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Phytochemical screening and antibacterial properties of "duro" leaves (*Nephelium ramboutan-ake* (Labill.) Leenh. 1986) against *Escherichia coli* and *Staphylococcus aureus*

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ABSTRACT

From the hinterlands of southern Palawan, Philippines, resides one of the oldest tribes in the country, the Palaw'an tribe. As mountain dwellers, they have developed unique healing practices, including the use of the "duro" *Nephelium ramboutan-ake* (Labill.) Leenh. 1986 plant to treat diarrhea. This study aimed to characterize the leaf morphology of "duro," screen for the phytochemicals present, and assess the antibacterial properties of the "duro" leaf extract using the Kirby-Bauer test. Leaves of "duro" were collected from Sitio Sumurum, Brgy. Ransang, Rizal, Palawan. Samples were verified, dried, and extracted before being subjected to phytochemical screening and antibacterial susceptibility test. The morphological characteristics of "duro" leaves include smooth, green-colored, paripinnate compound leaves with an oblanceolate shape arranged oppositely. They have entire margins and pinnate venation, are attached through a petiolate, with the leaf base decurrent at the petiole, and have a mean size of 10.33 cm by 5.77 cm. Only alkaloids, flavonoids, saponins, and tannins were detected among the secondary metabolites tested, while cardiac glycosides and terpenoids were absent. The "duro" leaves extract exhibited moderate antibacterial properties against *Escherichia coli* (Migula, 1895) Castellani and Chalmers, 1919 and *Staphylococcus aureus* Rosenbach, 1884, with mean inhibition zones of 12 mm and 10.67 mm, respectively. The antibacterial properties and presence of phytochemicals suggest the therapeutic potential of "duro" leaves for treating diarrhea. This study addresses knowledge gaps regarding "duro," its medicinal use among the Palaw'an tribe, and promotes further research on indigenous plants.

Keywords: antibacterial susceptibility test, diarrhea treatment, Palaw'an tribe, secondary metabolites

INTRODUCTION

People across the province of Palawan have long used a variety of plants for medicinal purposes, with some of the earliest recorded studies dating back to 1901 (Aguirre et al. 2021; Hirota and Tsuji 2021). Indigenous communities, in particular, have long relied on the local flora and fauna to meet their needs, and this use extends far beyond mere sustenance (Lichtenstein et al. 2017; Menoro and Tablizo 2017). Vegetation plays a key role in enriching their culture, yielding practical and profitable materials, and providing medicinal resources (Paing et al. 2022). Healing practices are uniquely designed for each group and are rooted in indigenous knowledge passed down through generations (Villanueva 2021).

One of the oldest, yet rarely studied tribes in the Philippines is the Palaw'an tribe (Perez 2014). This tribe, located in Southern Palawan, is composed of excellent farmers and foragers with vast knowledge of wild plants that they use in their daily lives (Bernadas and Peralta 2017). Like other indigenous groups, they rely on their traditional healers who use indigenous plants—wildcrafted plants consumed by native people or introduced to them long ago (Cogill 2015). One of these plants is the pulasan *Nephelium ramboutan-ake* (Labill.) Leenh. 1986 or "duro," as called by the Palaw'an tribe; they utilize it to treat diarrhea by preparing a decoction of its leaves for oral administration.

"Duro" is a tropical plant under the family Sapindaceae that is native to some Southeast Asian countries, including Myanmar, Malaysia, and Thailand, and is rarely cultivated at low elevations in the Philippines (Murugan and Tan 2022). The fruit can be eaten fresh, and its seeds are also edible. The aril has a sweet flavor similar to that of its relative species, *Nephelium lappaceum* L., 1767, commonly known as Rambutan (Djuita et al. 2016). It also contains high levels of antioxidants and helps control blood sugar levels (Hairunisa et al. 2021; Tan et al. 2022). Like many other indigenous plants used for traditional medicine, "duro" is typically overlooked, with only limited studies and factual information available to the public (Maramba-Lazarte 2020; Tan et al. 2022).

Only a few studies on the antioxidant properties of "duro" have been conducted. The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging experiment showed that the 80% ethanol extract of its rind has a high antioxidant activity (Sopee et al. 2019). Subsequently, the ethanol extract of "duro" leaves showed very strong antioxidant activity with the value 20.99 µg/mL, while its seeds and peels exhibited no antioxidant activity (Hairunisa et al. 2021).

To learn more about "duro," further studies are needed to address knowledge gaps concerning its bioactivities. This study aimed to characterize the morphology of "duro" leaves and examine their efficacy as a diarrheal treatment through qualitative phytochemical screening of certain secondary metabolites with antidiarrheal properties (alkaloids, cardiac glycosides, flavonoids, saponins, tannins, and terpenoids), as well as an antibacterial susceptibility test against the diarrheagenic bacteria *Escherichia coli* (Migula, 1895) Castellani and Chalmers, 1919 and *Staphylococcus aureus* Rosenbach, 1884. These tests provide a new understanding of the potential medicinal value of the "duro" plant used by the Palaw'an tribe in Rizal, Palawan.

METHODS

Plant Collection, Authentication, and Morphology

A total of 3.5 kg of "duro" leaf samples collected at Sitio Sumurum, Barangay Ransang, Rizal, Palawan were used in the experimental research design. A Wildlife Gratuitous Permit No. 2022-37 from the Palawan Council for Sustainable Development (PCSD) and a letter of request addressed to the Brgy. Captain of Brgy. Ransang, Rizal, Palawan were secured prior to plant collection. The obtained "duro" leaves were verified by a botanist at the Palawan Herbarium of the Palawan State University. Their morphological characteristics were described using the PlantNET (2024). Figure 1 presents the research procedures of the study.

Plant Preparation and Extraction

"Duro" leaves from the middle portion of the stems were used for plant extraction. Following the standard extraction guide by Phaiboon et al. (2019), 1.8 kg of leaves were thoroughly washed with distilled water to remove impurities, air-dried at room temperature for seven days, and oven-dried at 40°C for five hours. The dried samples were pulverized with a laboratory grinder, yielding 793.92 g of "duro" leaf powder. For every 250 mL of 95% ethyl alcohol, 25 g of "duro" leaf powder was soaked at a 1:10 ratio; 12 flask samples were prepared using 300 g of "duro" leaf powder in 3,000 mL of 95% ethyl alcohol. The samples were stored in a fume hood for seven days and filtered using sterile coffee filters to remove solid particles. The residual ethyl alcohol was evaporated using a rotary evaporator at 40°C. The evaporation process was repeated six times for eight hours each, processing 500 mL of sample per batch in the rotary evaporator. Eighteen grams (18 g) of "duro" leaf crude extract were obtained. The percent yield of the extract after evaporation was 6%, computed using the formula by Gonfa et al. (2020):

$$Extraction\ yield\ (\%) = \frac{Weight\ of\ crude\ extract}{Weight\ of\ powdered\ sample} x\ 100$$

Qualitative Phytochemical Screening

The standard screening procedures by Bargah (2015), Guevara (2005), Parekh and Chanda (2007), and Raju et al. (2021) were performed for the qualitative phytochemical screening of secondary metabolites such as alkaloids, cardiac glycosides, flavonoids, saponins, tannins, and terpenoids. The volume of the crude extract obtained was used as the stock solution since no additional dilution with any solvent was performed. The aliquot represented the volume of the extract specified for each phytochemical test.

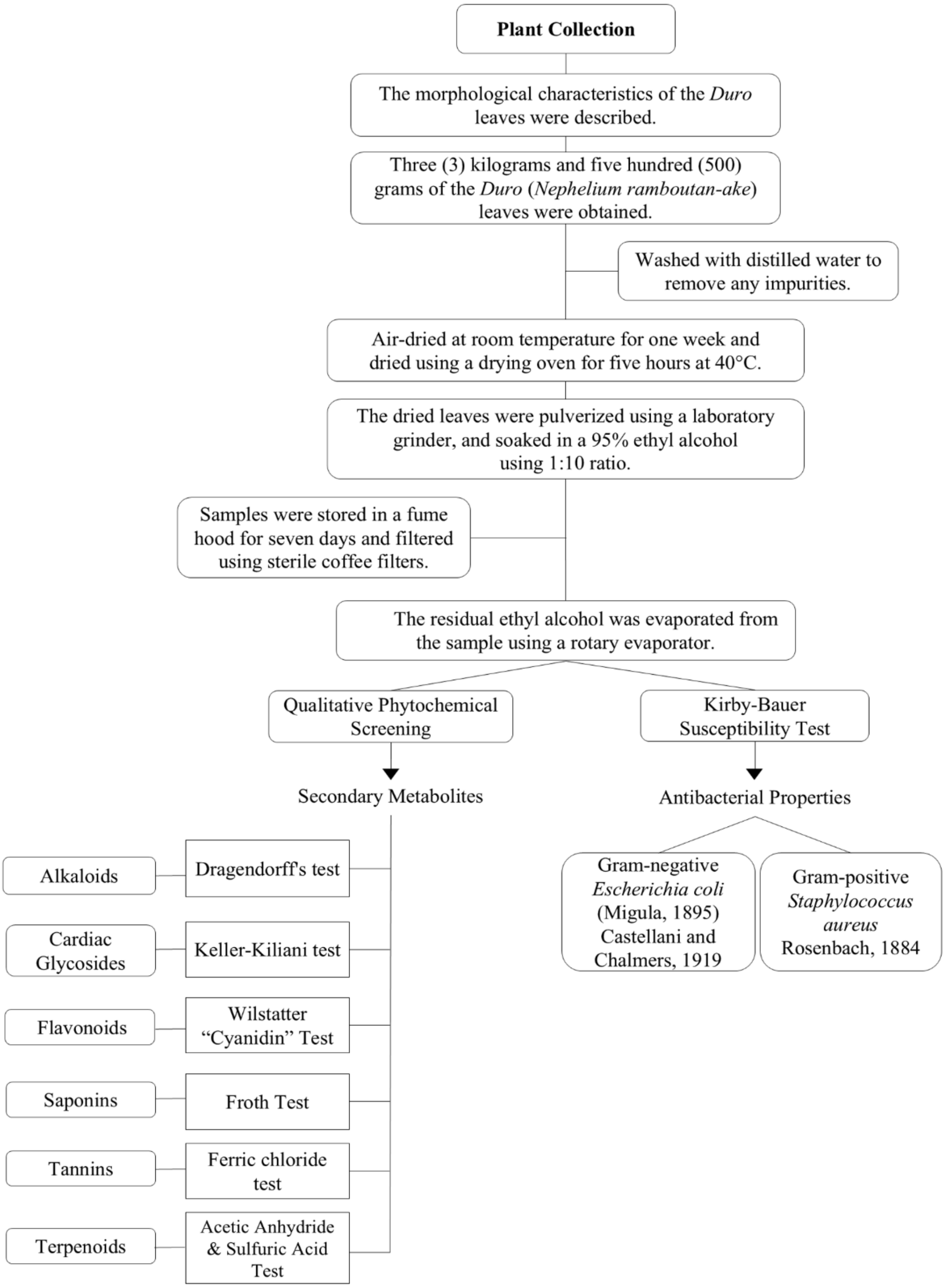

Figure 1. Schematic diagram of the research procedure.

Alkaloids. The Dragendorff's test was carried out, where 2 mL of concentrated hydrochloric acid (HCl) was added to 2 mL filtrate and 1 mL of Dragendorff's reagent. An orange, red, or orange-red precipitate indicated the presence of alkaloids (Raju et al. 2021).

Cardiac Glycosides. The Keller-Kiliani test was performed by adding 1 mL of glacial acetic acid (CH3COOH), 1 mL of ferric chloride (FeCl3), and 1 mL of concentrated sulfuric acid (H2SO4) to 2 mL of filtrate; a green-blue color indicated their presence (Parekh and Chanda 2007).

Flavonoids. The Wilstatter "cyanidin" test was conducted by adding 2 mL of concentrated hydrochloric acid (HCl) to 2 mL of filtrate and adding three to four strips of magnesium turnings or ribbons.

A pink to tomato-red color indicated their presence (Guevara 2005).

Saponins. The Froth test was performed by adding 5 mL of distilled water to 5 mL of filtrate. The solution was agitated for 30 seconds, and the formation of 1.5 cm of stable froth persisting for ten minutes indicated the presence of saponins (Bargah 2015).

Tannins. The Ferric Chloride test was performed by adding 2 mL of ferric chloride ($FeCl_3$) to 2 mL of filtrate, in which the presence of a blue-black precipitate indicates its presence (Parekh and Chanda 2007).

Terpenoids. Two milliliters (2 mL) of acetic anhydride ($C_4H_6O_3$) were added to 2 mL of filtrate, followed by a few drops of concentrated sulfuric acid (H_2SO_4). A blue-green ring indicated the presence of terpenoids (Raju et al. 2021).

The presence of secondary metabolites in the “duro” leaf ethanolic extract was classified as high (+++), moderate (++), low (+), or absent (-) (Nadayag et al. 2019).

Kirby-Bauer Susceptibility Test

The diarrheagenic pathogens *E. coli* (BIOTECH 1634) and *S. aureus*–obtained from the University of the Philippines Los Baños, Philippine National Collection of Microorganisms–were used for the Kirby-Bauer susceptibility test (De Las Llagas et al. 2014) at the Clinical Laboratory of Palawan Adventist Hospital (PAH). *Escherichia coli* and *S. aureus* were cultured in Mueller-Hinton broth and adjusted with a densitometer until the turbidity matched the 0.45 sodium chloride (NaCl) turbidity standard. Sterile cotton swabs were used to spread each inoculum on separate Mueller-Hinton (MH) agar plates; triplicates were prepared for both diarrheagenic pathogens. Three sterile paper discs were dipped in the “duro” ethanolic extract and placed on the first MH agar plate inoculated with *E. coli*. Another three sterile paper discs were dipped in the “duro” ethanolic extract and placed on the second MH agar plate inoculated with *S. aureus*. One side of the third MH agar plate was inoculated with *E. coli,* and the other side with *S. aureus*. On the side inoculated with *E. coli*, a sample-free disc for negative control and a 30 mcg ceftriaxone antibiotic disc for positive control were placed, while on the side inoculated with *S. aureus*, a sample-free disc for negative control and a 2 mcg clindamycin antibiotic disc for positive control were placed. All three plates were incubated at 36°C for 24 hours. After incubation, the diameter (or radius, if overlapping) of inhibition zones around the paper discs was measured using a caliper.

The antibacterial properties of the “duro” leaves ethanolic extract were classified as strong (≥ 15 mm), moderate (≥ 7 mm), or inactive (≤ 6 mm) according to the zone of inhibition measurements (Iikasha et al. 2017).

Statistical Analysis

The qualitative phytochemical screening and Kirby-Bauer susceptibility test were conducted in triplicate ($n = 3$). The mean, standard deviation, and statistical significance between the two bacterial strains in the Kirby-Bauer susceptibility test were analyzed using a paired t-test at $P < 0.05$ with IBM SPSS Statistics version 30.0 (Panpaliya et al. 2019).

RESULTS

Morphological Characteristics

Table 1 and Figure 2 depict the morphological characteristics of the “duro” leaves collected from Sitio Sumurum, Brgy. Ransang, Rizal, Palawan. “Duro” is an evergreen tree that grows upright in tropical forests (Figure 2A). Its green, oblanceolate leaves exhibit pinnate venation and are paripinnate, oppositely arranged leaflets without a terminal leaf, with a mean size of 10.33 cm by 5.77 cm (Figure 2B). Additionally, the smooth-textured leaves possess entire margins and are attached to a petiole, with the leaf base decurrent along the petiole, presenting a lighter green shade and noticeable secondary veins on the abaxial surface (Figures 2C and 2D). Flowers in the budding stage were also documented (Figures 2E and 2F).

Table 1. Morphological characteristics of the “duro” leaves.

Characteristics	Description
Type	Compound
Compound	Paripinnate
Color	Green
Texture	Smooth
Arrangement	Opposite
Margin	Entire
Shape	Oblanceolate
Venation	Pinnate
Attachment	Petiolate
Petiole	Decurrent
Mean Size	10.33 cm by 5.77 cm

Phytochemical Contents

Table 2 shows the results of the qualitative phytochemical screening of the “duro” leaf ethanolic extract. Only alkaloids, flavonoids, saponins, and tannins were detected. Alkaloids and tannins were consistently present in all trials, while flavonoids and saponins were detected in two out of three trials.

Antibacterial Properties

Figure 3 and Table 3 display the inhibition zones of the “duro” leaf ethanolic extract, showing its efficacy in inhibiting the growth of selected diarrheagenic bacteria *E. coli* (Figure 3A), *S. aureus* (Figure 3B), along with their positive and negative controls (Figure 3C). The test yielded inhibition zones of more than 7 mm for both diarrheagenic bacteria in all trials. However, no significant difference was observed between *E. coli* and *S. aureus* (P = 0.423). Furthermore, for *E. coli*, the positive control (ceftriaxone) produced a 20 mm inhibition zone, while the negative control showed no inhibition. Similar results were observed in *S. aureus*, where the positive control (clindamycin) produced a 25 mm inhibition zone, and no inhibition was observed in the negative control.

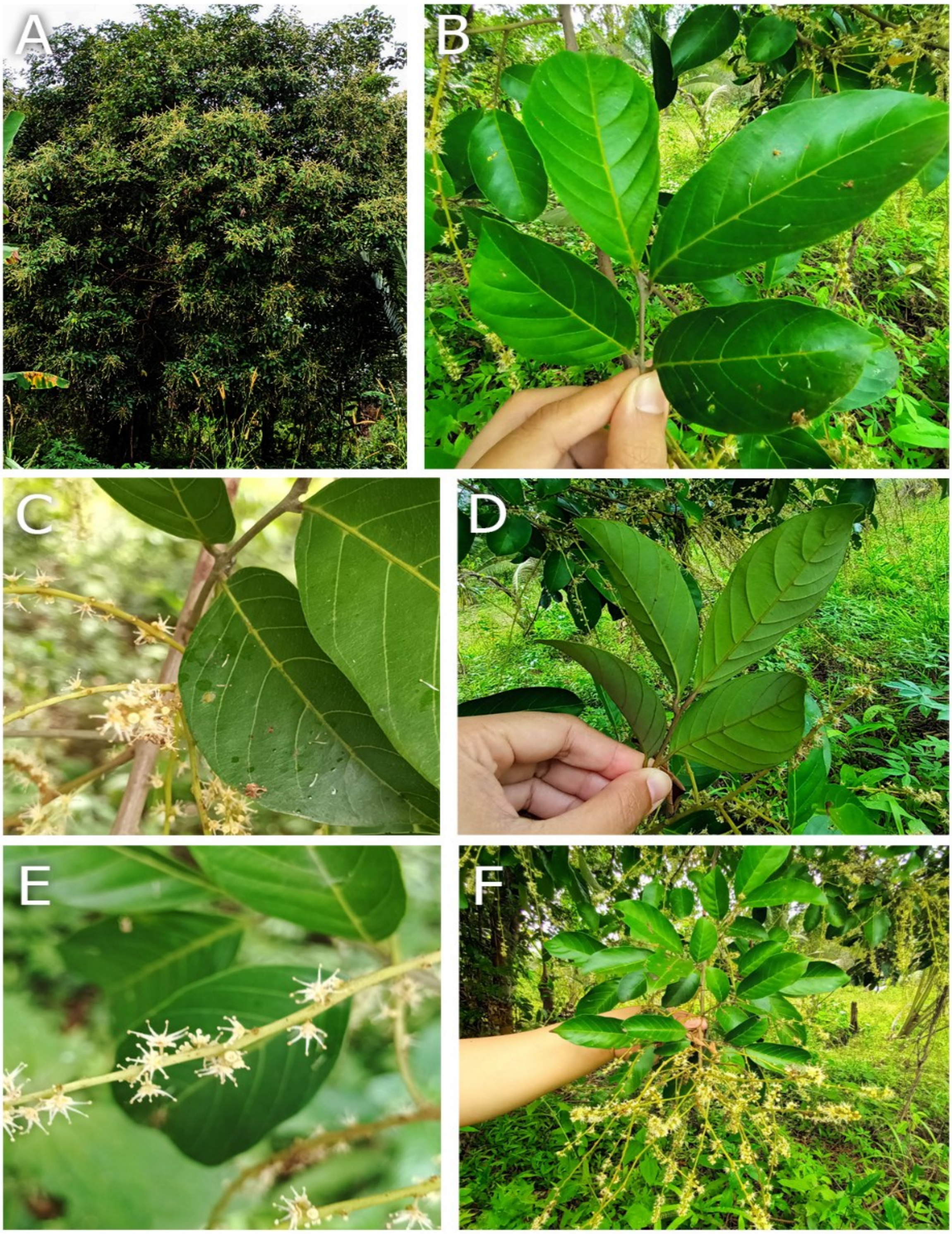

Figure 2. Morphological characteristics of the "duro" leaves.

Table 2. Secondary metabolites identified in "duro" leaves, classified by Nadayag et al. 2019 as highly present (+++), moderately present (++), low (+), and absent (-).

Secondary Metabolites	Methods	Trials*			Interpretation
		1	2	3	
Alkaloids	Dragendorff's Test	+	+	+	Highly Present
Cardiac Glycosides	Keller-Kiliani Test	-	-	-	Absent
Flavonoids	Wilstatter "Cyanidin" Test	+	-	+	Moderately Present
Saponins	Froth Test	+	+	-	Moderately Present
Tannins	Ferric Chloride Test	+	+	+	Highly Present
Terpenoids	Acetic Anhydride and Sulfuric Acid Test	-	-	-	Absent

Figure 3. Susceptibility tests of selected bacteria against "duro" leaves.

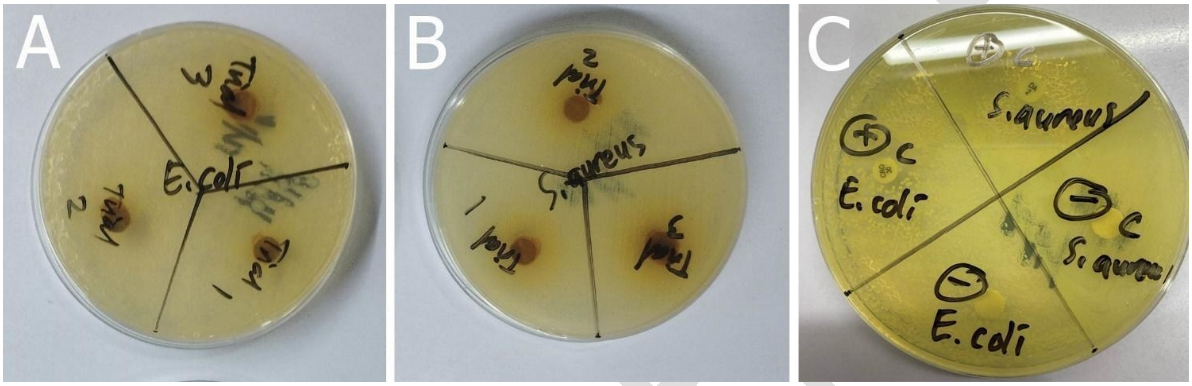

Table 3. Antibacterial activities of the "duro" leaves against *E. coli* and *S. aureus,* classified by Iikasha et al. 2017 as (≥ 15 mm): strong; (≥ 7 mm): moderate; (≤ 6 mm): inactive. (Note: *Mean ± Standard Deviation values represent the three trials, [a]30 mcg of ceftriaxone for positive control of *E. coli,* [b]2 mcg of clindamycin for positive control of *S. aureus,* [c]sample-free discs for negative controls).

Diarrheagenic Bacteria	Zones of Inhibition (mm)					
	Trial 1	Trial 2	Trial 3	Mean ± SD*	Control (+)	Control (-)[c]
Escherichia coli	11	13	12	12.00 ±1.000	20[a]	0
Staphylococcus aureus	11	9	12	10.67 ± 1.528	25[b]	0

DISCUSSION

Morphological Characteristics

The morphological characteristics of the "duro" leaves, as observed in this study, showed both similarities and variations. Most of the traits–such as color, shape, type of compound leaf, arrangement, and texture–closely matched the description provided by Lim (2013) and Morton (1987), which characterized the leaves as green, elliptic to obovate-lanceolate, paripinnate, oppositely arranged, and having a smooth or glabrous texture. However, slight differences were noted in Morton's (1987) observation, which indicated that a terminal leaf may also be present, making the "duro" leaves either paripinnate or imparipinnate (odd-pinnate) compound leaves.

A more contrasting observation was recorded by the National Parks Flora & Fauna Web (2024), which noted that variants exhibit wavy leaf margins, an elliptic shape, spirally arranged leaves, and a leathery to papery texture at maturity. These discrepancies could be attributed to climatic variations, soil conditions, or genetic adaptations across different regions (Everingham et al. 2024).

Phytochemical Contents

The results suggest that the "duro" leaves have antidiarrheal potential due to the high presence of alkaloids and tannins, as well as the moderate presence of flavonoids and saponins. Diarrhea is commonly caused by intestinal irritation and inflammation, which increase intestinal motility and water-electrolyte secretion (Alemu et al. 2022). To inhibit intestinal secretion and peristalsis, alkaloids deactivate the nitric oxide pathway, reducing small intestine volume (Sisay et al. 2017); tannins regulate calcium currents to promote muscle relaxation (Yacob et al. 2016); flavonoids inhibit arachidonic acid secretion to lessen gut inflammation (Al-Khayri et al. 2022); and saponins suppress spasmogenic activity to prevent excessive muscle contraction (Mishra et al. 2016). The properties of these phytochemicals collectively contribute to the antidiarrheal effects of the “duro” leaves.

A similar finding was reported in the study by Putri et al. (2021), who detected alkaloids, flavonoids, tannins, and saponins in the ethanol extract of "duro" leaves. Other members of the Sapindaceae family, such as Lychee (*Litchi chinensis* Sonn.) and Rambutan, also exhibit similar phytochemicals, including alkaloids, flavonoids, and tannins (Aktar et al. 2022; Perumal et al. 2021).

Antibacterial Properties

Data indicate that the “duro” leaves exhibit moderate antibacterial properties against selected diarrheagenic bacteria (Iikasha et al. 2017). Furthermore, *E. coli* and *S. aureus* showed similar susceptibility, with mean inhibition zones of 12 mm and 10.67 mm, respectively, despite the difference in their Gram properties. However, the difference between the antibacterial properties of the two bacteria was not statistically significant ($P = 0.423$).

In comparison, ethanolic extracts of other “duro” plant parts, such as the peels, demonstrated strong antibacterial properties against *E. coli* and *S. aureus,* with inhibition zones of 29.78 mm and 34.33 mm, respectively, while the seeds exhibited moderate antibacterial properties against the same bacteria, with inhibition zones of 6.11 mm and 8.78 mm, respectively (Fatisa 2013). Similar results were observed in the leaves of a closely related species, Rambutan, which also displayed moderate antibacterial properties against *E. coli* and *S. aureus,* with inhibition zones of 13.3 mm and 12 mm, respectively (Chigurupati et al. 2019). The moderate antibacterial properties of “duro” leaves can be attributed to the presence of secondary metabolites (high alkaloids and tannins, and moderate presence of flavonoids and saponins).

Overall, some inconsistencies were observed in the morphological description of “duro,” particularly in its leaf characteristics compared with existing references. The secondary metabolites identified in the “duro” leaves were alkaloids, tannins, flavonoids and saponins. It is notable that while the “duro” leaves showed antibacterial properties, they were less effective than the antibiotics used in the test. Therefore, further studies on the secondary metabolites of “duro” leaves—and their comparison with related species within the Sapindaceae family—should be prioritized, as this could help understand how their shared properties act against diarrhea and potentially aid in developing treatments. Likewise, investigating the leaf morphology and environmental conditions of the "duro" plant from various municipalities of Palawan, as well as in other regions and countries, may provide deeper insights into its ecological adaptations and variations under different environmental conditions.

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GENERATIVE AI STATEMENT

No part of this study was AI-generated.

ETHICAL CONSIDERATIONS

The collection of plant samples in this study was conducted with permission obtained from the Palawan Council for Sustainable Development (Wildlife Gratuitous Permit No. 2022-37) and the Brgy. Captain of Brgy. Ransang, Rizal, Palawan.

DECLARATION OF COMPETING INTEREST

The authors declare no conflicts of interest.

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