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## Original Research Article

### Exploring the Phytochemistry of *Hanguana malayana* (Jack) Merr. from Terengganu Freshwater Swamp in Malaysia

Razanah Ramya<sup>1\*</sup>, Rashidi Othman<sup>2</sup>, Farah Ayuni Mohd Hatta<sup>3</sup><sup>1</sup>Institute of the Malay World and Civilization, The National University of Malaysia, 43600 UKM Bangi, Selangor, Malaysia<sup>2</sup>Herbarium Lab Unit, Department of Landscape Architecture, Kulliyyah of Architecture and Environmental Design, International University of Malaysia, 53100 Kuala Lumpur<sup>3</sup>Institute of Islam Hadhari, The National University of Malaysia, 43600 UKM Bangi, Selangor, Malaysia

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#### ABSTRACT

A freshwater shrub produces a wide range of bioactive compounds, and interest in utilizing its phytochemistry is growing rapidly. Meanwhile, exploiting phenolic compounds for the natural product industry could lead to more sustainable biochemistry studies. This study aimed to assess the phytochemistry of *Hanguana malayana* (Jack) Merr. from Terengganu freshwater swamps as potential pharmaceutical ingredients. Volatile compounds (GCTOF-MS analysis), phenolic acid profiles (HPLC analysis), and antimicrobial activities were determined in *H. malayana* leaf samples collected from freshwater swamp or heath forest in Terengganu, Malaysia. Using different solvents such as hexane, butanol, ethyl acetate, and ethanol extract, alkaline extraction was carried out. The GCTOF-MS findings determined 20 compounds from various chemical groups; nonetheless, just two phenolic compounds were found in the ethyl acetate (phenol and 2,4-bis(1,1-dimethylethyl)- (C<sub>14</sub>H<sub>22</sub>O)) and ethanol extracts (phenol (C<sub>6</sub>H<sub>6</sub>O)). The total phenolic compound content was 477.98±6.02 µg GAE/g DW. The highest phenolic acids were also identified, namely caffeic acid (3375.23 µg/g DW), coumaric acid (794.68 µg/g DW), ferulic acid (1333.33 µg/g DW), 2-coumaric acid (124.43 µg/g DW), as well as 3-coumaric acid (100.45 µg/g DW). The findings demonstrated that *H. malayana* antibacterial has strong antibacterial effects against *S. aureus*, *S. epidermidis*, *E. coli*, MRSA, and *P. aeruginosa*, as well as its antifungal activity against *C. albicans*, *Fusarium* sp., and *M. gypsum*. Therefore, *H. malayana* may have the potential to act as a source of valuable pharmaceuticals due to its phytochemical ingredients, which, at the same time, will be a vital aspect of ecological resilience.

**Keywords:** *Hanguana malayana*, Freshwater, Aquatic plants, Phenolic, Heath forest, Antimicrobe, Swamp

#### Introduction

*Hanguana malayana* (Jack) Merr., also known as Bakong, is a perennial herbaceous species indigenous to Sri Lanka, Southeast Asia, and Palau.<sup>1</sup> It is an exclusive genus within the family Hanguanaceae.<sup>2</sup> This genus must be utilized for a prevalent, extensive, helophytic, and stoloniferous species.<sup>3</sup> *H. malayana* is a sizable colonial dioecious helophyte that generates enormous spongy stolons and reproduces through both sexual and asexual means. It is a flora species found in open lowlands adjacent to the muddy banks of substantial rivers, the peripheries of freshwater bodies, and freshwater swamp forests.<sup>4</sup> In 2010, *H. malayana* was redefined as a habitat-specific colonial freshwater helophyte, with fourteen additional species identified from Peninsular Malaysia, Sarawak, Sabah, and Singapore. Meanwhile, the *Hanguana thailandica* species was identified in Thailand,<sup>5</sup> which markedly differs from *H. malayana*, as it is a forest mesophyte lacking stolons. Meanwhile, *H. malayana* is a semiaquatic marginal species characterized by an extensive stolon network.

\*Corresponding author. E mail: [razanah.ramya@ukm.edu.my](mailto:razanah.ramya@ukm.edu.my)

Tel: +60-135978577

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In Indonesia,<sup>2</sup> two new species of Hanguana were identified: *Hanguana karimatae* from Karimata Island in the West Kalimantan province and *Hanguana nana* from the Central Kalimantan province. Finally, *Hanguana nitens* was documented for the first time in Singapore and is undoubtedly indigenous.<sup>3</sup>

Past studies have documented various uses of *H. malayana*, particularly by local indigenous tribes worldwide. Aside from utilizing Bakong fibers as an alternative pulp source for papermaking,<sup>1</sup> many indigenous tribes have been adopting the plant for medicinal purposes.<sup>6</sup> For instance,<sup>7</sup> reported the use of *H. malayana* as an antimalarial and antibacterial drug in Papua Island, Indonesia, and the Philippines. Meanwhile, the Tambaka (Kadazandusun) and Murut tribes in Sabah, Malaysia, use the entire *H. malayana* plant for treating hemophilia and fungal skin infections<sup>7,8</sup>, as well as reducing bone pain.<sup>9</sup>

Hydroxybenzoic acids, hydroxycinnamic acids, and flavonoids are classifications of phenolic compounds derived from plants. These are the main sources of antioxidants in our diet, which are absorbable in the gut. Phenolic acids are a category of plant-based phenolics characterized by a phenol group and a structure that can stabilize through resonance, which facilitates hydrogen atom donation and imparts antioxidant capabilities through a radical scavenging mechanism.<sup>10</sup> Polyphenols in these medications are considered the principal agents involved for their biological efficacy and therapeutic effects.<sup>11</sup> Plant phenolics provide a significant opportunity, since over fifty percent of all anticancer pharmaceuticals recorded worldwide from the 1940s to 2006 originated from natural chemicals or their related compounds, with several clinical trials in progress.<sup>12</sup> Phenolic acids can be divided into two main groups: hydroxybenzoic acids and hydroxycinnamic acids.<sup>13</sup> The four most common hydroxycinnamic

acids are ferulic, caffeic, p-coumaric, and sinapic acids, whereas the four principal hydroxybenzoic acids are p-hydroxybenzoic, protocatechuic, vanillic, and syringic acids. Phenolic acids are in high demand in various industries because they serve as precursors for other vital bioactive substances that are frequently required in the healthcare, cosmetic, and food sectors. They are primarily employed as antioxidants, anticancer, antibacterial, dermatological, anti-inflammatory, food preservatives, antidiabetics, neuroprotectives, and in animal husbandry.<sup>10</sup>

Wetlands offer a distinctive environment for numerous medicinal flora. Wetland macrophytes serve as a significant source for the identification of therapeutically pertinent natural compounds. The majority of heath forest vegetation comprises plant families abundant in secondary metabolites and significant in ethnobotanical applications, particularly in medicinal uses, like Moraceae, Myrtaceae, Rubiaceae, Podocarpaceae, Sapotaceae, Guttiferae, Dipterocarpaceae, and Fagaceae.<sup>14</sup> The term “heath” (known as *kerangas* in Malaysia) originates from the Iban Dayak language, which signifies areas unsuitable for rice cultivation. The designation arises from the nutrient-poor soil composition that constitutes heath woods. Heath forest is designated as such due to its unique vegetation in Sarawak.<sup>15</sup> The hue of heath forest soil is light grey, which is attributed mostly to organic matter and iron oxides as the principal pigmenting agents. The heath forest soil is sandy, nutrient-deficient, and highly acidic. The pH level of waters in heath woods is approximately 4, which often establishes adverse living conditions, particularly for aquatic ecosystems.<sup>14</sup> Notwithstanding these benefits, the health-enhancing phytochemicals and bioactivities of several macrophytes are still predominantly uninvestigated. Heath forests demonstrate vulnerability and responsiveness to ecological and anthropogenic disruptions.<sup>16</sup> Agricultural operations are prohibited on heath forest lands. The ecology is susceptible to damage and challenging to restore once disrupted. The exposure of heath woods will lead to the development of arid savannahs.<sup>15</sup> Nevertheless, minimal pharmacological research has been conducted to substantiate the capabilities. Thus, the study's objective was to evaluate the phytochemistry of *Hanguana malayana* (Jack) Merr. from Terengganu freshwater wetlands as a possible medicinal component and natural product for industry through the highlighted objectives: firstly, to identify compounds through Gas Chromatography-Time-of-Flight Mass Spectrometry (GCTOF-MS) qualitatively; secondly, to identify the total phenolic content of *H. malayana* leaf extract; and thirdly, to assess the antimicrobial properties of *H. malayana* leaf extract.

## Materials and Methods

### Preparation of plant samples

The leaves of *Hanguana malayana* (Jack) Merr. (*bakung air*) were collected from a heath forest in Kampung Jambu Bongkok, Marang, Terengganu, Malaysia, situated at 4°55'N, 103°21'E. The specimen was collected in October 2023. The specimen was dispatched and verified [HM\_HERB\_2017] by the Herbarium Lab Unit, Kulliyah of Architecture and Environmental Design at the International Islamic University Malaysia to confirm its details. The leaves were gathered at random, sanitized, and then dried using freeze-drying. Subsequently, they were pulverized into a fine powder and kept at -20 °C for future analysis.

### Alkaline filtration

Approximately 100 mL of 2 M NaOH was used to prepare 10 g of freeze-dried powdered substance for alkaline filtration. The sample was subjected to heating in an oven at 60 °C for 12 hours prior to chilling to 20 °C. The alkaline extract was pH-adjusted to 2 using hydrochloric acid (HCl), which made hemicellulose precipitation easier. After a few tweaks, including adding a funnel separator, the last leaf extracts were treated using hexane, butanol, ethyl acetate, and ethanol by following the approach of.<sup>17</sup> The extracts were later dried using a rotary evaporator set to 45 °C. For subsequent analysis, the *H. malayana* crude extract was mixed with 5 mL of methanol and then kept frozen at -20 °C.

### Sample evaluation by Gas Chromatography-Time-of-Flight Mass Spectrometry (GCTOF-MS)

The leaf extract of *H. malayana* was subjected to qualitative analysis using GCTOF-MS (Agilent 7890 system) equipped with a capillary column (30 m × 0.25 mm, 0.25 µm) utilizing the modified approach of.<sup>18</sup> Through a purge duration of one minute, a 1 µL sample was injected using the splitless approach. Four minutes was the specified solvent delay. Helium served as the carrier gas, with a flow rate of 1.0 mL min<sup>-1</sup>. The column temperature was initially established at 80 °C for 2 minutes, thereafter, elevated to 80 °C at a rate of 5 °C per minute, followed by an increase to 250 °C at a rate of 10 °C per minute. The input temperature was 220 °C, whereas the detector was calibrated to 340 °C. The time-of-flight mass spectrometer recorded data across a range of masses from 50 to 1000 m/z, taking 1 spectrum every second. The mass spectra with more than 90% matching were then contrasted to those found in the NIST library (NIST 14) and other published data to determine the peaks.

### Sample evaluation of total phenolic content

The total phenolic content was assessed with the Folin-Ciocalteu method.<sup>19</sup> A solution was formed by combining 90 µL of Folin-Ciocalteu reagent with 20% deionized water. The sample was divided into wells using a 96-well flat-bottom microplate. Next, a 1.0 mg/g DW specimen was incubated at ambient temperature for five minutes after being diluted in distilled water (1000 µg/mL). Furthermore, 90 µL of sodium carbonate was mixed with 7.5% w/v deionized water, and the mixture was left at room temperature for 2 hours. A TECAN microplate reader measured the absorption of the extract, with the standards established at a wavelength of 725 nm and using a blank sample as a reference.

### Sample evaluation by High-Performance Liquid Chromatography (HPLC)

Utilizing the Agilent 1200 series LC quick resolution system, the HPLC analysis of phenolic acids was performed (Agilent Technologies, Palo Alto, CA, USA). The design was derived from<sup>20</sup> and included a diode array detector (DAD), compact vacuum chambers for air extraction, a temperature-regulated zone for the column, and an automatic injection system with a dual-pump mechanism. The diode array detector was used together with a Zorbax SB-C18 column (Eclipse 100 × 2.1 mm, 1.8 µm). For the analysis, two mobile phases were utilized: 1% formic acid in water/acetonitrile (90:10 v/v) designated as Phase A and acetonitrile as Phase B. A linear gradient elution was used as follows: from 0 to 20 minutes, there was a linear increase from 0% B to 40% B; from 20 to 25 minutes, there was a linear increase from 40% B to 60% B; from 25.10 to 35 minutes, there was a constant 100% B; and from 35.10 to 40 minutes, there was an isocratic condition maintained at 0% B. A temperature of 25 °C was established for the column, with a flow rate of 0.4 mL/min and an injection volume of 20 µL. Sigma-Aldrich provided the phenolic acid standards, which included trans-p-coumaric acid, caffeic acid, ferulic acid, 2-coumaric acid, 3-coumaric acid, vanillic acid, and 4-hydroxybenzoic acid.

### Sample evaluation of antimicrobial activity

The antibacterial capabilities of *H. malayana* were assessed in relation to five gram-negative bacterial strains: *Staphylococcus aureus*, Methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, and *Escherichia coli*. The Microbiology Laboratory of the International Islamic University Malaysia supplied the test isolates. Muller Hinton (MH) broth medium was utilized as a nutrition source to promote bacterial growth for inoculum production. The strains were painstakingly inoculated on the MH agar medium using the agar well diffusion method.<sup>21</sup> The minimal inhibitory concentration (MIC) was found for extracts that exhibited growth inhibition exceeding 7 mm. It was subsequently reassessed after incubating these zones at 37 °C for 24 hours. The sample's MIC value was defined as the lowest concentration that impeded development.

### Statistical analysis

The concentration of the phenolic acid extract was checked three times, and the results are shown as mean ± standard deviation values. The

XLSTAT-Pro (2014) software (Addinsoft, Paris, France) was used to run a one-way variance test called ANOVA and to carry out the Turkey test. These tests showed that the differences in the mean were statistically significant at 99% confidence levels ( $p<0.0001$ ).

## Results and Discussion

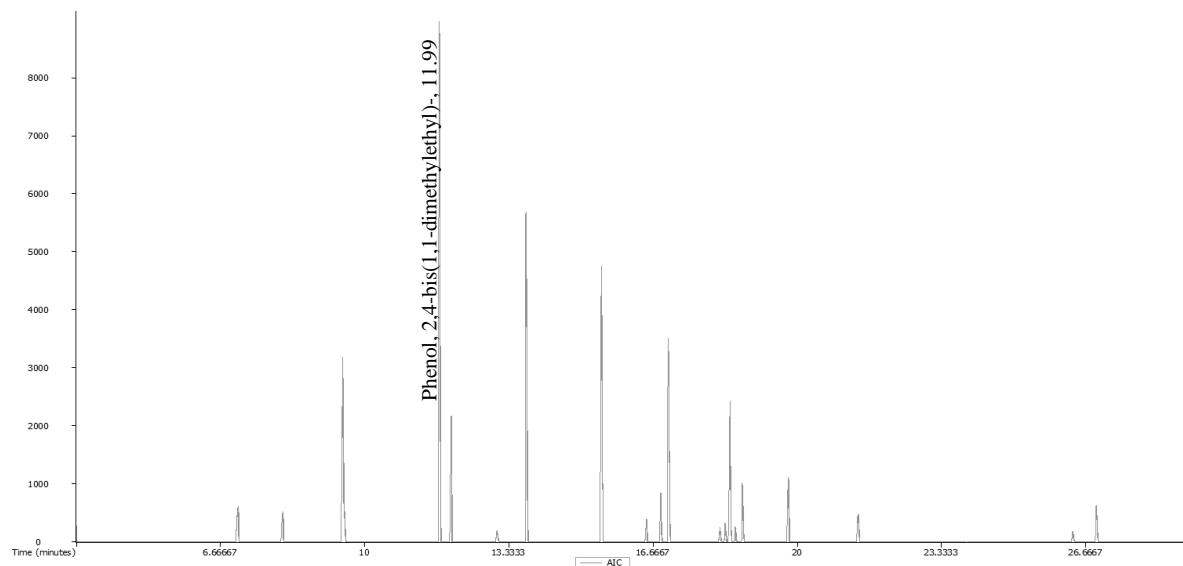
### Examination of volatile chemicals using Gas Chromatography-Time-of-Flight Mass Spectrometry (GCTOF-MS)

The GCTOF-MS analysis of *H. malayana* leaves identified 20 compounds from various chemical groups, including their specific

mass, formula, and retention time, by referencing the library data of the National Institute of Standards and Technology (NIST). Table 1 presents the findings, specifying a few extracts identified in petroleum ether, ethyl acetate, and ethanol. The result was established based on a resemblance surpassing 90% with NIST library data. No phenolic compounds were identified in any of the extracts (hexane, ethyl acetate, and butanol), with just two phenolic compounds recognized in ethyl acetate (phenol, 2,4-bis(1,1-dimethylethyl)- ( $C_{14}H_{22}O$ )) and ethanol (phenol ( $C_6H_6O$ )) from *H. malayana* (Figure 1).

**Table 1:** Volatile compounds identified in different solvents of *H. malayana* leaves extract

Solvent extract	Compound	Exact mass	Formula	Retention time (min)
Hexane	Isobutylamine	73.139	$C_4H_{11}N$	21.40
	2-Decanol	158.285	$C_{10}H_{22}O$	10.81
	Pentadecane	212.421	$C_{15}H_{32}$	11.75
Ethyl acetate	Phenol, 2,4-bis(1,1-dimethylethyl)-	206.167	$C_{14}H_{22}O$	11.99
Ethanol	Tetronic acid	100.07	$C_4H_4O_3$	11.93
	Oxalacetic acid	132.07	$C_4H_4O_5$	8.36
	6-Aminouracil	127.10	$C_4H_5N_3O_2$	6.51
	Phloretin	274.27	$C_{15}H_{14}O_5$	14.83
	2,4,4-Trimethyl-1-pentanol	130.231	$C_8H_{18}O$	14.11
	Glycylglycine	132.11	$C_4H_8N_2O_3$	18.42
	2-Acetyl-2-methyltetrahydrofuran	128.17	$C_7H_{12}O_2$	9.24
	Allyl glycidyl ether	114.14	$C_6H_{10}O_2$	15.47
	Diethyl phthalate	222.24	$C_{12}H_{14}O_4$	13.03
	Phenol	94.11	$C_6H_6O$	4.82
	n-Hexadecanoic acid	256.43	$C_{16}H_{32}O_2$	16.88
	Furyl hydroxymethyl ketone	126.11	$C_6H_6O_3$	6.24
	9,12,15-Octadecatrienal	262.43	$C_{18}H_{30}O$	18.60
	Oleic Acid	282.46	$C_{18}H_{34}O_2$	18.57
	2-Furancarboxaldehyde, 5-methyl-	110.11	$C_6H_6O_2$	4.66
	Isophytol	296.53	$C_{20}H_{40}O$	16.75



**Figure 1:** GCTOF-MS Spectra from *H. malayana* ethyl acetate extract

Other compounds were also found in the hexane extract (isobutylamine, 2-decanol, and pentadecane) (Figure 2). Finally, fifteen compounds were found in ethanol extract (Tetronic acid, Oxalacetic acid, 6-Aminouracil, Phloretin, 2,4,4-Trimethyl-1-pentanol, Glycylglycine, 2-

Acetyl-2-methyltetrahydrofuran, Allyl glycidyl ether, Diethyl phthalate, Phenol, n-Hexadecanoic acid, Furyl hydroxymethyl ketone, 9,12,15-Octadecatrienol, Oleic Acid, 2-Furancarboxaldehyde, 5-methyl-, and Isophytol) (Figure 3).

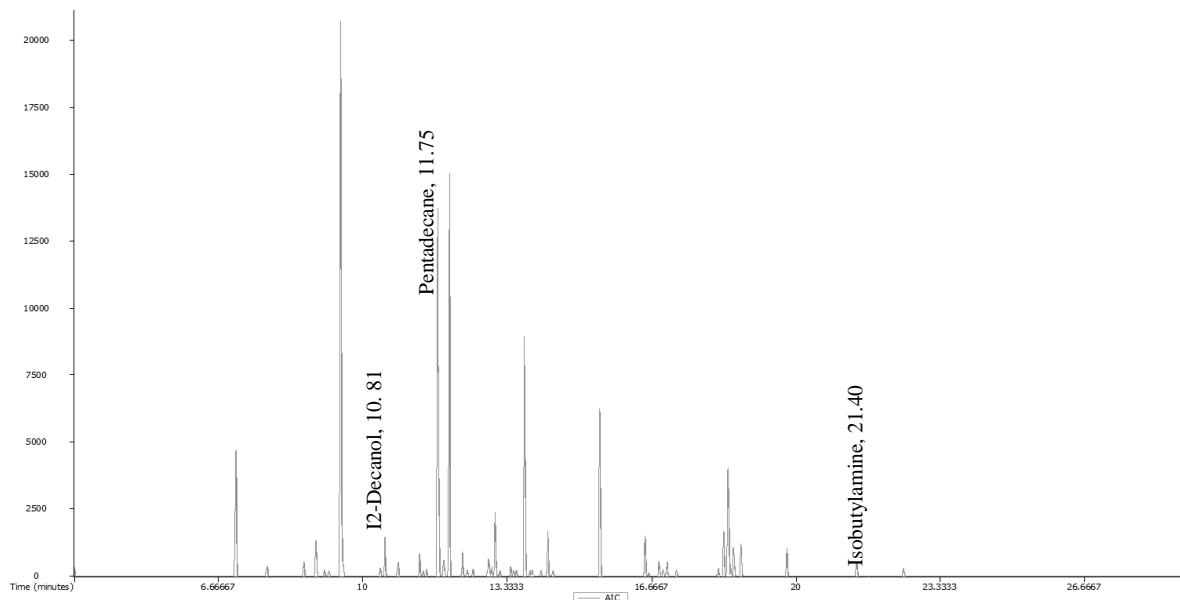


Figure 2: GCTOF-MS Spectra from *H. malayana* hexane extract

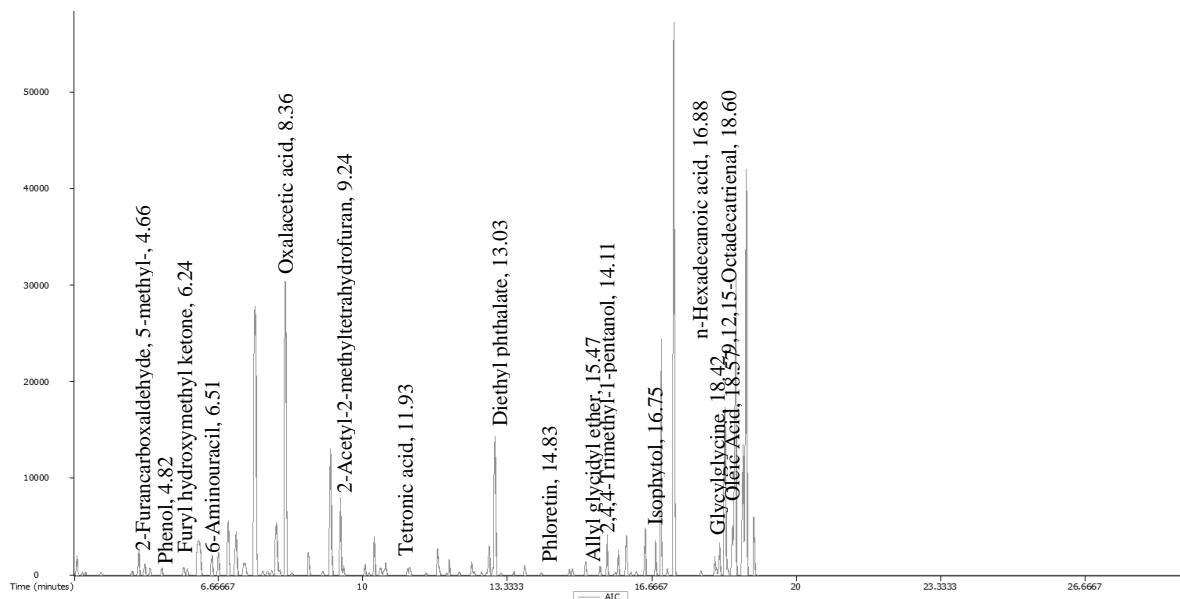


Figure 3: GCTOF-MS Spectra from *H. malayana* ethanol extract

#### Assessment of total phenolic and specific phenolic acids content

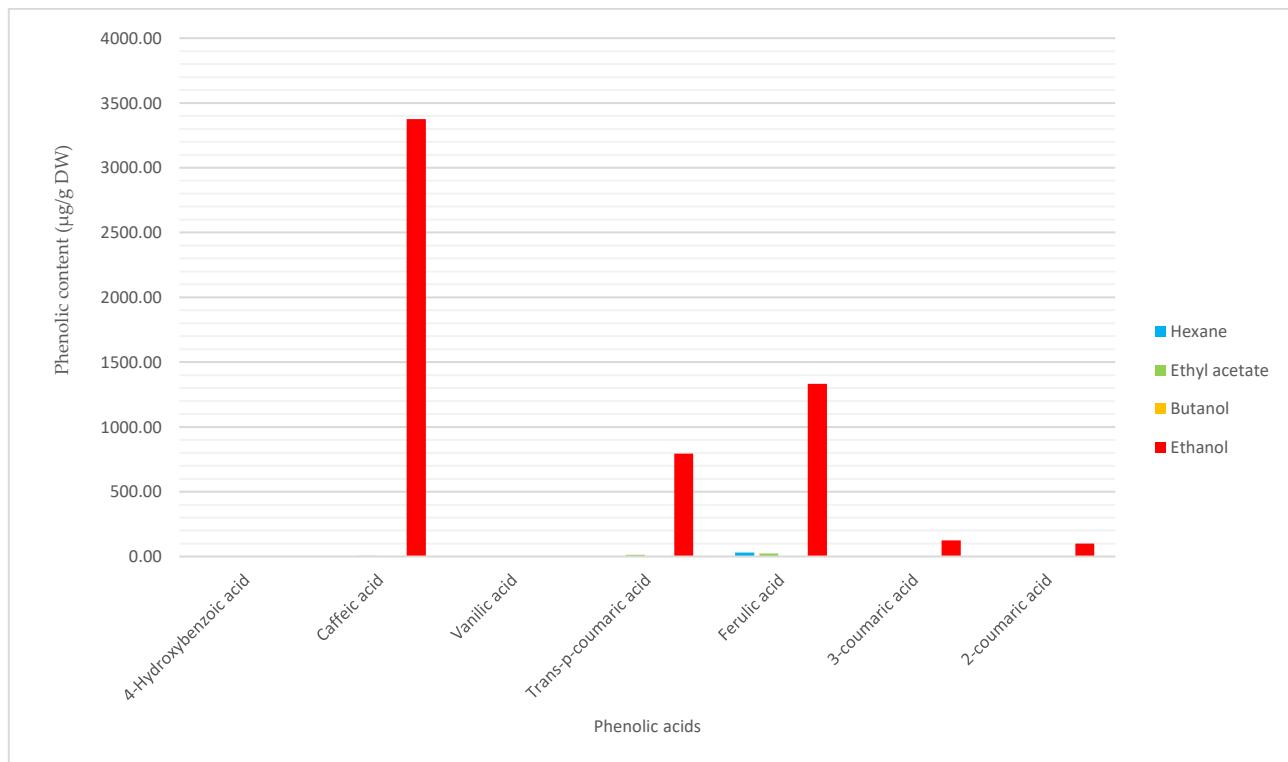
To ascertain the total phenolic content (TPC) of *H. malayana*, the Folin-Ciocalteau technique was employed. The TPC value showed that *H. malayana* had  $477.98 \pm 6.02$   $\mu\text{g GAE/g DW}$ . Figure 4 illustrates the HPLC graph of the *H. malayana* alkaline extract which demonstrates that ethanol has the most phenolic compounds compared to butanol, hexane, and ethyl acetate. The findings reveal that the predominant phenolic acids were caffeic acid (3375.23  $\mu\text{g/g DW}$ ), coumaric acid (794.68  $\mu\text{g/g DW}$ ), ferulic acid (1333.33  $\mu\text{g/g DW}$ ), 2-coumaric acid (124.43  $\mu\text{g/g DW}$ ), and 3-coumaric acid (100.45  $\mu\text{g/g DW}$ ). Four phenolic acids were detected in the butanol extract, namely

hydroxybenzoic acid (3.64  $\mu\text{g/g DW}$ ), vanillic acid (4.46  $\mu\text{g/g DW}$ ), coumaric acid (1.50  $\mu\text{g/g DW}$ ), and ferulic acid (3.54  $\mu\text{g/g DW}$ ). Hexane and ethyl acetate also exhibited the presence of analogous phenolic acids, including caffeic acid, coumaric acid, and ferulic acid. Generally, three predominant phenolic acids were identified in *H. malayana* leaves: coumaric acid, caffeic acid, and ferulic acid across all solvents.

The primary benefit of utilizing phenolic acids lies in their capacity for metabolism by natural bacteria, thus offering a crucial alternative to synthetic compounds that are detrimental to the environment.<sup>10</sup> Moreover, n-hexadecanoic acid has been reported to possess multiple

properties: enzyme inhibition, antibacterial, antiatherosclerotic, antiandrogenic, anticancer, antitumor, anti-inflammatory, antifungal, antioxidant, antiandrogenic, hypocholesterolemic, hemolytic, nematicidal, pesticidal, 5-inhibitory, powerful mosquito larvicidal, rheumatic symptom alleviation, antiandrogenic and antimicrobial activities.<sup>22</sup> Oleic acid can lower blood pressure and possesses antibacterial, antimicrobial, and anti-inflammatory effects.<sup>23</sup> The phytochemical examination of the extracts indicated that phenolic and flavonoid chemicals might suppress bacterial protein production.<sup>24</sup>

The TPC was calculated to be  $13.94 \pm 0.97$  mg GAE/g extract. The TFC was determined to be  $5.81 \pm 0.18$  mg CAE/g extract. In GC-MS analysis, 20 compounds were identified in ethanolic leaf extract.<sup>6</sup> A similar result was observed in this study whereby twenty compounds were found; however, only two phenolic chemicals were spotted in ethyl acetate, specifically phenol and 2,4-bis(1,1-dimethylethyl)-(C<sub>14</sub>H<sub>22</sub>O), and in ethanol, namely phenol (C<sub>6</sub>H<sub>6</sub>O), from *H. malayana*.



**Figure 4:** Individual phenolic acid content from *H. malayana* leaves extract

<sup>25</sup>indicated that *H. malayana* possesses significant levels of ferulic acid and sinapic acid, which demonstrate antioxidant, anti-inflammatory, and anti-aging properties. This suggests that *H. malayana* warrants increased consideration as an alternate treatment for rheumatoid arthritis. Ferulic acid has been reported to facilitate the development process by mitigating the effects of harmful free radicals on the skin; it is also said to protect the skin from sun damage. Another notable aspect is its apparent synergistic effect alongside other cancer prevention agents such as vitamins C and E. 4-Hydroxybenzoic acid serves as the foundation for the synthesis of its esters, commonly referred to as parabens. Parabens are widely used in cosmetic and medicinal products. Vanillic acid is a type of phenolic acid that can be found in some types of vanilla and other plant extracts, which could serve as a flavoring or fragrance ingredient that produces a tempting, smooth smell. It can also be a metabolic byproduct during the two-step bioconversion process of turning ferulic acid into vanillin. Additionally, vanillic acid stops 5'-nucleotidase from working in a specific way.<sup>7</sup>

Preserving riparian habitats is crucial for enhancing water quality and sustaining ecological integrity. A robust river ecology further facilitates the protection of species. Nonetheless, environmental degradation caused by chemical contaminants and physical alterations renders river ecosystems increasingly susceptible to decline and loss of biodiversity.<sup>25</sup> The number of phenolic compounds that plants make varies with the type of stress they are under and how they react. Plants make phenolic acids and flavonoids when they experience a lot of light stress. At first, temperature stress prompts plant cells to produce

osmoprotective compounds. Subsequently, thermal stress induces plant cells to synthesize antioxidant enzymes and chemicals, including flavonoids, tannins, and phenolic acids. Reactive oxygen species are produced when plants are exposed to salt, which causes oxidative stress.<sup>26</sup>

#### Analysis Antimicrobial Activity

The MIC values of *H. malayana* leaf extract against *S. aureus*, *S. epidermidis*, *E. coli*, MRSA, and *P. aeruginosa* are displayed in Table 2 meanwhile, the agar plate of antibacterial activity presented in Figure 5 and agar plate of antifungal activity presented in Figure 6.<sup>27,28</sup> A general overview of the results indicates that the most effective solvents for all solvent extracts were hexane and ethyl acetate. *S. aureus*, *E. coli*, and MRSA were detected with clear antimicrobial activity between the 4 and 10 mm inhibition zones. Meanwhile, the butanol extract detected *S. aureus* and *S. epidermidis* with inhibition zones ranging from 4 to 10 mm. *P. aeruginosa* showed less of an inhibition zone in all solvent extracts. The inhibition zone by fungal strains is presented in Table 3. All the extracts showed a slight effect on *C. albicans*, *Fusarium sp.*, and *M. gypseum*, with inhibition zones ranging from 1 to 3 mm. Except for butanol extract, there were moderate effects of antifungal activity for *C. albicans* (3-4 mm), and hexane extract for *M. gypseum* (3-4 mm). Based on the antimicrobial activity studied, both bacteria and fungi showed different effects on the *H. malayana* leaf and solvent extract. However, all the tests showed the inhibition effect.

**Table 2:** Antibacterial activity from *H. malayana* leaf

Solvents	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>E. coli</i>	<i>MRSA</i>	<i>P. aeruginosa</i>
Hexane	++	~	++	++	~
Ethyl acetate	++	~	++	++	~
Butanol	++	++	~	~	-

Notes<sup>27,28</sup>:

- : No antimicrobial activity, inhibition zone of sample &lt; inhibition zone of ethanol +1 mm

~ : Slight antimicrobial activity, inhibition zone of sample 1-3 mm &gt; inhibition zone of ethanol

+ : Moderate antimicrobial activity, inhibition zone of sample 3-4 mm &gt; inhibition zone of ethanol

++ : Clear antimicrobial activity, inhibition zone of sample 4-10 mm &gt; inhibition zone of ethanol

+++ : Strong antimicrobial activity, inhibition zone of sample +10 mm &gt; inhibition zone of ethanol

**Table 3:** Antifungal activity from *H. malayana* leaf

Solvents	<i>C. albicans</i>	<i>Fusarium sp.</i>	<i>M. gypseum</i>	<i>P. chrysosporium</i>	<i>A. niger</i>
Hexane	~	~	+	-	-
Ethyl acetate	~	~	-	-	-
Butanol	+	~	~	-	-

Notes<sup>27,28</sup>:

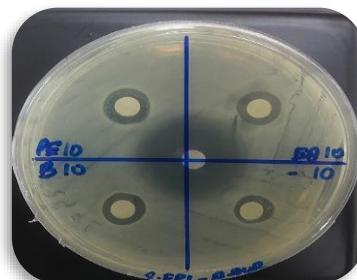
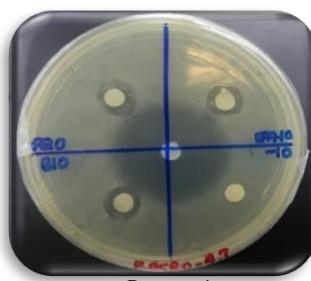
- : No antimicrobial activity, inhibition zone of sample &lt; inhibition zone of ethanol +1 mm

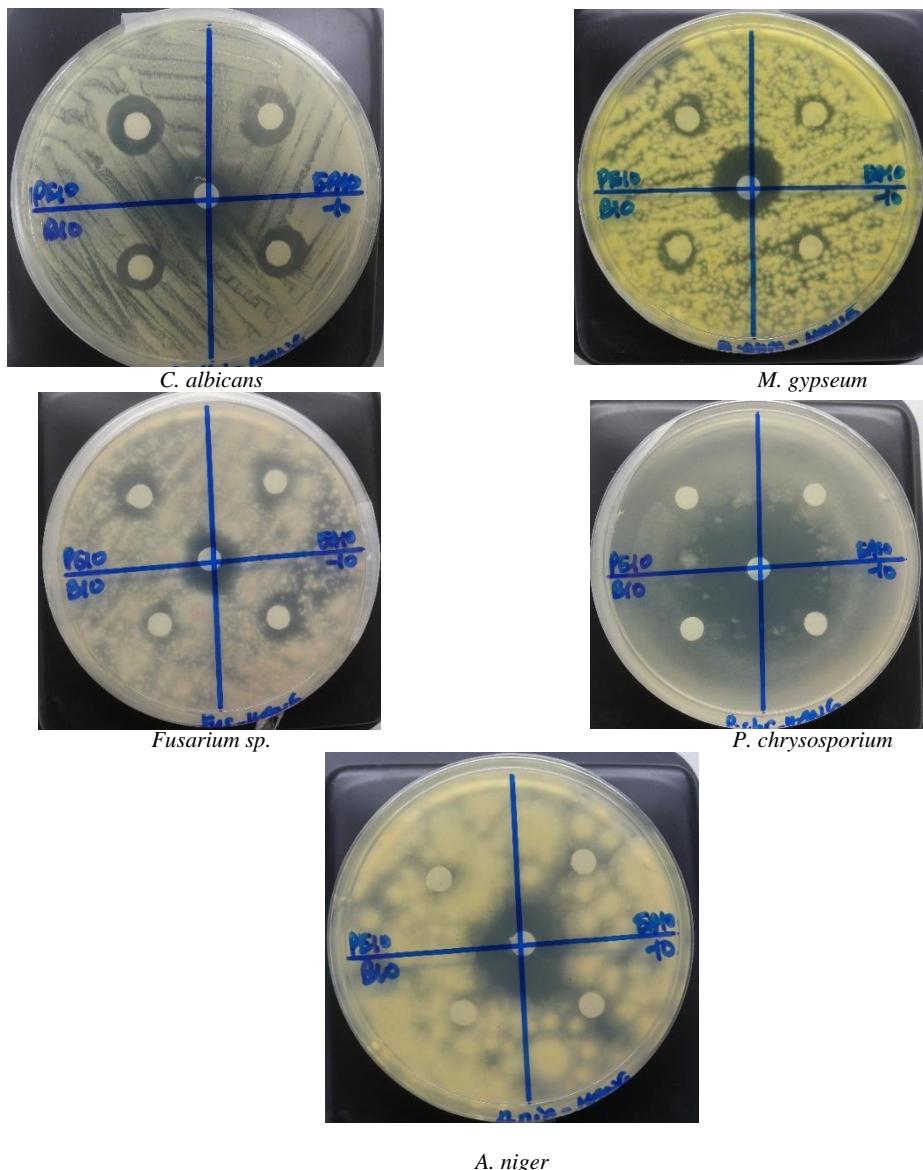
~ : Slight antimicrobial activity, inhibition zone of sample 1-3 mm &gt; inhibition zone of ethanol

+ : Moderate antimicrobial activity, inhibition zone of sample 3-4 mm &gt; inhibition zone of ethanol

++ : Clear antimicrobial activity, inhibition zone of sample 4-10 mm &gt; inhibition zone of ethanol

+++ : Strong antimicrobial activity, inhibition zone of sample +10 mm &gt; inhibition zone of ethanol

*S. aureus**S. epidermidis**E. coli**Methicillin-resistant Staphylococcus aureus (MRSA)**P. aeruginosa***Figure 5:** Agar well diffusion assay showing the antibacterial activity of *H. malayana* leaf



**Figure 6:** Agar well diffusion assay showing the antifungal activity of *H. malayana* leaf

## Conclusion

The current study concluded that *Hanguana malayana* (Bakong) possesses the potential to serve as a source of valuable pharmaceuticals due to its antibacterial efficacy against *S. aureus*, *S. epidermidis*, *E. coli*, MRSA, and *P. aeruginosa*, in addition to its antifungal properties against *C. albicans*, *Fusarium* sp., and *M. gypseum*. The quantification of biologically active compounds in the extracts, including predominant caffeic acid (3375.23 µg/g DW), coumaric acid (794.68 µg/g DW), ferulic acid (1333.33 µg/g DW), 2-coumaric acid (124.43 µg/g DW), and 3-coumaric acid (100.45 µg/g DW), substantiated the existence of antimicrobial phytochemicals in these plants. This species may offer natural ingredients to substitute the synthetic compounds presently employed to treat disorders resulting from infections transmitted by water. Henceforth, the present study illustrates that aquatic plants may serve as a significant source of extracts and bioactive chemicals for the development of novel products. Additional study is necessary to investigate these pigments' possible uses, especially in food systems, to help improve and make better use of them in food, medicine, and clothing industries.

## Conflict of Interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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