GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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Phytochemical screening, antioxidant, antibacterial and antifungal activities of ethanolic extract of *Artemisia vulgaris* L. harvested in Thai Nguyen, Vietnam

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GSC Biological and Pharmaceutical Sciences, 2025, 33(02), 467-473

Publication history: Received 16 October 2025; revised on 22 November 2025; accepted on 24 November 2025

Article DOI: https://doi.org/10.30574/gscbps.2025.33.2.0469

Abstract

Wormwood (*Artemisia vulgaris*) is a widely used medicinal herb worldwide. Traditionally, it has been employed to treat various ailments such as musculoskeletal disorders, pain relief, pregnancy support, and hemostasis. Additionally, wormwood exhibits diverse biological activities including anti-inflammatory, anticancer, and antioxidant effects. This study reports the phytochemical composition and in vitro antibacterial, antifungal and antioxidant activities of the ethanolic leaf extract of *A. vulgaris*. Qualitative phytochemical analysis of the ethanol extract from *Artemisia vulgaris* leaves collected in Thai Nguyen revealed the presence of four major classes of bioactive compounds: alkaloids, flavonoids, tannins, and saponins. The *A. vulgaris* extract demonstrated strong antioxidant activity with an IC_{50} value of 7.20 µg/mL. Moreover, the extract inhibited the growth of three bacterial strains (*Staphylococcus aureus*, *Helicobacter pylori*, *Citrobacter freundii*) and three fungal strains (*Aspergillus brasiliensis*, *Aspergillus flavus*, *Candida albicans*) at a concentration of 50 µg/mL. These findings highlight the potential of *Artemisia vulgaris* as a source of natural compounds that could serve as alternatives to synthetic antibiotics and antioxidants, contributing significantly to modern medicine and the conservation of traditional medicinal plants.

Keywords: *Artemisia Vulgaris*; Antioxidant; Antibacterial; Antifungal

1. Introduction

Wormwood (*Artemisia vulgaris* L. var. *indica* (Willd.) DC.), also known as wild mugwort is a herbaceous plant belonging to the Asteraceae family, widely distributed in temperate and subtropical regions worldwide. In Vietnam, this species grows vigorously and is commonly found in the midland and mountainous areas of the northern region, including provinces such as Thai Nguyen, Lang Son, and Cao Bang.

The chemical composition of *Artemisia vulgaris* contains a complex array of bioactive compounds, notably flavonoids (such as quercetin, luteolin, and apigenin), polyphenols, organic acids, coumarins, sesquiterpenes, and volatile essential oil constituents [1]. These compounds have been demonstrated to exhibit diverse pharmacological properties, including antioxidant, antibacterial, anti-inflammatory, enzyme inhibitory, and cytoprotective activities, indicating broad potential applications in natural-based pharmaceuticals and cosmetics. Ethanol extracts from five species of the *Artemisia* genus analyzed using modern chromatographic methods revealed the presence of monoterpenes, sesquiterpenes, flavonoids, flavonoid glycosides, coumarins, and phenolic acids. The extracts exhibited notable antibacterial and antifungal activities at low concentrations (≥150 µg/mL), and certain species also demonstrated inhibitory effects on the nematode *Caenorhabditis elegans*, suggesting potential applications in biological control [2].

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The chemical composition and biological activities of *A. vulgaris* vary depending on geographical location, climate, and genetic sources. Essential oils rich in iso-artemisia ketone and piperitone show strong antibacterial activity. Both essential oils and ethanol extracts exhibit antioxidant and antibacterial effects, with some samples also showing cytotoxic activity against cancer cells [3].

In Vietnam, studies on the chemical composition and biological activities of *A. vulgaris* have also been conducted in different regions. Trinh et al. analyzed the essential oil of *A. vulgaris* from Tien Giang and identified δ-elemene as a characteristic compound, rarely found in samples from other regions (Trinh và cs, 2024a) . This essential oil exhibited broad-spectrum antibacterial and significant anti-inflammatory activity by inhibiting nitric oxide (NO) and TNF-α production. Furthermore, Trinh et al. evaluated the antioxidant, anti-inflammatory, and xanthine oxidase inhibitory activities of *A. vulgaris* ethanol extracts. Their study isolated five flavonoid compounds with effective xanthine oxidase inhibition, highlighting potential applications in gout therapy [4].

Although previous studies have confirmed the chemical diversity and biological potential of *A. vulgaris*, there has been no in-depth investigation of its biological activities in the midland and northern mountainous regions of Vietnam, which possess distinct ecological conditions. Therefore, the present study aims to evaluate antioxidant activity (DPPH), antibacterial and antifungal (agar well diffusion), and to perform qualitative phytochemical screening. The results are expected to provide a scientific database and clarify the pharmacological potential of this species, paving the way for its application in the development of natural medicinal products.

2. Materials and methods

2.1. Materials

Fresh leaves of *Artemisia vulgaris* were collected in August 2023 from Thai Nguyen province, Vietnam. The collection site is situated at an elevation of 597 meters above sea level, with geographic coordinates of 21°39.69′N, 105°90.88′E. The plant materials were taxonomically identified by Associate Professor Danh Thuong Sy, Head of the Botany Department, Faculty of Biology, Thai Nguyen University of Education. Voucher specimens were prepared and deposited at the Biological Museum of the Faculty of Biology, Thai Nguyen University of Education, under accession numbers TNUE-AV2023-001 and TNUE-AV2023-002. Healthy plants free from pests and diseases were manually harvested, thoroughly washed with distilled water to remove impurities, and dried at 55°C for 72 hours until a constant weight was achieved. The dried leaves were ground into a fine powder and stored in airtight containers at room temperature until extraction.

The microbial strains used in this study included three bacterial strains (*Staphylococcus aureus*, *Helicobacter pylori*, *Citrobacter freundii*) and three fungal strains (*Aspergillus brasiliensis*, *Aspergillus flavus*, *Candida albicans*). All strains were provided and authenticated by the Department of Biology, Thai Nguyen University, Vietnam, and maintained under standard laboratory conditions prior to experimentation.

2.2. Methods

Preparation of Ethanol Extract: Fresh *A. vulgaris* leaves were washed with distilled water to remove impurities and then dried at 55°C in a convection oven until a constant weight was reached. The dried leaves were mechanically ground into a fine powder. A total of 100 g of leaf powder was macerated in 99% ethanol (1:10 w/v) at room temperature for 72 hours, with periodic shaking 200 rpm to enhance extraction efficiency. The mixture was filtered through Whatman No. 1 filter paper to remove solid residues. Ethanol was then evaporated under reduced pressure using a rotary evaporator at 40°C to obtain a concentrated crude extract. The crude extract was stored in airtight containers at 4°C for subsequent analyses.

Qualitative Phytochemical Analysis: Qualitative analysis of the chemical constituents in the ethanol extract of *A. vulgaris* was performed using standard identification tests [5]. Secondary plant metabolites were detected as follows: alkaloids (Wagner's and Dragendorff's reagents), flavonoids (cyanidin reaction and NaOH test), tannins ($FeCl_3$ solution), and saponins (frothing with hot water). All assays were conducted under standard laboratory conditions, with each test performed in triplicate to ensure accuracy and reproducibility. The presence of chemical compounds was confirmed based on characteristic color changes or precipitate formation according to published standards.

Antibacterial Activity Assay: The antibacterial activity of the ethanol extract was assessed using the agar well diffusion method. Three bacterial strains (*Staphylococcus aureus*, *Helicobacter pylori*, *Citrobacter freundii*) were cultured on LB

(Luria-Bertani) agar plates. The extract was prepared at concentrations of 30, 50, and 70 µg/mL. Each 6 mm diameter well in the agar was filled with 50 µL of extract solution. Plates were kept at 4°C for 1 hour to allow diffusion, then incubated at 37°C for 24 hours. Antibacterial activity was evaluated by measuring the inhibition zone diameter. Dimethyl sulfoxide (DMSO) served as the negative control, while ampicillin at 50 µg/mL was used as the positive control. All experiments were performed in triplicate. Inhibition zones were measured using an electronic caliper and reported as mean ± standard deviation (SD).

Antifungal Activity Assay: Antifungal activity was evaluated using the agar well diffusion method on Potato Dextrose Agar (PDA). Three fungal strains (*Aspergillus brasiliensis*, *Aspergillus flavus*, and *Candida albicans*) were tested. The extract was prepared at concentrations of 30, 50, and 70 µg/mL. Each 6 mm diameter well was filled with 50 µL of extract solution. Plates were incubated at 30°C for 72 hours. Antifungal activity was assessed by measuring the inhibition zone diameter after 3 days. DMSO served as the negative control, and *Amphotericin B* at 50 µg/mL was used as the positive control. All experiments were performed in triplicate, and inhibition zones were measured with an electronic caliper and reported as mean ± SD.

Antioxidant Activity Assay: The antioxidant activity of the ethanol extract was determined using the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay as described by Abramovič et al. [6]. DPPH is a stable free radical with a characteristic purple color that turns pale yellow (1,1-diphenyl-2-picrylhydrazine) upon reaction with hydrogen-donating substances. A DPPH 0.1 mM solution was prepared in methanol. The extract and the positive control (vitamin C) were diluted in methanol to various concentrations. The reaction mixture, consisting of 40 µL of DPPH solution and 960 µL of extract or vitamin C solution, was incubated in the dark at room temperature for 30 minutes. Absorbance was measured at 517 nm using a UV-Vis spectrophotometer. The percentage of inhibition (I%) was calculated using the formula: $I\% = \frac{A_0 - A_i}{A_0} \times 100$ where I% is the inhibition percentage, Ai is the absorbance of the sample, and A0 is the absorbance of the DPPH solution without sample. Each assay was performed in triplicate to ensure accuracy and reproducibility.

Statistical Analysis: All experiments were performed in triplicate (n = 3). Data were expressed as mean ± standard deviation (SD). Statistical significance was evaluated using one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$).

3. Results and discussion

3.1. Qualitative Phytochemical Analysis

The results of qualitative analysis of the ethanol extract from *Artemisia vulgaris* leaves are summarized in Table 1. The analysis indicates that the extract contains the major classes of secondary metabolites, including flavonoids, tannins, saponins, and alkaloids. These secondary metabolites are commonly found in plants and are known to be associated with various biological activities, such as antioxidant, antibacterial, anti-inflammatory, and cytoprotective effects.

Table 1 Qualitative analysis of secondary metabolites in *Artemisia vulgaris* leaf extract

No.	Compound Class	Specific Test	Observation	Result
1	Alkaloids	Wagner's reagent	Dark brown precipitate	(+)
		Dragendorff's reagent	Orange precipitate	(+)
2	Flavonoids	Cyanidin reaction	Orange-red solution	(+)
		NaOH test	Dark yellow solution	(+)
3	Tannins	$FeCl_3$ solution	Light brown-green precipitate	(+)
4	Saponins	Frothing with hot water	Foam formation	(+)

The qualitative results (Table 1) confirm that the ethanol extract from *A. vulgaris* leaves contains flavonoids, tannins, saponins, and alkaloids. These classes of compounds are widely recognized for their biological activities, including antioxidant, antibacterial, and anti-inflammatory effects [4].

Compared with previous studies, Bisht et al. (2021) reported that *A. vulgaris* also contains terpenoids, carotenoids, and polysaccharides [7]. This difference is primarily attributed to the choice of extraction solvent. Ethanol, with intermediate polarity, can dissolve both polar compounds (flavonoids, tannins) and moderately polar compounds (alkaloids, saponins), resulting in a diverse chemical profile [8]. In contrast, non-polar solvents (e.g., hexane) preferentially extract terpenoids and lipids, while highly polar solvents (e.g., water, methanol) enrich polysaccharides and phenolic acids [7]. Therefore, ethanol was chosen as the extraction solvent in this study, as it ensures efficient recovery of bioactive compounds while being safer for applications in herbal medicine and food products compared to many other organic solvents.

3.2. Evaluation of Antioxidant Activity

The antioxidant activity of the wormwood ethanol extract was assessed using the DPPH assay. The results demonstrated a clear dose-dependent relationship between extract concentration and the percentage of free radical scavenging (Figure 1).

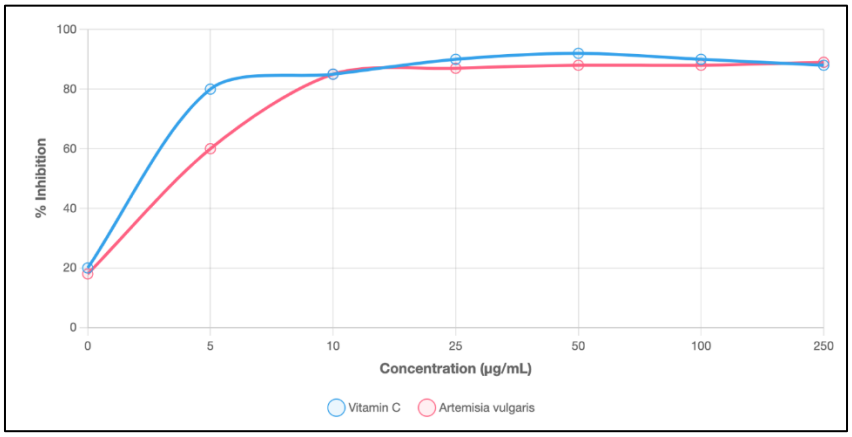


Figure 1 Graph illustrating the relationship between extract concentration and DPPH radical scavenging (%) of *Artemisia vulgaris* leaf extract and Vitamin C.

The results showed that the leaf extract of *Artemisia vulgaris* exhibited an IC_{50} of 7.20 µg/mL (Vitamin C: 2.52 µg/mL), indicating significant antioxidant activity. According to the DPPH activity classification criteria (IC_{50} < 50 µg/mL = very strong or strong; IC_{50} 50–100 µg/mL = moderate activity; IC_{50} 100–250 µg/mL = weak), the *Artemisia vulgaris* extract belongs to the strong or very strong activity group, although it does not reach the level of Vitamin C [9]. However, the DPPH assay only reflects the direct free radical scavenging mechanism and is not sufficient to determine the overall mechanism of action or to confirm superiority over Vitamin C [10]. Major flavonoids such as luteolin, apigenin, quercetin, and some other phenolics (e.g., chlorogenic acid derivatives) may participate in free radical stabilization and inhibit lipid peroxidation; however, additional biochemical assays are required to fully elucidate the mechanism of action.

3.3. Assessment of Antibacterial Activity

Table 2 Antibacterial activity of *Artemisia vulgaris* leaf extract

Sample	Concentration (µg/mL)	Zone of inhibition (mm)		
		S. aureus	*H. pylori*	*C. freundii*
Ampicillin	50	23 ± 0.3	25.0 ± 0.4	24.3 ± 0.3
Extract	30	16 ± 0.6	15.3 ± 0.6	0
	50	20 ± 0.5	20.3 ± 0.6	17.3 ± 0.4
	70	26 ± 0.4	27.3 ± 0.5	20.2 ± 0.6

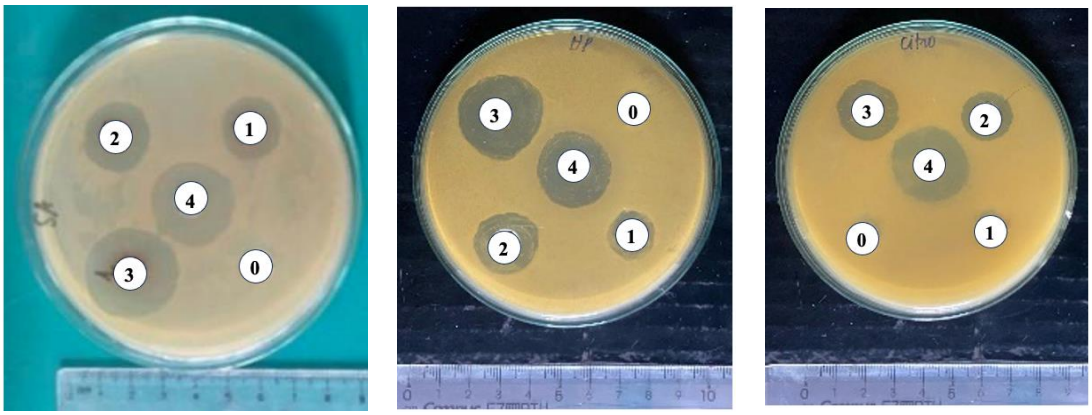
Note: Values are expressed as mean ± SD (n = 3).

The antifungal activity of *Artemisia vulgaris* extract against three bacterial strains (*S. aureus*, *H. pylori*, and *C. freundii*) is presented in Table 2 and Figure 2.

The tests on *S. aureus*, *H. pylori*, and *C. freundii* showed that the ethanol extract of *Artemisia vulgaris* exhibited dose-dependent antibacterial activity. For *S. aureus*, the inhibition zone was 16 mm at 30 µg/mL (lower than Ampicillin: 23 mm), increasing to 20 mm at 50 µg/mL and 26 mm at 70 µg/mL, surpassing Ampicillin. Similar results were observed for *H. pylori*: 15.3 mm at 30 µg/mL, 20.3 mm at 50 µg/mL, and 27.3 mm at 70 µg/mL, also exceeding Ampicillin at higher concentrations. In contrast, *C. freundii* was less sensitive: 0 mm at 30 µg/mL, 17.3 mm at 50 µg/mL, and 20.2 mm at 70 µg/mL, still lower than Ampicillin (24.3 mm).

This indicates selective antibacterial activity, with strong efficacy against Gram-positive bacteria (*S. aureus*) and spirochetes (*H. pylori*), but limited activity against Gram-negative bacteria (*C. freundii*), likely due to cell wall structure and specific resistance mechanisms. Compared to Ampicillin, higher concentrations of the extract are required to achieve comparable or superior effects. However, the extract offers advantages as a natural source with minimal potential side effects and the possibility of multi-target mechanisms (e.g., cell membrane disruption, enzyme inhibition, DNA interference).

These results are consistent with previous studies on *Artemisia*, where essential oils and polyphenols exhibited broad-spectrum antibacterial activity. Overall, *Artemisia vulgaris* extract shows potential as a natural compound source to replace or complement antibiotics, especially in the context of increasing antimicrobial resistance. Optimization of concentration and possible combination with other agents may enhance efficacy, particularly against Gram-negative bacteria.



S. aureus *H. pylori* *C. freundii*

Figure 2 Antibacterial activity of ethanol extract of wild *A. vulgaris*

Note: 0: 2% DMSO; 1: extract at 30 µg/mL; 2: extract at 50 µg/mL; 3: extract at 70 µg/mL; 4: Ampicillin 50 µg/mL.

Our results are consistent with previous studies [11]. Another study in 2023 on the pharmacology and antibacterial mechanisms of Artemisia species indicated that bioactive compounds in the plant exhibit antibacterial activity through cell membrane disruption and DNA interference [12]. Previous studies also reported that compounds such as terpenoids and flavonoids play important roles in antibacterial, antifungal, and antioxidant activities [13].

3.4. Assessment of Antifungal Activity

The antifungal activity of Artemisia extract was evaluated against three fungal strains, with results presented in Table 3 and Figure 3.

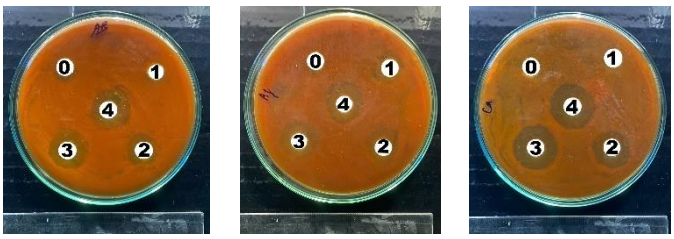
At 30 µg/mL, the extract did not inhibit *A. brasiliensis* or *C. albicans* (0 mm), and showed a weak, selective activity against *A. flavus* (6.1 mm), indicating a low initial effect, likely due to insufficient concentration to inhibit yeast (*C. albicans*) or mold (*A. brasiliensis*). At 50 µg/mL, the extract exhibited activity against all three fungi, with improved efficacy: 7.4 mm for *A. brasiliensis*, 9.3 mm for *A. flavus*, and 10.2 mm for *C. albicans*, although still lower than *Amphotericin B* (10.7 mm, 12.0 mm, and 15.0 mm, respectively). At 70 µg/mL, the extract reached maximal efficacy: 10.2 mm for *A. brasiliensis* (approaching *Amphotericin B*), 10.3 mm for *A. flavus*, and 16.4 mm for *C. albicans* (exceeding *Amphotericin B*), confirming dose-dependent activity and strong potential against *C. albicans*.

Table 3 Antifungal activity of wild *A. vulgaris* extract

Sample	Concentration (µg/mL)	Zone of inhibition (mm)		
		A. brasiliensis	*A. flavus*	*C. albicans*
Amphotericin B	50	10.7 ± 0.4	12.0 ± 0.4	15.0 ± 0.3
Extract	30	0.0	6.1 ± 0.6	0.0
	50	7.4 ± 0.5	9.3 ± 0.4	10.2 ± 0.5
	70	10.2 ± 0.4	10.3 ± 0.5	16.4 ± 0.6

Note: Values are expressed as mean ± SD (n = 3).

Overall, the antifungal activity increased with concentration, showing highest efficacy against *C. albicans*, a common pathogenic yeast, and lower activity against *A. brasiliensis*, possibly due to differences in action mechanisms such as cell membrane disruption or enzyme inhibition. Compared to *Amphotericin B*, the extract was less effective at equivalent concentrations but offers advantages as a natural product with lower risk of resistance and multi-target mechanisms. These findings highlight the potential application of Artemisia extract as a natural antifungal agent, particularly at concentrations ≥50 µg/mL, and suggest further optimization or combination with other agents to enhance efficacy, in line with the trend toward using natural products to combat drug-resistant fungal infections.



A. brasiliensis *A. flavus* *C. albicans*

Figure 3 Antifungal activity of ethanol extract of wild *A. vulgaris*

Note: 0: 2% DMSO; 1: extract at 30 µg/mL; 2: extract at 50 µg/mL; 3: extract at 70 µg/mL; 4: Amphotericin B 50 µg/mL.

These results are consistent with previous studies [2]. Research on the pharmacological properties of the *Artemisia* genus has indicated that compounds such as sesquiterpene lactones (artemisinin) and monoterpenes (eucalyptol, camphor) contribute to the observed biological activities [7].

4. Conclusion

This study qualitatively identified flavonoids, tannins, saponins, and alkaloids in wild *A. vulgaris* extract and evaluated its antioxidant, antibacterial, and antifungal activities. The antibacterial activity of the extract increased strongly with concentration, showing particularly high efficacy against *S. aureus* and *H. pylori* at 70 µg/mL, while activity against *C. freundii* remained limited, demonstrating selective antibacterial properties. Regarding antifungal activity, the extract exhibited outstanding efficacy against *C. albicans* at 70 µg/mL (16.4 mm). In terms of antioxidant activity, wild *A. vulgaris*extract achieved nearly 100% free radical scavenging at 100 µg/mL, with an IC_{50} of 7.2 µg/mL, which is lower than that of Vitamin C (2.52 µg/mL).

Compliance with ethical standards

Acknowledgments

This study was privately funded by the authors and no special financial support was obtained from any funding agency in the public, commercial, or not-for-profit sectors.

Disclosure of conflict of interest

All the authors hereby declare that no conflicts of interest exist related to this article.

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