its importance as a key biodiversity area in Palawan. However, research efforts over the past 25 years have been uneven, with heavy emphasis on species discovery in select habitats while large geographic, taxonomic, and ethnobotanical gaps remain. The high number of

threatened and potentially at-risk species highlights the urgent need for expanded surveys, comprehensive assessments, and conservation actions. Strengthening research in understudied areas, integrating traditional ecological knowledge of the Tagbanua people, and prioritizing ultramafic forests are essential for building a more complete understanding of VAMR's flora and ensuring its long-term conservation.

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## GENERATIVE AI STATEMENT

The authors are the ones who conceptualized, analyzed, and wrote this review paper. Generative AI tool was used solely for grammar and language refinement.

## ETHICAL CONSIDERATIONS

There are no human or animal subjects in this article and informed consent is not applicable.

## DECLARATION OF COMPETING INTEREST

The authors declare that there are no competing interests among the authors.

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## REFERENCES

- Abdullah A and Khan SM. 2023. Traditional ecological knowledge sustains due to poverty and lack of choices rather than thinking about the environment. *Journal of Ethnobiology and Ethnomedicine*, 19(1). <https://doi.org/10.1186/s13002-023-00640-1>
- Acero L. 2020. Towards environmental sustainability: Beliefs and livelihood practices of Tagbanuas in Buong Narra Palawan, Philippines. *IOP Conference Series: Earth and Environmental Science*, 505(1). <https://doi.org/10.1088/1755-1315/505/1/012034>
- Arriola AH, Cobangkiat AB and Alejandro GJ. 2017. *Psydrax multiflora* sp. nov. (Rubiaceae) from Palawan, Philippines. *Nordic Journal of Botany*, 35(2): 182-184. <https://doi.org/10.1111/njb.01255>
- Bautista MA, Mangussad V, Cervancia J and Bustamante RA. 2024. *Psychotria pendens* (Rubiaceae, Psychotriaceae), a new ultramafic species from central Palawan, Philippines. *Phytotaxa*, 649(3): 252-260. <https://doi.org/10.11646/phytotaxa.649.3.2>
- Beijnen JV and Jose ED. 2020. Botanical observations from a threatened riverine lowland forest in Aborlan, Palawan, Philippines. *The Palawan Scientist*, 12: 1-19. <https://doi.org/10.69721/TPS.J.2020.12.1.01>
- BGCI (Botanic Gardens and Plant Conservation). 2024. ThreatSearch online database. Botanic Gardens Conservation International. Richmond, UK. [https://tools.bgci.org/threat\\_search.php](https://tools.bgci.org/threat_search.php). Accessed on 07 April 2025.
- Cruz RM, Villafuerte-van den Beukel D, Lacerna-Widmann I, Schoppe S and Widmann P. 2007. Wildlife trade in southern Palawan, Philippines. *BANWA Archives* (2004-2013), 4(1): 12-26.
- Cheek M and Jebb M. 1999. *Nepenthes* (Nepenthaceae) in Palawan, Philippines. *Kew Bulletin*, 54(4): 887. <https://doi.org/10.2307/4111166>
- De Castro ME, Carandang VI JS and Agoo EM. 2020. Floristic study of an ultramafic formation in Sitio Magarwak, Sta. Lourdes, Puerto Princesa city, Palawan Island, Philippines. *Biodiversitas Journal of Biological Diversity*, 21(8). <https://doi.org/10.13057/biodiv/d210844>
- Deng J, Fang S, Fang X, Jin Y, Kuang Y, Lin F, Liu J, Ma J, Nie Y, Ouyang S, Ren J, Tie L, Tang S, Tan X, Wang X, Fan Z, Wang Q, Wang H and Liu C. 2023. Forest understory vegetation study: Current status and future trends. *Forestry Research*, 3(1). <https://doi.org/10.48130/fr-2023-0006>
- Diesmos A and Palomar N. 2004. The status of biological diversity in the Palawan Corridor. Surublien: Strategies to Conserve Palawan's Biodiversity. Palawan Council for Sustainable Development, Department of the Environment and Natural Resources, NGO Network, and Conservation International, Puerto Princesa City, Palawan, 1-7.
- Dressler W. 2005. Disentangling Tagbanua lifeways, swidden and conservation on Palawan Island. *Research in Human Ecology*, 12(1).
- Fici S. 2016. A new narrow-leaved species of *Capparis* (Capparaceae) from central Palawan, Philippines. *Phytotaxa*, 267(2): 146. <https://doi.org/10.11646/phytotaxa.267.2.7>
- Fidenci P and Castillo R. 2009. Some data on the distribution, conservation status and protection of freshwater turtles in the Palawan Island Group, Philippines. *Testudo*, 7(2): 76-87.
- Fleischmann A, Robinson A, McPherson S, Heinrich V, Gironella E and Madulid D. 2011. *Drosera ultramafica* (Droseraceae), a new sundew species of the ultramafic flora of the Malesian highlands. *Blumea - Biodiversity, Evolution and Biogeography of Plants*, 56(1): 10-15. <https://doi.org/10.3767/000651911x560907>
- Galey ML, Van der Ent A, Iqbal MC and Rajakaruna N. 2017. Ultramafic geoecology of South and Southeast Asia. *Botanical Studies*, 58(1). <https://doi.org/10.1186/s40529-017-0167-9>
- Hoffmann P, Baker AJ, Madulid DA and Proctor J. 2003. *Phyllanthus Balgooyi* (euphorbiaceae S.L.), a new nickel-hyperaccumulating species from Palawan and Sabah. *Blumea - Biodiversity, Evolution and Biogeography of Plants*, 48(1): 193-199. <https://doi.org/10.3767/000651903x686178>
- Lacuna-Richman C. 2004. Subsistence strategies of an indigenous minority in the Philippines: Nonwood forest product use by the Tagbanua of Narra, Palawan. *Economic Botany*, 58(2): 266-285. [https://doi.org/10.1663/0013-0001\(2004\)058\[0266:ssoaim\]2.0.co;2](https://doi.org/10.1663/0013-0001(2004)058[0266:ssoaim]2.0.co;2)
- Maclang RJ. 2023. Puerto Princesa to join inter-LGU Victoria-anepahan mountain range management. *Palawan News*. <https://palawan-news.com/city-enro-pushes-puerto-princesa-to-be-part-of-inter-lgu-agreement-on-victoria-anepahan-mountain-range-management/#:~:text=Victoria%2DAnepahan%20is%20a%2>

- [014%2C000.are%20of%20high%20conservation%20signific](#)  
[ance](#). Accessed on 07 May 2024.
- Madulid DA. 2002. A pictorial guide to the noteworthy plants of Palawan. Palawan Tropical Forestry Protection Programme.
- Magdayao AG. 2021. Move to declare Victoria-anepahan range a new protected area gains ground. Palawan News. [https://palawan-news.com/move-to-declare-victoria-anepahan-range-a-new-protected-area-gains-ground/#google\\_vignette](https://palawan-news.com/move-to-declare-victoria-anepahan-range-a-new-protected-area-gains-ground/#google_vignette). Accessed on 07 May 2024.
- McPherson S. 2010. *Nepenthes palawanensis*: Another new species of giant pitcher plant from the Philippines. Carnivorous Plant Newsletter, 39(3): 89-90. <https://doi.org/10.55360/cpn393.sm928>
- McPherson S, Bourke G, Cervancia J, Jaunzems M, Gironella E, Robinson A and Fleischmann A. 2010. *Nepenthes leonardo* (Nepenthaceae), a new pitcher plant species from Palawan, Philippines.
- Pelser PB, Barcelona JF and Nickrent DL (eds.). 2011 onwards. Co's Digital Flora of the Philippines. [www.philippineplants.org](http://www.philippineplants.org). Accessed on 07 April 2024.
- Quakenbush JP, Malabrigo Jr PL, Umali AG, Tobias AB, Magarce-Camangeg L, Ang YP and Bustamante RA. 2020. Notes on the *Medinilla* (Melastomataceae) of Palawan, Philippines, Including Two New Species: *M. simplicymosa* and *M. ultramacicola*. Systematic Botany, 45(4), 862-872. <https://doi.org/10.1600/036364420x16033962925376>
- Recuenco M, De Luna JR, Magallano N and Salamane K. 2020. Phytochemical screening, total phenolics, and antioxidant and antibacterial activities of selected Philippine Indigenous fruits. Philippine Journal of Science, 149(3-a). <https://doi.org/10.56899/149.3a.02>
- Reyes G. 2019. PCSD pushes for joint management of Victoria Anepahan mountain range. Palawan Daily News. <https://palawandailynews.com/environment/pcsd-pushes-for-joint-management-of-victoria-anepahan-mountain-range/>. Accessed on 07 April 2024.
- Robinson AS, Fleischmann AS, Mcpherson SR, Heinrich VB, Gironella EP and Peña CQ. 2009. A spectacular new species of *Nepenthes* L. (Nepenthaceae) pitcher plant from central Palawan, Philippines. Botanical Journal of the Linnean Society, 159(2): 195-202. <https://doi.org/10.1111/j.1095-8339.2008.00942.x>
- Robinson AS, Gironella EP and Cervancia JM. 2016. Two new orchid species of *Sigmatodactylus* (Orchidoideae; Diurideae) and a new record of *Cryptostylis carinata* from central Palawan, Philippines. Phytotaxa 252 (2): 99-113. <https://doi.org/10.11646/phytotaxa.257.1.8>
- Sandalo RM and Baltazar T. 1997. The Palawan Biosphere Reserve, Philippines (No. 19). SouthSouth Cooperation Programme on Environmentally Sound Socio-Economic Development in the Humid Tropics.
- Sopsop LB. 2010. Human-Forest Interaction in Aborlan Guba System, Palawan Island, Philippines and Implications for Conservation and Management [Unpublished doctoral dissertation]. University of the Philippines Los Baños.
- Sopsop LB and Buot IE. 2009. Endangered plants of Palawan Island, Philippines. Asia Life Sciences, 18(2): 251-279.
- Sopsop LB and Buot IE. 2011. The importance of non-wood forest products in the household economy of the direct users of Aborlan Guba system, Palawan Island, Philippines. Journal of Environmental Science and Management, 14(2): 50-59.
- Sopsop LB and Buot IE. 2013. The forest types in Aborlan Guba system, Palawan Island, Philippines. IAMURE International Journal of Ecology and Conservation, 7(1). <https://doi.org/10.7718/ijec.v7i1.729>
- Supsup A and Asis C. 2018. Identifying important habitats of endemic and threatened species in Puerto Princesa, Palawan. Our Palawan, 4: 1-20.
- Tamayo MN, Marcos-Langbao A, Bustamante RA, Ang YP, Schubert D, Mustaqim W, Fritsch P and Robinson A. 2023. *Rhododendron astrophorum* (Ericaceae, rhododendron sect. Schistanthe), a new micro-endemic species from central Palawan, Philippines. Edinburgh Journal of Botany, 80: 1-14. <https://doi.org/10.24823/ejb.2023.1979>
- Tamayo MN, Bustamante RA and Fritsch PW. 2021. *Vaccinium exiguum* (Ericaceae, Vaccinieae), a new species from the ultramafic summit of Mt. Victoria, Palawan Island, Philippines. PhytoKeys, 179: 145-154. <https://doi.org/10.3897/phytokeys.179.68323>
- Tandang DN, Bustamante RA, Ferreras U, Hadsall AS, Pym-lyon S and Robinson AS. 2020. *Corybas circinatus* (Orchidaceae), a new species from Palawan, the Philippines. Phytotaxa, 446(2): 135-140. <https://doi.org/10.11646/phytotaxa.446.2.7>

**ROLE OF AUTHORS:** JSA – conceptualized the study, gathered, analyzed and interpreted the data; MCBH – conceptualized the study, supervised the study, reviewed the analysis and interpretation of data, edited the paper.

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## GUIDE FOR AUTHORS

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- c. International System of Units of measurements must be used but separated from the value and the unit of measure (e.g. 5 mm, 3 mL, 5 L, 25 g, 30 m<sup>3</sup>, 100 μm, 9 ind ha<sup>-1</sup>, 10 sacks ha<sup>-1</sup>, 2 kg h<sup>-1</sup>, 2 kg h<sup>-1</sup> day<sup>-1</sup>) and probability (*P*) is in upper cased and italicized (e.g. *P* > 0.05; *P* < 0.05; *P* = 0.01). To fix a single space between the value and its unit of measure, use the MS word command “CTR+SHIFT+SPACE BAR” to provide a space between the value and its unit of measure.
- d. There is a single space between numbers and the following mathematical signs: ±, =, ×, -, +, ÷, (e.g. 92 ± 0.092; 5 × 6).
- e. Numbers less than 10 should be spelled out (for example: eight trees, 10 fish) except when followed by a unit of measure (for example: 9 cm, not nine cm). Number mentioned at the start of the statement should be spelled-out (e.g. Nine fishermen not 9 fishermen or Six degrees Celsius not 6°C).
- f. The symbol for Degree (°) should be inserted using the insert symbol option and not zero (0) or alphabet (o) superscript.
- g. Do not separate a percent sign and degree of temperature with the number (example: 5% and 8°C).
- h. Write dates in this manner: day-month-year (example: 20 October 2012).
- i. Use a 24-h system for time (example: 1300 instead of 1:00 pm). To express a measured length of time, abbreviations for hour (h), minutes (min) and seconds (sec) should be used (example: 2 h and 30 min; or 2.5 h).
- j. Include apostrophes in years (example: 2014's).
- k. No periods in acronyms (example: UNESCO not U.N.E.S.C.O.; CITES not (C.I.T.E.S.))
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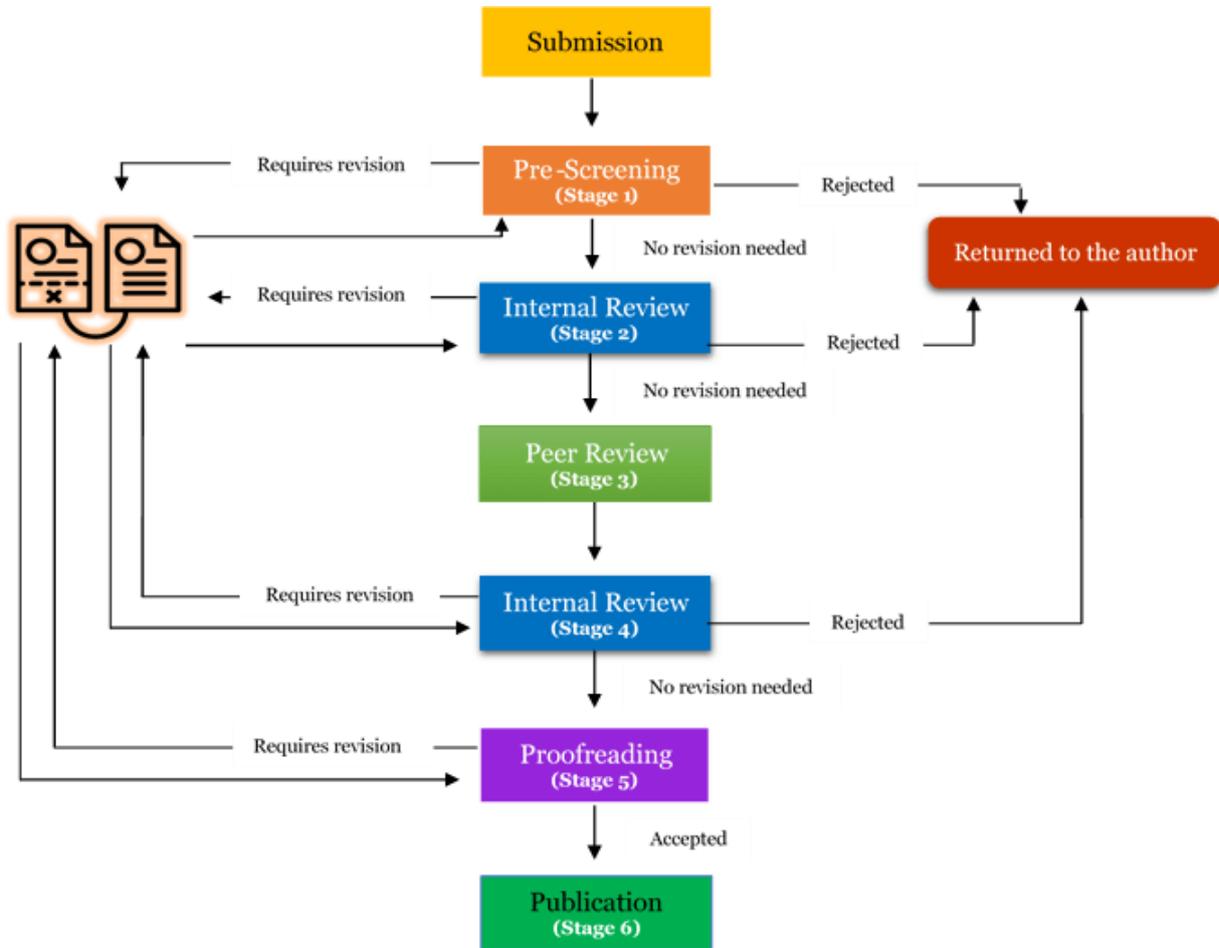
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