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Proximate Analysis, Phytochemical Screening, and Antibacterial Activity of Wild Sampalok-Sampalukan (*Moeroris Amara*) In Selected Barangays of Aliaga, Nueva Ecija

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Abstract. The global challenge of microbial resistance to antimicrobial drugs has prompted the search for new agents from natural sources like medicinal plants. This study focuses on Moeroris amara, a traditional Indian medicinal plant, to assess its antibacterial potential against *Staphylococcus aureus* (ATCC 6538) and *Escherichia coli* (ATCC 25922), common causes of skin and urinary tract infections, respectively. Samples were collected from Aliaga, Nueva Ecija, and ethanol (95%) was used to extract the plant components. Proximate analysis determined the nutritional composition, while phytochemical screening assessed Total Phenolic Content (TPC) and Total Flavonoid Content (TFC). Disk diffusion method was used to assess the antibacterial activity. The analysis revealed *Moeroris amara* leaves contain 7.6% ash, 5.4% crude fat, 15.0% crude fiber, 13.6% moisture and 19.30% crude protein, suggesting potential health benefits. High levels of phenolic and flavonoid compounds were found, with TPC at 260.65 mg GAE/g and TFC at 193.65 mg RHE/g. The ethanolic leaf extracts exhibited significant inhibitory activity against both bacterial strains. In conclusion, *M. amara* shows promise as a natural source for antimicrobial agents due to its significant nutritional and antibacterial properties. Its high phenolic and flavonoid content contribute to antioxidant and antibacterial activities. Further research is recommended to explore its potential as functional foods and natural antimicrobial agents.

Keywords: Antibacterial activity; *Moeroris amara; Phyllanthus amarus;* Phyotchemical screening; Proximate composition

1. Introduction

The accelerating threat of antibiotic resistance, coupled with a sluggish pipeline for new antimicrobial agents, has been a persistent concern (Elisha et al., 2017). *Staphylococcus aureus*—especially MRSA—frequently causes dangerous skin

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infections and poses significant treatment challenges (Taylor & Unakal, 2023). Meanwhile, *Escherichia coli* continues to be a leading culprit behind diarrheal illness, urinary tract infections, bloodstream infections, and neonatal meningitis (Poolman, 2017). The 2022 WHO Global Antimicrobial Resistance and Use Surveillance System (GLASS) report underscores rising resistance, notably to third-generation cephalosporins in *E. coli* and methicillin in *S. aureus*. Alarmingly, by 2020, one-fifth of urinary infections caused by *E. coli* had decreased responsiveness to commonly used antibiotics such as ampicillin, co-trimoxazole, and fluoroquinolones.

Moeroris amara (*Phyllanthus amarus* Schum. & Thonn.), known locally as "Sampalok-sampalukan," is a tropically widespread medicinal plant traditionally employed to combat bacterial infections. Research, such as that by Mazumder et al. (2006), confirms its methanolic extract exhibits concentration-dependent antibacterial activity, particularly against gram-negative bacteria, largely attributed to the compound phyllanthin. This study aims to evaluate the antibacterial efficacy of *Moeroris amara* while also profiling its nutritional and phytochemical components—thereby underpinning its traditional uses and exploring its potential role in countering antibiotic resistance.

2. Methodology

2.1. Collection, Identification and Preparation of Plant Sample

Moeroris amara specimens were collected in the vicinity of Barangay Santa Monica and Magsaysay in Aliaga, Nueva Ecija. The collected plants were examined based on their morphological characteristics and were subsequently sent to Central Luzon State University in Science City of Muñoz, Nueva Ecija, for formal identification. Each sample was meticulously rinsed under running tap water to ensure the thorough removal of contaminants. The leaves were then carefully separated, subjected to an additional rinse, and strained. Following this, the leaves underwent an air-drying process for three days before being pulverized into a fine powder with the aid of a blender.The maceration and extraction followed the method of Buhian et al. (2016) with modifications. Crushed leaves (100 g) were soaked in 95% ethanol (1:10 w/v) for 72 hours. The mixture was decanted, filtered, and the filtrate concentrated under reduced pressure at 45 °C using a rotary evaporator at the Philippine Rice Research

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Institute, yielding about 80 mL of extract. The extract was then refrigerated for preservation.

2.2. Proximate Analysis

To determine the proximate composition of M. amara, powdered leaf samples (200 g) were submitted to the Department of Agriculture in San Fernando, Pampanga, for the quantification of crude fat, crude fiber, moisture, and ash content. In addition, a separate aliquot (10 g) was forwarded to the Philippine Rice Research Institute for the determination of crude protein content.

2.3. Phytochemical Screening

A 10 mL extract of M. amara leaves was sent to the Philippine Rice Research Institute for phytochemical screening. Total phenolic content (TPC) was determined using the modified Singleton et al. (1999) method, with absorbance measured at 760 nm and results expressed as mg gallic acid equivalent per gram of sample (mg GAE/g). Total flavonoid content (TFC) was assessed via a modified Bao et al. (2005) method, with absorbance read at 415 nm and results expressed as mg rutin hydrate equivalent per gram (mg RHE/g).

2.3. Antibacterial Activity

A 50 mL extract was sent to the Industrial Technology Development Institute (DOST), Taguig City, for antimicrobial testing using the disk diffusion method. *Staphylococcus aureus* (ATCC 6538) with Oxacillin and *Escherichia coli* (ATCC 25922) with Amikacin served as test organisms and positive controls. Bacteria were cultured in Tryptic Soy Broth, adjusted to 0.5 McFarland standard, and plated on Mueller-Hinton Agar. After pre-incubation, 10 µL of the extract was applied to sterile 10-mm filter paper discs, then placed on the plates alongside antibiotic discs. Plates were incubated overnight at 35 °C, and zones of inhibition were observed and recorded.

2.4.1. Reactivity Rating

The extent of antimicrobial activity was evaluated based on the diameter of the inhibition zone. A rating of 0 indicated no detectable zone around or under the specimen. A rating of 1 denoted slight activity, with some malformed or degenerated cells observed under the specimen. A rating of 2 signified mild activity, with the zone limited to the area beneath the specimen. Moderate activity, rated as 3, showed a zone extending 5 to 10 mm beyond the specimen,

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while severe activity, rated as 4, indicated a zone extending more than 10 mm beyond the specimen. Inhibitory activity was also qualitatively rated as follows: (+++) for complete inhibition, (++) for partial inhibition, (+) for slight inhibition, and (−) for no inhibitory effect.

3. Results and Discussion

3.1. Collection and Identification

The collected specimens were examined and provisionally identified based on their morphological characteristics, referencing the Florida Plant Atlas and relevant botanical literature. Notably, the classification of *Phyllanthus amarus* has been revised: in 2022 it was reclassified under the genus *Moeroris*, and is now accepted as *Moeroris amara*, with *Phyllanthus amarus* regarded as its basionym. Field observations indicated that *M. amara* thrives in shaded environments characterized by well-drained, moderately moist soils. Specimens were collected from Barangay Magsaysay and Barangay Santa Monica in Aliaga, Nueva Ecija. The plant presents as a small herbaceous species with slender, often basally branching stems exhibiting angular morphology. Leaves are alternately arranged, oblong to elliptical in shape, and range in coloration from green to light green. Both leaf surfaces and margins are smooth. Flowering structures bear six tepals surrounding seed-containing fruits, while the root system is distinctly woody and stout.

Figure 1. *Moeroris amara*

3.2. Proximate Analysis

The nutritional components of *M. amara* leaves were analyzed for crude fat, crude fiber, moisture, ash content, and crude protein.

Table 1. Nutritional composition of *Moeroris amara* leaves

COMPONENTS	CONTENT (g/100g)
% Crude fat	5.4 ± 0.4
% Crude fiber	15.0 ± 0.9
% Moisture content	13.6± 0.3

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% Ash content	7.6 ± 0.1
% Crude protein	19.30 ± 0.0

Lipids are essential energy sources, providing about 9 kcal/g. The leaves of *M. amara* showed a low crude fat content (5.4%), aligning with Igwe et al. (2010) at 6.03%, and higher than the 1.5% reported in *P. gomphocarpus* leaves (Bahari et al., 2014). The crude fiber content in *M. amara* leaves was 15.0%, significantly higher than the 2.40% from Achikanu et al. (2022), and differing from values by Umoh et al. (2011) and Igwe et al. (2010), possibly due to variations in drying methods. Moisture content was 13.6%, within the 8–14% range recommended by the British Pharmacopoeia (2011). This is slightly higher than the 10.10% reported by Okwute et al. (2015) and 9.57% in *P. niruri* (Adebisi et al., 2021). *M. amara* had a 7.6% ash content, indicating moderate mineral presence—lower than 10.58% (Achikanu et al., 2022), close to 6.80% (Igwe et al., 2010), and much lower than the 40% in *P. fraternus* (Ananias, 2021). Protein content was 19.30%, slightly lower than 23.97% (Achikanu et al., 2022), but far higher than the 0.88% in *P. fraternus* leaves and stems (Ananias, 2021), highlighting *M. amara*'s potential as a nutritional protein source (Okiki et al., 2015).

3.3. Phytochemical Screening

The average Total Phenolic Content (TPC) of *M. amara* leaf extract was determined to be 260.65 milligrams of Gallic Acid Equivalent per gram (mg GAE/g). Similarly, the average Total Flavonoid Content (TFC) of the sample was measured to be 193.65 milligrams of Rutin Hydrate Equivalent per gram (mg RHE/g). This reflects the concentration of phenolic and flavonoid compounds present in the sample, which are also recognized for their antioxidant properties and potential health-promoting effects.

Table 2. Total Phenolic Content and Total Flavonoid Content of *Moeroris amara* leaf extract

SAMPLE	VALUES	
	Total Phenolic Content (mg GAE/g)	Total Flavonoid Content (mg RHE/g)
Average	260.65	193.65

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Phenolic compounds offer various health benefits, including reduced risks of cancer, heart disease, and diabetes, and exhibit antibacterial, antiviral, anti-inflammatory, and anti-allergenic properties (Shahidi & Ambigaipalan, 2015). Bioactive compounds like phenols, flavonoids, and alkaloids are commonly found in herbs (Nahar et al., 2011). The study found that *M. amara*'s ethanolic leaf extract contains a high total phenolic content (260.65 mg GAE/g), exceeding those of other *Phyllanthus* species using different solvents (Zain & Omar, 2018; Rao, 2021), suggesting ethanol is more effective for extraction. Flavonoids, known for their antioxidant and therapeutic effects (Saeed et al., 2012; Panche et al., 2016), were also abundant in the extract (193.65 mg RHE/g). This value is higher than *P. emblica* fruit extracts (Li et al., 2022) and aqueous extracts reported by Nguyen et al. (2017), though lower than their methanolic extract. These findings highlight *M. amara*'s potential as a rich source of phenolics and flavonoids, depending on the extraction method used.

3.4. Antibacterial Activity

Phytochemical constituents, serving as secondary metabolites in plants, function as a defence mechanism against various microorganisms, insects, and herbivores. These bioactive compounds operate through diverse mechanisms and demonstrate antimicrobial properties (Thangavel et al., 2012). Flavonoids, for instance, are capable of inhibiting or eradicating numerous bacterial strains, inhibiting key viral enzymes like reverse transcriptase and protease, and eliminating certain pathogenic protozoans (Havsteen, 2002).

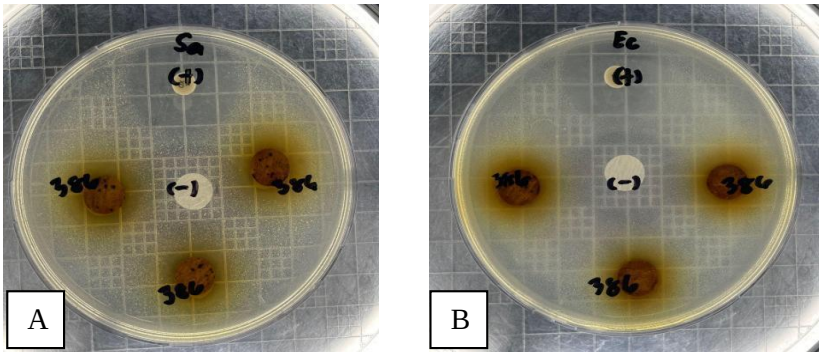

Figure 2. Disk diffusion assay for (A) *Staphylococcus aureus* (ATCC 6538) and (B) *Escherichia coli* (ATCC 25922)

The ethanolic leaf extract of *M. amara* showed complete inhibitory activity with moderate reactivity against *Staphylococcus aureus* (ATCC 6538), though less effective than Oxacillin. This aligns with Ospina et al. (2016), who observed

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similar inhibition using *P. salviifolius* essential oils. Against *Escherichia coli* (ATCC 25922), the extract also showed complete inhibition with mild reactivity, consistent with findings by Ogunlesi et al. (2009) and Senjobi et al. (2017). Notably, it outperformed *P. niruri* and *P. guajava* extracts, which showed no inhibition (Valle et al., 2015). These results suggest *M. amara* has notable antimicrobial potential, particularly against *S. aureus* and *E. coli*. Its pharmacological benefits—including antiviral and chemoprotective effects—have been noted by Patel et al. (2011). The high total phenolic (260.65 mg GAE/g) and flavonoid content (193.65 mg RHE/g) may contribute to its antibacterial activity (Cushnie & Lamb, 2005).

4. Conclusions

It is concluded that the proximate analysis of *Moeroris amara* leaves revealed a promising nutritional profile with moderate moisture (enhancing shelf stability), moderate lipids, significant dietary fiber, and notably high crude protein—suggesting its potential as a valuable dietary supplement. The ethanolic leaf extract stood out, showing elevated total phenolic and flavonoid contents known for antioxidant benefits. It also exhibited strong antibacterial activity against *Staphylococcus aureus* (ATCC 6538) and *Escherichia coli* (ATCC 25922), likely due to its phenolic and flavonoid compounds, indicating potential as a natural antioxidant and antibacterial agent.

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