

Research Article

TAXONOMIC DESCRIPTION AND ANTIBACTERIAL ACTIVITY OF *Dillenia* sp. AGAINST *Escherichia coli*

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ARTICLE HIGHLIGHTS

- The study first reported taxonomic study of *Dillenia* sp. in Malita, Davao, Occidental Philippines.
- This is the first reported findings of the bacterial activity of the bark extract of *Dillenia* sp. against *Escherichia coli*.

ABSTRACT

The study aimed to provide a taxonomic description of *Dillenia* sp. and evaluate its antibacterial activity against *Escherichia coli*. The taxonomic classification was carried out by using taxonomic keys and field guides. The antibacterial activity of *Dillenia* sp. was screened using the Kirby-Bauer disk diffusion assay. Taxonomic description resulted from this study confirmed *Dillenia* sp. to be an angiosperm flowering seed plant native to the Philippines, characterized by its round-shaped growth and evergreen foliage. The antibacterial activity of *Dillenia* sp. bark extracts against *E. coli* was assessed using different concentrations (100 ppm, 500 ppm, and 1,000 ppm) and different extraction methods (decoction, ethanolic, and aqueous). Significant differences in the antibacterial activity observed among treatments within the decoction extracts were observed, indicating varying antibacterial activities. Post-hoc analysis revealed that concentrations of 100 ppm and 500 ppm were significantly more effective in inhibiting bacterial growth compared to concentration of 1,000 ppm within the decoction treatment. Each treatment showed distinct patterns of antibacterial activities, with ethanolic and aqueous extracts displaying relatively consistent activities across different concentrations, while the decoction extract exhibited concentration-dependent antibacterial activities. The study provided evidence of the antibacterial potential of *Dillenia* sp. bark extracts against *E. coli*, with implications for further research and potential practical applications in combating bacterial infections. Recommendations include exploring lower concentration ranges, considering different treatment formulations, investigating combination treatments, and assessing antimicrobial mechanisms to enhance efficacy and guide the development of novel antibacterial drugs.

Keywords:

antibacterial activity, *Dillenia*, endemic, Philippines

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INTRODUCTION

The World Health Organization (WHO) highlights a global health threat, i.e., the increasing prevalence of multi-drug-resistant *Escherichia coli* strains (WHO 2023). This bacterium, commonly found in human or animal waste, causes severe symptoms, such as diarrhea, vomiting, stomach pains, and cramps (Colina 2021). *Escherichia coli* is a part of the human gut microbiota and a leading cause of various infections, from urinary

tract infections to more severe systemic conditions. The emergence of multi-drug-resistant strains emphasized the urgent need for new antibacterial agents (Mueller & Tainter 2023).

Dillenia sp., a plant endemic to the Philippines, has emerged as a potential source of novel antibacterial compounds. This interest stems from the presence of diverse phytochemicals within the plant, known for their activity against various bacterial strains (Dante *et al.* 2019). However, there remain uncertainties

regarding the exact taxonomic classification of *Dillenia philippinensis*, highlighting the need for further investigation. The bark of *D. philippinensis* presents a particularly intriguing avenue for exploration due to its rich phytochemical profile (Sabandar *et al.* 2017).

Furthermore, the first description of a *Dillenia* sp. in the Philippines is attributed to Rolfe (Stuart 2020). Interestingly, there are currently no documented occurrences of *Dillenia* within Malita Municipality, Davao Occidental Province.

Despite the traditional use of *Dillenia* sp. for its anti-microbial, anti-inflammatory, analgesic, and antidiabetic properties, there was a limited systematic exploration into the taxonomic classification and antibacterial activity of *Dillenia* sp. against clinically significant bacteria like *Escherichia coli* (Patra 2012).

The study of Ragasa *et al.* (2009) reported that the air-dried leaves of *Dillenia philippinensis*, commonly known as “katmon” contain betulinic acid and 3-oxoolean-12-en-30-oic acid based on assessment by using silica gel chromatography. Those 2 acidic compounds exhibited moderate activity against the fungus *Candida albicans* and slight activity against the bacteria *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus subtilis*. The antibacterial activity of *Dillenia* sp. leaves have been reported, but studies on the antibacterial properties of the bark extract are still lacking.

Therefore, this research aimed to elucidate the taxonomic identity of *Dillenia* sp. and evaluate its ability to inhibit *E. coli* bacteria. The specific focus was on the potential of *Dillenia* sp. bark extract as a source for developing new and effective antibacterial agents (Stuart 2020).

MATERIALS AND METHODS

In this study, the descriptive aspect focused on the taxonomic classification of *Dillenia* sp. The experimental aspect evaluated the antibacterial activity of *Dillenia* sp. against *E. coli*. Plant extracts were obtained from the collected samples and subjected to antimicrobial disk susceptibility testing using standard laboratory techniques.

Research Locale

Samples of *Dillenia* sp. were collected at Sitio Maylaya, Barangay Kilalag, Malita Municipality, Davao Occidental Province (6°21” N; 125°29” E). Sitio Maylaya had a total land area of 20 ha and is a remote area in Malita Municipality, located along the upland area of the municipality, and was chosen due to the occurrence of mature *Dillenia* sp. in the area.

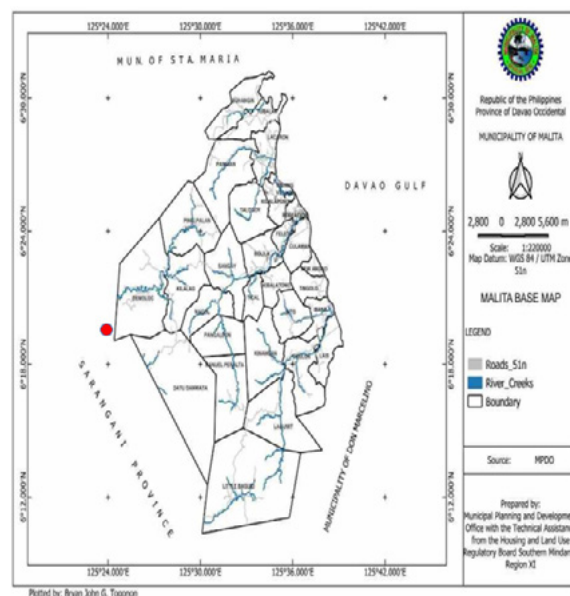


Figure 1 Base map of Malita Municipality, Davao Occidental Province, Philippines

Pre-Implementation Phase

Necessary permits for taking samples of *Dillenia* sp. were secured from pertinent authorities and landowners. Approval from the Department of Environment and Natural Resources (DENR), Research and Laboratory Services Center (RLSC), landowner, Barangay Captain, and the Dean of the Institute of Teacher Education and Information Technology (ITEIT) were also secured.

Collection and Identification of *Dillenia* sp.

Samples of *Dillenia* sp. bark were collected from mature trees located in Sitio Maylaya. The samples were carefully excised from healthy bark sections using sterilized tools, ensuring minimal damage to the plant. The collected bark samples were clearly labeled with the date, location, and any other relevant information. Subsequently, the collected bark samples were washed thoroughly to remove any dirt or contaminants and then allowed to air dry. The air-dried bark samples were then ground into fine powder by using a blender.

Measurements of *Dillenia* sp. tree were taken to determine the taxonomy of the plant, which include tree height, growth form, trunk description, leaf sizes, leaf shapes and arrangement, leaf margins, leaf venation and petiole, flower color and symmetry, fruit color, fruit classification, fruit type, and roots. These measurements were used to make comparisons in relation to the taxonomic keys. A field Guidebook on Native Trees within the Quirino Forest Landscape (Carig 2020), published literature by Stuart (2020), and published literature on Philippine *Dillenia* - *Dillenia philippinensis* (Orate & LaFrankie 2011) were used to determine *Dillenia* sp. taxonomy.

Preparation of Culture Media and Processing of *Dillenia* sp. Bark

LS broth medium of 17.8 g was dissolved in 500 mL distilled water and mixed thoroughly using magnetic stirrer. Six test tubes were each filled with 10 mL of the prepared broth. The test tubes were autoclaved at 121 °C for 15 minutes and then allowed to cool.

In the meantime, alcohol lamp, wire loop, and contaminated water were prepared and placed in a biosafety cabinet. The wire loop was heated in the alcohol lamp until turning red, then was allowed to cool for 5 seconds before being placed in the contaminated water. The wire loop with contaminated water was then placed inside the test tube that had been filled with LS Broth medium. This procedure was repeated for the other test tubes. These test tubes containing samples were placed in an incubator at 37 °C for 48 hours.

Dillenia sp. bark was powdered using a mortar and pestle and prepared 100 g powdered bark sample. Three methods of bark extraction were then carried out: (1) decoction, (2) aqueous, and (3) ethanolic.

To prepare bark decoction, 40 g of bark were used for every 200 mL of water which was boiled for 20 minutes. After boiling, bark decoction was allowed to cool, and the extract was filtered using filter paper. Subsequently, the extract was evaporated using the wash bath and allowed to cool before further analysis.

In preparing the aqueous extract, 40 g of powdered bark was mixed with 200 mL of distilled water for one and a half minutes (1:30 min). The mixture was then simmered on low heat for 15-20 minutes until the desired concentration was achieved. Afterward, the bark extract was evaporated using water bath and allowed to cool slightly before further processing.

The ethanolic extract was prepared by pouring 200 mL of ethanol over 40 g of powdered bark until completely soaked. The bark was allowed to soak in ethanol for 24 hours to 72 hours, with gentle agitation of the container from time to time to facilitate extraction. The ethanol extract was filtered with filter paper to remove plant debris, and the filtered extract was collected in a clean vial. Subsequently, the extracted solution was stored in a clean Erlenmeyer flask and allowed to evaporate in a water bath.

Antibacterial Assessment

Escherichia coli, as test organism, was cultivated by using standard protocol following Ogodo *et al.* (2022). Mueller-Hinton agar was used to cultivate *E. coli*. The Mueller-Hinton agar was prepared following the supplier's instructions (Tankeshwar 2013).

There were 4 treatments of *Dillenia* sp. bark extracts concentrations: 1) 100 ppm; 2) 500 ppm; 3) 1,000 ppm, and 4) control group, with 3 replicates for each treatment.

Antibacterial petri dishes were divided into four parts and labeled according to concentration, replicates, and treatment. The antibacterial petri dishes were then soaked in the aqueous, ethanolic and decoction solutions with a specific concentration of the *Dillenia* sp. extract, allowing the disks to air dry in a sterile environment. The plates were then incubated at 37 °C for 24 hours, allowing the bacteria to grow and potentially be inhibited by the extract. Zones of inhibition were observed after incubation.

Ethical Guidelines

Ethical guidelines were practiced in obtaining the samples of *Dillenia* sp. from the research

locale by securing the necessary permits from the DENR, Barangay Captain and the landowners. Decontamination protocol in laboratory was strictly adhered to in preparing the test organism and the antibacterial assessment assay.

Data Analysis

The zone of inhibition was calculated using the formula following Bhargay *et al.* (2016):

$$\pi r^2$$

where:

r = radius of the inhibition zone

Statistical Analysis

Mean, standard deviation, minimum, maximum, and quartiles for each concentration level were used in the study. One-way ANOVA and post-hoc test were used to interpret statistical significance of the different treatments. The statistics were computed using the SPSS software.

RESULTS AND DISCUSSION

Taxonomic study on *Dillenia* sp.

Taxonomic study conducted in this research showed that *Dillenia* sp. is a flowering seed plant with two cotyledons (dicotyledons), classified within the division of angiosperms. *Dillenia* sp. grows as a small tree, reaching heights between 6 m and 17 m. The tree is autotrophic with rounded shape, contributing to its aesthetic appeal. *Dillenia* sp. is native to the Philippines and thrives in terrestrial habitats within tropical climate zones. *Dillenia* sp. is a tree reaching a height of 6 m to 15 m, smooth or nearly so (Stuart 2020; Carig 2020). The trunk of *Dillenia* sp. exhibits a stout and sturdy demeanor, often reaching considerable heights in its natural habitat (Fig. 2).

Bark of *Dillenia* sp. appears as shallow fissures and showcases hues ranging from greyish-brown to reddish-brown tones (Fig. 3). The bark not only provides a protective layer for the tree's internal tissues, but also contributes to its aesthetic appeal. The shallow fissures add texture to the bark, giving it a rugged yet visually intriguing appearance.

The leaf structure of *Dillenia* sp. is characterized by a leathery texture, imparting a robust and durable quality to withstand various environmental conditions. Their surface exhibits a glossy sheen, adding to their visual appeal and potentially serving functional purposes, such as water repellency or light reflection. The shape of *Dillenia* sp. leaf ranges from ovate to elliptic or oblong-ovate, showcasing a broad yet elongated form that enhances their efficiency in capturing sunlight for photosynthesis, with lengths typically spanning from 12 cm to 25 cm (Fig. 4). Along the margins of the leaf, coarse teeth are present, providing a serrated appearance.

Dillenia sp. tree produces bisexual flowers with radial symmetry (Fig. 5). The flowers exhibit a variety of colors, including red and white. Their large size, ranging from 6 cm to 8 cm in diameter, commands attention and makes them a prominent feature in the surrounding foliage. The petals of *Dillenia* flowers are soft and fleshy to the touch. This softness not only adds to the tactile allure of the flowers, but also underscores their ephemeral beauty.

Mature fruit of *Dillenia* sp. exhibits a vibrant green coloration, signaling their ripeness and readiness for dispersal (Fig. 6). The fruit is classified as simple, fleshy fruits, possessing a single-seeded structure encapsulated within a soft and pliable outer layer.

The morphology of the studied *Dillenia* sp. collected in Malita Municipality, Davao Occidental Province, Philippines, and the *Dillenia* sp. reported by Stuart (2020) shows a similarity in terms of tree height of 6 m to 15 m. The leaves are leathery, shining, ovate, elliptic or oblong-ovate, with 12 cm to 25 cm leaf length, and coarsely toothed at the margins. The flowers are white, large, soft, fleshy, and green, with diameter of 6 cm to 8 cm, having large fleshy sepals tightly enclosing the true fruit. Based on the morphology, these characteristics are key features of *Dillenia* sp. Stuart (2020) further corroborated its taxonomic classification and distinctive characteristics.



Figure 2 Tree of *Dillenia* sp.



Figure 3 Trunk and bark of *Dillenia* sp.



Figure 4 Leaf structure of *Dillenia* sp.



Figure 5 Flower structure of *Dillenia* sp.

Figure 6 Fruit of *Dillenia* sp.Table 1 Zone of Inhibition (ZOI) of different concentrations of *Dillenia* sp. bark extract against *Escherichia coli*

Concentration	Zone of inhibition (ZOI) of each bark extract concentration and extraction method				
	Replicates	Ethanolic (mm ²)	Aqueous (mm ²)	Decoction (mm ²)	Control (mm ²)
100 ppm	R1	40.69	42.99	50.24	21.23
	R2	60.79	50.24	63.59	58.06
	R3	8.01	13.50	14.13	11.30
Mean of ZOI		36.50	35.58	42.65	30.20
500 ppm	R1	33.17	38.47	50.24	52.78
	R2	50.24	50.24	50.24	60.79
	R3	10.99	13.82	13.19	14.13
Mean of ZOI		31.47	34.18	37.89	42.57
1,000 ppm	R1	19.63	38.47	50.24	50.24
	R2	39.19	50.24	55.39	38.47
	R3	10.05	10.99	13.19	12.56
Mean of ZOI		22.96	33.23	39.61	33.76

Antibacterial Activity of *Dillenia* sp. Bark Extract

The highest Zone of Inhibition (ZOI) observed in this study was 42.65 mm² at 100 ppm by using decoction extraction method, while the lowest was 22.96 mm² at 1,000 ppm by using ethanolic

extraction method (Table 1). The lower the concentration of the extract, the larger the zone of inhibition observed in this study. This variation indicated a concentration-dependent response, with higher concentrations of *Dillenia* sp. bark extract generally leading to smaller ZOI.

Table 2 Statistical analysis indicating significant differences among zones of inhibition of different concentrations of *Dillenia philippinensis* extracts against *E. coli*

Extracts	Concentration (ppm)	Mean	P value	Interpretations
Decoction	100	42.65 ^a	0.00	Significant
	500	37.89 ^a		
	1,000	39.61 ^b		
Aqueous	100	35.58	0.99	Not Significant
	500	34.18		
	1,000	33.23		
Ethanollic	100	36.50	0.74	Not Significant
	500	31.47		
	1,000	22.96		
Control	100	30.20	0.80	Not Significant
	500	42.57		
	1,000	33.76		

Note: Numbers having the same superscripts has no significant difference at P value 0.05.

According to the study of Wani *et al.* (2022), microorganisms have an extremely short generational span, are able to quickly adapt to survive in high levels of antimicrobials and are able to pass the resistance around in a population. This suggests that *Dillenia* sp. bark extract has a dose-independent antibacterial effect on *E. coli*.

Analysis conducted in this study suggested that multiple factors influence the ZOI. The lower concentrations tended to exhibit larger ZOI, possibly due to increased active molecule presence inhibiting bacterial growth. Additionally, methods of extraction (ethanollic, aqueous, decoction) may affect extract potency, influencing the ZOI. Moreover, other factors, such as bacterial growth phase, culture medium, and measurement method (e.g., agar well diffusion assay) could also be an influencing factor.

The study of Gajic *et al.* (2022) demonstrated a similar concentration-dependent relationship between plant extract concentration and antibacterial activity. Furthermore, Garcia *et al.* (2021) highlighted the impact of extraction solvent on extract potency, aligning with our observations.

Statistical analysis indicating significant differences among zones of inhibition (ZOI) of different concentrations of *Dillenia* sp. extracts against *E. coli* is presented in Table 2.

The analysis of variance (ANOVA) results indicated significant differences among different concentrations of *Dillenia* sp. bark extract within the decoction extraction method. This suggested that at least one extract concentration had a significantly different mean affecting the zone of inhibition compared to the others.

These subsequent post-hoc test results confirmed that the antibacterial activity indicated by Zone of Inhibition varied significantly with different concentrations of the extract. Specifically, the lower concentrations (100 ppm and 500 ppm) in the decoction extraction method produced significantly larger zones of inhibition compared to the 1,000 ppm treatment. This finding suggested that the antibacterial activity of the extracts is concentration-dependent, with 100 ppm and 500 ppm exhibiting stronger antibacterial effects against *E. coli* than the 1,000 ppm treatment. This suggested a non-linear relationship between concentration and antibacterial activity, wherein higher concentrations may not necessarily yield stronger inhibition. The plausible explanations for this phenomenon could include saturation effects, where the antibacterial agents may reach a plateau in their effectiveness at higher concentrations or even potential cytotoxicity at excessively high doses.

According to the study of Aladejana *et al.* (2024), the antibacterial activity of *Dillenia* sp. could be attributed to the presence of bioactive compounds, such as tannins, flavonoids, alkaloids, and phenolic compounds. These compounds possess antimicrobial properties that inhibit the growth of bacteria through various mechanisms.

Tannins are known for their ability to precipitate proteins, disrupting the cell membranes of bacteria and leading to cell death. Similarly, flavonoids and phenolic compounds exhibit antimicrobial effects by interfering with bacterial enzymes and metabolic processes crucial for their survival and replication. Some alkaloids found in *Dillenia* sp. likely contributed to its antibacterial activity by disrupting bacterial cell membranes or inhibiting specific cellular processes.

Moreover, the antibacterial properties of *Dillenia* sp. are inherent in this genus. Previous study conducted by Yakop *et al.* (2020) reported that *Dillenia suffruticosa* contains diethyl ether and ethyl acetate extracts exhibited by higher total phenolic content (TPC) and total flavonoid content (TFC), as well as superior antioxidant activities compared to other fractions. Furthermore, the methanol extract and its fractions displayed antibacterial activity against *Staphylococcus aureus*, with the diethyl ether fraction demonstrating comparable efficacy to the standard antibiotic streptomycin. In contrast, inhibition against *Bacillus subtilis* was observed only in certain fractions, while no inhibition was detected against *E. coli* and *Pseudomonas aeruginosa* (Yakop *et al.* 2020). In a study by Ilori *et al.* (2022), on *Dillenia indica* and *Ficus exasperata* extracts, it was found that both species exhibited antibacterial activity against *E. coli*, *S. aureus*, and *Streptococcus pyogenes*. However, the fact that *Dillenia* sp. demonstrated antibacterial activity against *E. coli* at different concentrations, suggesting its potential as a source of bioactive compounds, was first reported in this study.

CONCLUSION

This study confirmed that *Dillenia* sp. is a flowering plant indigenous to the Philippines, elucidating key characteristics, such as morphology and habitat. *Dillenia* sp. investigated in this study was identified as *Dillenia philippinensis*, which bark extracts possess antibacterial activity against *Escherichia coli*, with varying effectiveness depending on concentration and treatment method. Lower concentrations generally exhibited a larger Zone of Inhibition (ZOI), indicating a concentration-dependent response.

Statistical analysis revealed significant differences among different concentrations of bark extract in the decoction extraction method, indicating varying mean effects. Post-hoc test further confirmed that concentrations of 100 ppm and 500 ppm were significantly more effective than 1,000 ppm in the decoction extraction method, suggesting an optimal range of effectiveness between 100 ppm and 500 ppm.

The study highlighted the potential of *Dillenia* sp. bark extracts as antibacterial agents against *E. coli*, with concentrations between 100 ppm and 500 ppm. The findings from this study support the utilization of *Dillenia* sp. as a source of alternative medicine by the indigenous people in the Philippines.

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