

## CHEMICAL CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF *Mallotus nanus* AIRY SHAW (EUPHORBIACEAE)

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### ABSTRACT

This study characterizes the phytochemical profile and antibacterial activity of ethanolic leaf, stem, and root extracts of *Mallotus nanus* (*M. nanus*), a traditional medicinal plant endemic to Vietnam's Central Highlands. Leaf extract showed the highest total phenolic content ( $239.00 \pm 0.94$  mg GAE/g) and total flavonoid content ( $72.01 \pm 0.13$  mg QE/g), followed by stem and root extracts. UHPLC analysis identified eleven bioactive constituents (eight flavonoids and three phenolic acids) with organspecific distribution, with leaves enriched in phenolic acids and stems, and roots richer in flavonoids. All extracts inhibited *Staphylococcus aureus* and *Escherichia coli* in a concentration dependent manner, with leaf extract producing the largest inhibition zones at 100 mg/mL ( $21.00 \pm 0.00$  mm and  $22.25 \pm 0.35$  mm, respectively). Leaf extract also exhibited the strongest MIC/MBC values ( $6.25/25.00$  mg/mL for both strains), exceeding the activities reported previously for other *Mallotus* species. These findings provide the first UHPLC based profiling of *M. nanus* and support its leaves as a promising source of phenolic and flavonoidrich extracts for development of plantderived antibacterial agents.

**Keywords:** *Mallotus nanus*, antibacterial activity, flavonoid, phenolic acids, UHPLC.

### 1. INTRODUCTION

The rise in antibiotic-resistant bacteria and the adverse effects associated with many synthetic drugs have intensified the global focus on plant-derived natural products as promising alternatives for antimicrobial drug development (Okla *et al.*, 2021; Porras *et al.*, 2021; Ashraf *et al.*, 2023). Numerous medicinal plants demonstrate potent antimicrobial activities attributed to diverse phytochemicals. A wide array of plant metabolites—including flavonoids, phenolic acids, alkaloids, terpenoids, tannins, and coumarins—exhibit antibacterial properties through multiple mechanisms such as membrane disruption and enzyme inhibition (Adamczak, Ożarowski and Karpiński, 2020); Liang *et al.*, 2022). In Asian traditional medicine, plant preparations continue to play an important role in the prevention and treatment of various ailments, which underscores the need to validate and expand the pharmacological understanding of local medicinal flora (Niazi and Monib, 2024).

The genus *Mallotus* (Euphorbiaceae), known locally as “Ba bét” in Vietnam, is among the richest and most diverse genera within the family and comprises about 40 species endemic or native to Vietnam (Rivière *et al.*, 2010; Anh *et al.*, 2022). Species of this genus are widely used in traditional medicine for an array of indications including chronic hepatitis, gastrointestinal disorders, and

skin diseases. Extensive phytochemical studies have revealed that *Mallotus* species are rich in polyphenols, flavonoids, phloroglucinols, coumarins, steroids, terpenoids, tannins, saponins, and alkaloids, many of which exhibit potent antioxidant, anti-inflammatory, antimicrobial, cytotoxic, anticancer, and immunoregulatory properties (Rivière *et al.*, 2010; Van Kiem *et al.*, 2010; Phan Thị Hoa, 2020; Anh *et al.*, 2022). Notably, the biological activity of these compounds has prompted sustained interest in the pharmacological investigation and drug development potential of this genus.

*Mallotus nanus*, endemic to Vietnam's Central Highlands, is traditionally used by ethnic minorities for acne treatment and shows antioxidant activity (ORAC assay) (Van Kiem *et al.*, 2010; Phan Thị Hoa, 2020). Despite its ethnomedicinal application, little is known about the chemical and antibacterial properties of *M. nanus*.

The study aims to comprehensively analyze the chemical profile (total phenolic and flavonoid content, UHPLC profiling) and evaluate the antibacterial activity of ethanolic extracts from the leaf, stem, and root of *M. nanus*. The extracts were tested against representative Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria to assess their potential as sources for natural antimicrobial agents and future therapeutic development.

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## 2. MATERIALS AND METHODS

### 2.1. Materials

The samples of *M. nanus* Airy Shaw, including leaves, stems, and roots of the medicinal plant were collected from Buon Don District, Dak Lak Province, Vietnam, in March 2024. This medicinal plant was identified by Thi Huong Tran, a botanist, Tay Nguyen University, and a voucher specimen (Code: MN 01) was deposited at the Institute of Biotechnology and Environment, Tay Nguyen University, Dak Lak 630000, Vietnam. The herbs including leaf, stem, root were dried to a constant weight, vacuum-sealed in polyethylene bags, and labeled BBL-DL (Ba Bet Lun-Dak Lak). We stored these specimens at 0-4°C until extraction. Pharmaceutical-grade ethanol was used as the solvent for extraction.

The standard chemicals for UHPLC analysis, including catechin, chlorogenic acid, epicatechin, epicatechin gallate, vitexin, salicylic acid, isovitexin, rutin, apigetrin, quercetin, luteolin, kaempferol and caffeine were procured from Sigma Chemical Co., USA.

*Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922) were used as reference strains, which were tested for antibacterial activity in the study. Both bacterial strains were obtained from the Institute of Biotechnology and Environment, Tay Nguyen University, Vietnam. Chloramphenicol (GoldBio, USA) and gentamicin (Sigma Aldrich, Germany) served as positive controls.

### 2.2. Methods

#### 2.2.1. Preparation of the extract from *M. nanus*

Herbal extracts were prepared using the percolation method. The herbal materials were moistened with 96% ethanol for approximately 12 hours before proceeding to the next stages. Additional solvent was added to cover the herbal material by about 1–2 cm, and the mixture was soaked for around 24 hours. The solvent-to-herbal-material ratio was 10:1. The combined extracts were filtered. The solution was filtered at room temperature using Whatman No.1 filter paper (Whatman International Ltd., Maidstone, UK). This filtrate was concentrated at 40°C under reduced vacuum using a rotary evaporator (Heidolph, Germany) and freeze-dried to obtain a dark brown extract, which was stored at -30°C before further use. The extraction yields were 15.10%, 7.03%, and 4.85% from 200 g of *M. nanus* leaf, stem, and root material, respectively, for each plant part.

#### 2.2.2. Phytochemical analysis

**The assay for determination of total polyphenol and flavonoid content:** The total polyphenol content is determined by the Folin-Ciocalteu method (1927) (Folin and Ciocalteu, 1927). A mixture of 1 mL *M. nanus* extracts and 5 mL Folin-Ciocalteu reagent was incubated for 5 minutes. Then, 4 mL of 7.5% sodium carbonate (w/v) was added into the mixture and incubate for 30 minutes at 20°C. Finally, measuring absorbance at 765 nm. All determinations were performed in triplicate. The results are expressed as mg gallic acid equivalent (GAE) per gram of extract. The total flavonoid contents were measured by the aluminum chloride colorimetric assay and modified slightly by Nguyen and Eun (Zhishen, Mengcheng and Jianming, 1999; Nguyen *et al.*, 2023). A volume 0.5 mL of *M. nanus* extract was added to a test tube containing 2.5 mL of distilled water. Sodium nitrite solution (0.075 mL, 5%) was then added to the mixture, followed by incubation for 5 min, then, 0.15 mL of 10% aluminum chloride was added. After 6 min, 0.5 ml of 1 M sodium hydroxide was finally added, after which the mixture was diluted with 0.275 ml of distilled water. The absorbance of the mixture at 510 nm was measured immediately in comparison to a standard curve prepared with quercetin. The results are expressed under mg quercetin equivalent (QE) per gram of extract.

**Chemical profiling by UHPLC analysis:** The phenolic and flavonoid constituents in *M. nanus* extracts were analyzed using an ultra-high-performance liquid chromatography (UHPLC) system (Thermo Scientific™ Ultimate 3000, Waltham, MA, USA). Chromatographic separation was carried out using a BDS Hypersil C18 column (250 × 4.6 mm, 5 µm particle size) maintained at 30 °C. The injection volume was 5 µL and each sample was injected in triplicate. The mobile phase consisted of MeOH (solvent A) and 0.1% phosphoric acid in water (solvent B), with a flow rate of 0.7 mL/min under the following gradient elution program: 0–0.5 min, 97% B; 0.5–8.0 min, 97%–83% B; 8.0–10.0 min, 83%–70% B; 10.0–15.0 min, 70%–55% B; 15–20 min, 55%–5% B; 20–22 min, 5%–97% B, and 22–23 min, 97% B. The chromatograms were monitored at 265 nm. We created calibration curves by comparing the size of the chromatographic peaks of each standard to the internal standard. A specific calibration curve, derived from the standards, enabled the quantification of phenolic compounds.

#### 2.2.3. Bioactivity assays

For the evaluation of antibiotic activity, several

assays were employed:

**Assessment of antibacteria assay:** The extract was evaluated using the agar diffusion method based on the Kirby-Bauer technique, as modified by Oonmetta-Aree, J., et al. (2006). The *M.nanus* extract dissolved with water to make different concentrations; then, 30  $\mu$ L of this mixture was soaked into a 6 mm round paper disc, allowed to dry, and used to assess its antibacterial efficacy. Using a micropipette, transfer 100  $\mu$ L of the bacterial solution containing  $10^6$  CFU/mL onto Nutrient agar supplemented with 1.5% NaCl. A sterile swab was used to spread it evenly and allowed to dry for 3–5 minutes. Place the filter paper discs impregnated with the extract onto the agar plates and incubate at 35°C for 24 hours. Antibiotic-impregnated paper discs (1 mg/mL) were used as positive controls: chloramphenicol (GoldBio, USA) for *Staphylococcus aureus* and gentamicin (Sigma-Aldrich, Germany) for *Escherichia coli*. The experiment was repeated three times. The antibacterial effect of the *M. nanus* extract was assessed by measuring the size of the area devoid of bacteria after 24 hours at 35 °C.

**Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC):** The resazurin-based turbidometric (TB) assay by Teh CH et al. (2017) was adopted to demonstrate the inhibition effects of *M. nanus* extracts. The extracts are diluted in two stages from an initial concentration of 100 mg/mL. The bacterial suspension was cultured overnight and diluted to a density of  $10^6$  CFU/mL. Add 50  $\mu$ L of bacterial suspension and 50  $\mu$ L of *M. nanus* extract solution at different dilution concentrations to each well. The control wells contain bacterial solution and medium. We repeat each experiment three times. Incubate at 37°C overnight. After 24 hours, add 30  $\mu$ L of 0.015% resazurin reagent to each well. Incubate at 37°C and observe the color change of the mixture in the well. The MIC value is the lowest concentration in the series of extracts that can inhibit bacterial growth (indicated by wells that do not change the color of resazurin).

The minimum bactericidal concentration (MBC) is found using the spot inoculation method, 50  $\mu$ L of the test solution from wells that did not change color with resazurin will be placed on BPW agar plates and kept at 37°C. After 24 hours, bacterial growth will be observed. The MBC value represents the lowest concentration in a series of extract concentrations that are capable of eliminating all bacteria (Teh et al., 2017) the medical arsenal now is experiencing shortage of effective drugs

to combat diseases, particularly against diseases caused by the dreadful multidrug-resistant strains, such as the methicillin-resistant *Staphylococcus aureus* (MRSA).

#### 2.2.4. Statistical Analysis

Data were analyzed using Microsoft Excel and SPSS software. The analysis involved the calculation of means and standard deviations (SD) to summarize the data. A p-value of <0.05 was considered statistically significant.

### 3. RESULTS AND DISCUSSIONS

#### 3.1. Chemical profiles of *M. nanus* extract

##### 3.1.1. Total phenolic and flavonoid content in *M. nanus* extracts

Results indicated that leaf extract exhibited the highest TPC ( $239.00 \pm 0.94$  mg GAE/g), while stem extract showed highest TFC ( $73.69 \pm 0.26$  mg QE/g vs.  $72.01 \pm 0.13$  mg QE/g in leaf). Root had lowest levels (TPC:  $169.67 \pm 1.26$  mg GAE/g; TFC:  $33.26 \pm 0.16$  mg QE/g) (Table 1). Compared to other *Mallotus* species, the TPC and TFC of *M. nanus* extracts were found to be higher. For instance, the fruit of *M. philippinensis* contains  $21.25 \pm 1.50$  mg GAE/g and  $43.26 \pm 2.66$  mg QE/g (Gangwar et al., 2014; Ali et al., 2024) antibacterial, wound healing activities, and so forth. So, present investigation was designed to evaluate the total antioxidant activity and radical scavenging effect of 50% ethanol fruit glandular hair extract (MPE; the stem of *M. repandus* contains  $136.30 \pm 0.78$  mg GAE/g and  $38.72 \pm 0.85$  mg QE/g mg QE/g (Mondal et al., 2020) itching, fever, rheumatic arthritis, and a variety of liver disorders. The aim of the present work was to evaluate the hepatoprotective activity and the antioxidant potential of the ethyl acetate stem extract of *M. repandus* (ESMR). High TPC and TFC, especially in leaf extract, hint at its potency as a source for natural antibacterial effect.

Phenolics and flavonoids are common phytochemical compounds present in herbal extracts, known to exert antibacterial effects through disruption of bacterial cell membranes and inhibition of nucleic acid and protein synthesis (Liang, Huang and Ma, 2022). Particularly, phenolic-rich extracts show strong correlations with antibacterial activity (Liang, Huang and Ma, 2022; Pozzo et al., 2023). Flavonoids display antibacterial activity primarily due to the flavonoids – membrane interaction that is related to their chemical structure, particularly the number and positions of methoxy and hydroxy groups (Liang, Huang and Ma, 2022).

**Table 1. The total phenolic and flavonoid contents of different parts of *M. nanus***

Sample	TPC <sup>a</sup> (mg GAE/g)	TFC <sup>a</sup> (mg QE/g)
Leaf	239.00 ± 0.94	72.01 ± 0.13
Stem	208.39 ± 0.39	73.69 ± 0.26
Root	169.67 ± 1.26	33.26 ± 0.16

<sup>[a]</sup>Values are the mean ± SD. Experiments were performed in triplicate (n = 3).

### 3.1.2. UHPLC chemical profile

In addition to previous investigations, the UHPLC analysis offered more information about the chemical compositions of *M. nanus* Airy Shaw extracts. The chromatogram results, presented in Table 2 and Figure 1, thoroughly showed clear separation of multiple peaks, with major phenolic and flavonoid compounds identified by comparison to standards and retention times.

A total of eleven bioactive compounds (**1-11**) were identified, comprising eight flavonoids and three phenolic acids. Flavonoids included epicatechin, epicatechin gallate, epigallocatechin gallate (EGCG), vitexin, isovitexin, rutin, myricetin and quercetin; the phenolic acids comprised gallic acid, chlorogenic acid, and salicylic acid. Among these, epicatechin, epicatechin gallate, rutin, myricetin, and quercetin were consistently detected across all plant parts, while vitexin and isovitexin were only in the stem and root. EGCG was unique to the ethanolic stem extract. Chlorogenic and salicylic acids were abundant in all three extracts; gallic acid was found in the leaf and root samples, reflecting an organ-specific metabolic partitioning.

Van Kiem et al. (2010) isolated five flavonoids (kaempferin, juglanin, quercitrin, myricitrin, rhoifolin) from methanolic *M. nanus* leaf extract. Similarly, Phan Thi Hoa (2020) identified mallonanoid A, β-sitosterol, stigmast-4-en-3-one, daucosterol, and palmitic acid from root extract. To the best of our knowledge, this UHPLC-based study represents the first profiling of 11 compounds (eight flavonoids, three phenolic acids) in *M. nanus*, substantially expanding its chemical characterization (Van Kiem et al., 2010; Phan Thi Hoa, 2020; Anh et al., 2022).

The phenolic acids detected in *M. nanus* extracts, including gallic, chlorogenic, and salicylic acids, are recognized as key antibacterial agents acting mainly through membrane dis-

ruption, metabolic inhibition, and biofilm suppression. Gallic acid exhibits strong bactericidal activity against *S. aureus* and *E. coli* (MIC 0.03–0.25 mg/mL) by increasing membrane permeability and inhibiting DNA repair enzymes, while chlorogenic acid induces oxidative stress and apoptosis-like cell death in Gram-negative bacteria (MIC 20 to 80 µg/mL) (Lou et al., 2011; Lee and Lee, 2018; Keyvani-Ghamsari, Rahimi and Khorsandi, 2023; Kiran and Patil, 2024). Salicylic acid shows moderate activity (MIC ≈ 4 mg/mL) primarily via destabilization of the cell wall and leakage of intracellular components (Song et al., 2022). Overall, phenolic acids constitute major contributors to the antibacterial efficacy of *M. nanus* extracts.

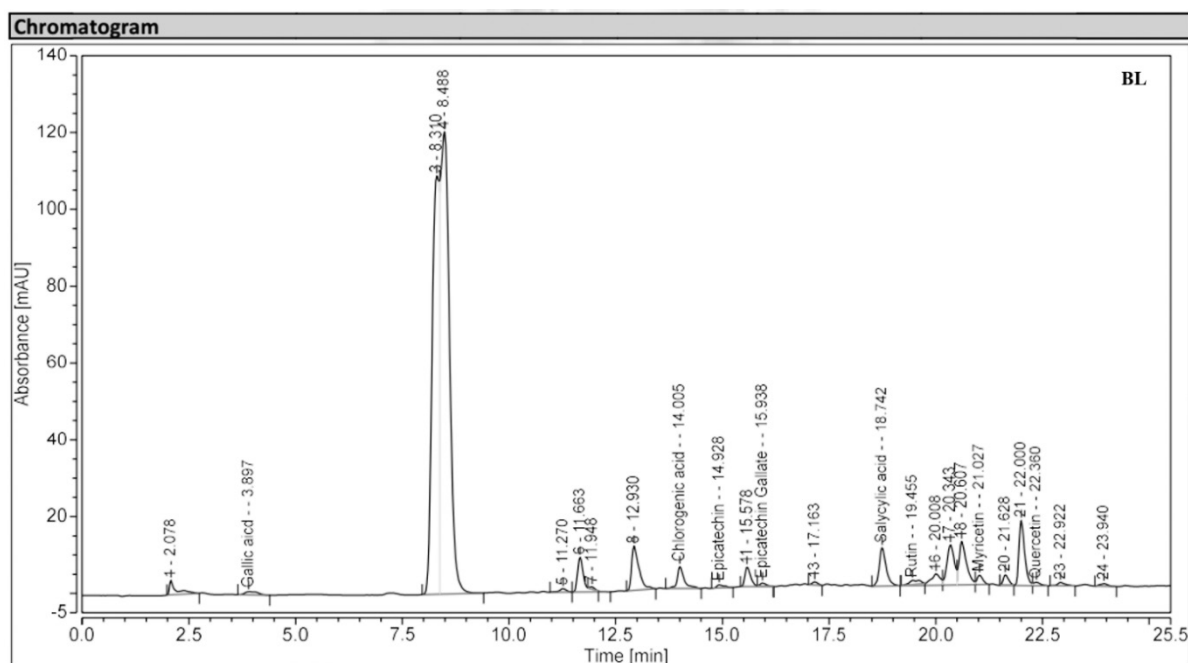
Flavonoids identified in this study exert antibacterial and anti-virulence effects mainly through membrane interaction, enzyme inhibition, and biofilm suppression. Quercetin and catechins (EGCG, ECG) display strong antibacterial activity against *S. aureus* and *E. coli* (MIC 16–128 µg/mL) by damaging membranes and inhibiting essential enzymes, whereas rutin shows weaker direct activity but enhances membrane permeability and antibiotic susceptibility (Arakawa et al., 2004; Jing et al., 2022; Nguyen and Bhattacharya, 2022; Yi et al., 2024; Teng et al., 2025). Myricetin, vitexin, and isovitexin mainly inhibit biofilm formation and bacterial virulence rather than bacterial growth (Das et al., 2022; Zeng et al., 2025) like vitexin, has long been in use for their antibacterial effect. The present work demonstrates the role of vitexin in modulating *Staphylococcus aureus* surface hydrophobicity while aggregating to form biofilm and pathogenesis in a host. In planktonic form, vitexin shows minimum inhibitory concentration at 252 µg/ml against *S. aureus*. Sub-MIC doses of vitexin and antibiotics (26 µg/ml of vitexin, 55 µg/ml of azithromycin, and 2.5 µg/ml of gentamicin). Collectively, flavonoids complement phenolic acids in shaping the antibacterial profile of *M. nanus* extracts.

Overall, UHPLC profiling reveals that the identified phenolic acids and flavonoids in *M. nanus* extracts collectively underpin their antibacterial

**Table 2. Compounds identified in ethanolic extracts of *M. nanus* Airy Shaw via UHPLC**

No/ Family	Compound	Ret. time (min)		
		Leaf	Stem	Root
<b>Flavonoid</b>				
1	Epicatechin	14.928	14.620/14.903	14.625/14.910
2	Epicatechin gallate	15.938	16.008/14.910	16.025
3	Epigallocatechin gallate (EGCG)	n.d.	14.357	n.d.
4	Vitexin	n.d.	17.902	17.908
5	Isovitexin	n.d.	18.855	18.842
6	Rutin	19.455	19.66	19.673
7	Myricetin	21.027	20.695	20.693
8	Quercetin	0.54	22.422	22.398
<b>Phenolic acid</b>				
9	Gallic acid	3.897	n.d.	3.897
10	Chlorogenic acid	14.005	13.788	13.782
11	Salicylic acid	18.742	18.593	18.602

*n.d.* Not detected.



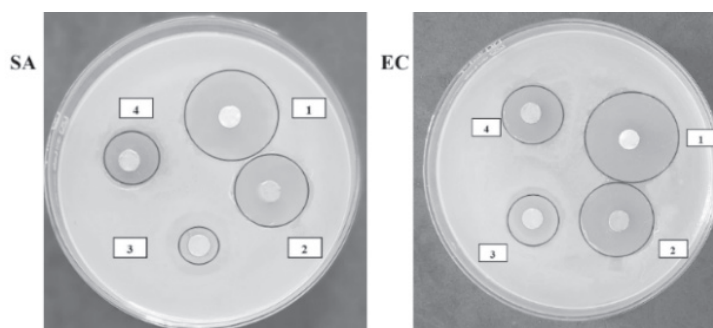
**Figure 1. Chromatogram of the ethanolic leaf extract of *M. nanus***

### 3.2. Antimicrobial activity of *M. nanus* extracts

#### 3.2.1. Antibacterial activity (disc diffusion assay)

In this study, the ethanolic extract was tested against the organisms, including *S. aureus* (Gram-positive) and *E. coli* (Gram-negative) in (Figure 2, Table 3). These are common disease-causing bacteria, such as those that cause diarrhea, food poisoning, skin infections, and ulcers (Domachowske, 2025; Sizar, Leslie and Unakal, 2025). The diam-

eter of the sterile zone increased with concentration of the crude extract (40 mg/mL, 60 mg/mL, 80 mg/mL, and 100 mg/mL) with total of three *M. nanus* extracts.



**Figure 2.** Inhibition zones (mm) of ethanolic *M. nanus* extracts against *S. aureus* (SA) and *E. coli* (EC) at 100 mg/mL. (1) Standard drugs (chloramphenicol, gentamicin); (2) leaf extract; (3) root extract; (4) stem extract.

**Table 3.** Antibacterial activity measured by zone of inhibition (mm) of *M. nanus* extracts at 100 mg/mL and positive controls at 1 mg/mL<sup>a</sup>

Samples/ Standard drugs	Microorganism	
	<i>Staphylococcus aureus</i> – Zone of inhibition (mm)	<i>Escherichia coli</i> – Zone of inhibition (mm)
Leaf	21.00 ± 0.00	22.25 ± 0.35
Stem	14.00 ± 0.00	18.50 ± 0.71
Root	2.00 ± 0.00	11.00 ± 0.00
Chloramphenicol	32.00 ± 0.00	Na.
Gentamicin	Na.	33.00 ± 0.00

<sup>[a]</sup> Values are means of triplicate determination (n=3) ± standard deviations; Na.: not assessed.

As shown in Figure 2 and Table 3, ethanolic extracts from leaf, stem, and root at 100 mg/mL produced clear inhibition zones against both *S. aureus* and *E. coli*. Leaf extract exhibited the strongest activity, with zones of 21.00 ± 0.00 mm for *S. aureus* and 22.25 ± 0.35 mm for *E. coli*, followed by stem and root extracts, whereas the standard drugs chloramphenicol and gentamicin produced zones of 32.00 ± 0.00 mm and 33.00 ± 0.00 mm, respectively. The inhibition zones increased with extract concentration (40–100 mg/mL), and *E. coli* was consistently more susceptible than *S. aureus*. This trend agrees with previous data for *M. philippinensis* fruit, where methanolic extract at 60 mg/mL afforded zones of 18.00 ± 0.27 mm (*S. aureus*) and 20.00 ± 0.37 mm (*E. coli*) (Gangwar, Kumar, Singh, et al., 2011). Taken together, these results indicate that *M. nanus* extracts, particularly the leaf extract, display stronger antibacterial effects than *M. philippinensis* fruit under comparable conditions.

### 3.2.2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extract

In this study, MIC and MBC values were determined with resazurin-based assays and bacterial culture on agar plates (Figure 3, Table 4). Leaf extract showed the strongest inhibitory

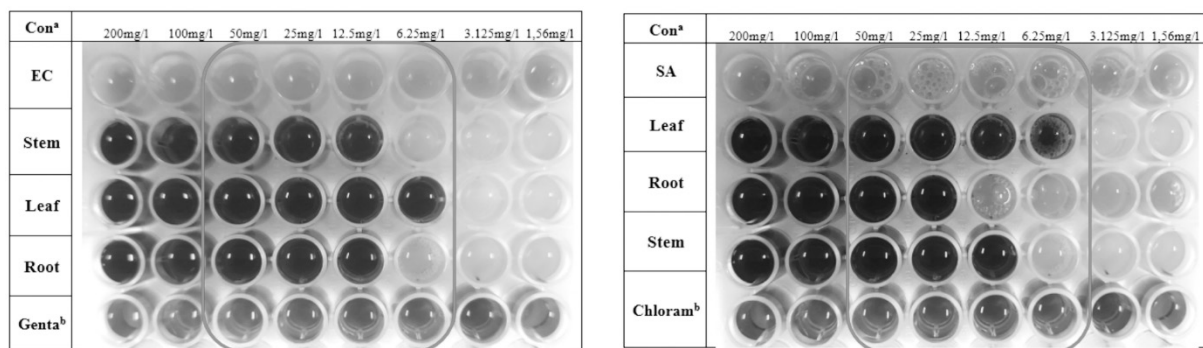
and bactericidal effects, with MICs of 6.25 mg/mL and MBCs of 25.00 mg/mL against both *S. aureus* and *E. coli*, compared with MICs of 12.50 mg/mL and MBCs of 50.00 mg/mL for the stem and root extracts. These data confirm the superior potency of the leaf extract against both Grampositive and Gramnegative bacteria.

In Vietnam, the antibacterial activity of *M. nanus* has only been reported by Phan Thị Hoa (2020) against *Propionibacterium acnes*, with a MIC of 8.8 mg/mL for the total root ethanolic extract. Root preparations at 10% (0.05 g herb/0.5 mL) and 20% (0.10 g herb/0.5 mL) also showed strong activity against clinical *P. acnes* isolates (Phan Thị Hoa, 2020). However, no previous study has evaluated the antibacterial activity of *M. nanus* against *S. aureus* and *E. coli*. Similar antibacterial mechanisms were observed in related *Mallotus* species, for example, the methanolic extract of *M. philippinensis* fruit was indicated *in vitro* antibacterial efficacy with MIC value of 15 mg/mL for *M. aureus* and 12 mg/mL for *E. coli* (Gangwar, Kumar, Tilak, et al., 2011). These findings demonstrate that *M. nanus* leaf extract possesses markedly stronger antibacterial activity than stem and root extracts, with MIC/MBC values of 6.25/25.00 mg/mL against both *S. aureus* and *E. coli*. Compared with previously reported

activity of *M. nanus* root against *P. acnes* (MIC 8.8 mg/mL) and the higher MICs of *M. philippinensis* fruit extracts, the present results highlight *M. nanus* leaves as a particularly promising source of plant-derived antibacterial agents.

We compared antimicrobial data for pure compounds isolated from several *Mallotus* species and found that purified constituents often exhibit stronger activity than crude extracts. For example, phenolic derivatives isolated from *M. oppositifolius* leaves showed inhibitory activity against four bacterial strains, including *E. coli* and *S. aureus*,

with MIC values ranging from 3.125 to 50 µM. Similarly, two pure phenolic derivatives from *M. philippinensis* fruits displayed significant antibacterial effects against *S. aureus*, *E. coli*, *M. luteus*, *S. mutans* and *B. cereus*, with MIC values of 3.8–15.5 µM (Cheenpracha *et al.*, 2019; Tchanguou *et al.*, 2020; Anh *et al.*, 2022). Together with our current data, these findings indicate that antibacterial potency in the genus *Mallotus* is strongly influenced by the plant species, plant part, extraction solvent and, in particular, the presence and concentration of specific active compounds.



**Figure 3.** Results of MIC determination for the extracts against *S. aureus* (SA) and *E. coli* (EC). <sup>(a)</sup> *M. nanus* extracts at different concentrations (mg/mL); <sup>(b)</sup> the standard drugs gentamicin and chloramphenicol.

**Table 4.** MIC and MBC values of the *M. nanus* extracts

Microorganism	MIC (mg/mL)			MBC (mg/mL)		
	Stem	Root	Leaf	Stem	Root	Leaf
<i>E. coli</i>	12.50	12.50	6.25	50.00	50.00	25.00
<i>S. aureus</i>	12.50	25.00	6.25	50.00	50.00	25.00

#### 4. CONCLUSIONS

This study provides the first UHPLC-based characterization of the phytochemical composition and antibacterial properties of *M. nanus* extracts. The leaf, stem, and root extracts were all rich phenolic and flavonoid compounds, with the leaf extract showing the highest TPC and the strongest antibacterial activity. UHPLC profiling identified eleven bioactive constituents (flavonoids and phenolic acids), several of which had not previously been reported in this species, expanding the known chemical diversity of *M. nanus*.

Ethanol extracts demonstrated significant, concentration-dependent inhibition against both

Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria, with MIC and MBC values supporting their potential as broad-spectrum, plant-derived antimicrobials. These findings validate the ethnomedicinal use of *M. nanus* and support its leaves as a promising source of plant-derived antibacterial agents, warranting further work on isolation and structural elucidation of active compounds, mechanism action studies, and formulation development.

#### Acknowledgment

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## NGHIÊN CỨU THÀNH PHẦN HÓA HỌC VÀ HOẠT TÍNH KHÁNG KHUẨN CỦA CAO CHIẾT *Mallotus nanus* AIRY SHAW (EUPHORBIACEAE)

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### TÓM TẮT

Nghiên cứu nhằm phân tích thành phần hóa học và đánh giá hoạt tính kháng khuẩn của cao chiết ethanol từ lá, thân và rễ cây *Mallotus nanus* Airy Shaw, một loài cây thuốc truyền thống của vùng Tây Nguyên. Kết quả nghiên cứu cho thấy cao chiết lá cho tổng polyphenol cao nhất ( $239,00 \pm 0,94$  mg GAE/g) và flavonoid gần cao nhất ( $72,01 \pm 0,13$  mg QE/g). Trong đó, hàm lượng tổng polyphenol cao hơn rõ rệt so với rễ củ. Phân tích UHPLC đã xác định được 11 hợp chất (8 flavonoid và 3 acid phenolic) với phân bố theo cơ quan: lá giàu acid phenolic (gallic, chlorogenic, salicylic), trong khi thân và rễ củ giàu flavonoid (vitexin, isovitexin, EGCG, rutin, myricetin, quercetin). Tất cả các cao chiết đều ức chế *Staphylococcus aureus* và *Escherichia coli* theo kiểu phụ thuộc nồng độ, trong đó cao lá tạo vòng vô khuẩn lớn nhất ở 100 mg/mL ( $21,00 \pm 0,00$  mm và  $22,25 \pm 0,35$  mm). Đồng thời, cao lá cũng có MIC/MBC thấp nhất (6,25/25 mg/mL cho cả hai chủng), mạnh hơn so với cao thân và rễ. Kết quả này góp phần cung cấp thêm cơ sở khoa học việc sử dụng truyền thống của loài *M. nanus* và gợi ý bộ phận lá là nguồn giàu phenolic–flavonoid tiềm năng để phát triển các tác nhân kháng khuẩn có nguồn gốc thực vật.

**Từ khóa:** *Mallotus nanus*, hoạt tính kháng khuẩn, flavonoid, acid phenolic, UHPLC.

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