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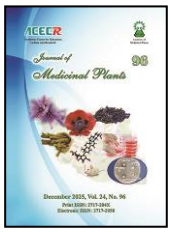

**Research Article**

# The essential oil of *Alpinia officinarum* Hance: chemical compounds, antioxidant, antimicrobial, and anti-inflammatory activities

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ABSTRACT

**Background:** *Alpinia officinarum* Hance (*A. officinarum*) roots are used as a treatment for various illnesses, including colds, stomachaches, inflammation, and discomfort. **Objective**: This work aimed to evaluate the anatomy, chemical compounds, antioxidant, antimicrobial, and anti-inflammatory activities of *A. officinarum* roots essential oil (EO). **Methods**: First, the plant's anatomy was studied by the alum-green iodine staining ‧method, followed by steam distillation to extract EOs. The determination of chemical compositions by Gas chromatography-mass spectroscopy (GC-MS). The antioxidant activities were conducted by free radical resistance by 1,1-diphenyl-2-picrylhydrazyl (DPPH), ABTS (2,2-Azino-bis-3-ethylbenzothiazoline-6-sulphonic acid), and FRAP (Ferric Reducing Antioxidant Power). The paper plate method was used to determine antimicrobial activity. Finally, the anti-denaturation of the Bovine serum albumin assay was measured for the anti-inflammatory properties of EO. **Results**: Anatomical results show that the EO of *A. officinarum* is distributed in the form of secretory sacs. By GC-MS, twenty-eight components were identified; the main compounds are Eucalyptol (24.57 %), *β*-Bisabolene (9.72 %), and *β*-Sesquiphellandrene (5.95 %). The $IC_{50}$ of FRAP, ABTS, and DPPH assays are 810.00 µg/mL, 1343.71 µg/mL, and 26953.21 µg/mL, respectively. In addition, the EO exhibited anti-inflammatory activity with an $IC_{50}$ of 2.55 mg/mL, compared to the standard Diclofenac (1.03 mg/mL). Additionally, the antimicrobial activity test demonstrated that the EO exhibited antimicrobial activity against various bacterial strains, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Bacillus cereus*, and *Listeria innocua*. **Conclusions:** These findings demonstrate that EOs can be utilized in the production of anti-inflammatory compounds and ferric-reducing additives.

## 1. Introduction

With 53 genera and 1300 species, the Zingiberaceae family, the Zingiberaceae family is considered the largest family in the Zingiberales family, including *Zingiber*, *Alpinia*, *Curcuma*, *Globba*, *Etlingera*, *Renealmia*, *Riedelia*, *Amomum*, *Aframomum*, *Boesenbergia*, *Meisteria*, *Hedychium*, and

*Abbreviations:* DPPH, 1,1-diphenyl-2-picrylhydrazyl; ABTS, 2,2-Azino-bis-3-ethylbenzothiazoline-6-sulphonic acid; BSA, Bovine serum albumin; D, diameter; EO, Essential oil; FRAP, Ferric Reducing Antioxidant Power; GC-MS, Gas chromatography-mass spectroscopy; $IC_{50}$, Half maximal inhibitory concentration

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*Hornstedia* [1]. The family is commonly found in the tropical regions of Africa, the Americas, and Asia, particularly in countries such as Egypt, Sri Lanka, Malaysia, India, Indonesia, and Thailand, due to their favorable agroecological conditions [2]. Most of the Zingiberaceae species, such as *Zingiber* and *Alpinia* species (*Z. officinale* Roscoe, *Z. zerumbet* (L.) Roscoe ex Sm., *Z. cassumunar* Roxb., *Alpinia galanga* Linn., *A. conchigera* Griff, A. *katsumadai* Hayata, *A. oxyphylla* Miq., *A. zerumbet* (Pers.) Burtt. et Smith, *A. purpurata* (Viell.) K. Schum., and *A. officinarum* Hance) have been employed in conventional medicine [1].

*Alpinia officinarum* Hance (*A. officinarum*), called galangal, belongs to the Zingiberaceae family, is utilized throughout Southeast Asia, particularly in Thailand and Indonesia, as a traditional culinary herb, and since it has antiviral, antibacterial, and antioxidant properties, it can be used as a treatment for a number of illnesses, such as colds, stomachaches, inflammation, and discomfort [2-4]. *A. officinarum* has long been utilized in Chinese, Ayurvedic, Thai, and Greek medicine to increase blood flow and decrease swelling, diarrhea, headaches, renal issues, ulcers, joint pain, and respiratory issues [5]. In addition to its reputation as a medicinal herb, *A. officinarum* is also utilized as a preservative and taste enhancer in cooking. In Asian cooking, it is a common pickling spice due to its distinct sweet and spicy scent, which enhances flavor and prolongs the expiry date [6]. The historic use of A. officinarum has been supported by recent investigations that have demonstrated its varied pharmacological properties. For example, Laksmitawati et al. (2022) revealed that *Alpinia* is an antioxidant agent due to its polyphenol and total flavonoid components [7]. There is evidence that *Alpinia* can be used to treat inflammatory pain because it inhibits p38 mitogen-activated protein kinases, nuclear factor-kappa B, nod-like receptor protein 3 signals, TRPV1 activation, regulates the expression of COX-2, and NF-κB/TNF-$\alpha$ [8, 9]. In addition, extracts from *A. officinarum* have demonstrated significant sun protection factor (SPF) and free radical scavenging properties, which enhance their efficacy as natural sunscreens [10]. Methanol extracts of *A. officinarum* also showed significant antibacterial activity against Gram-negative *Enterobacter sakazakii* and Gram-positive *Staphylococcus aureus* pathogens [11]. Besides, the antidiabetic effect is also shown by *Alpinia officinarum*, mainly through inhibiting the $\alpha$-glucosidase enzyme to decrease the conversion of carbohydrates to glucose [12]. Regarding anticancer activity, Yakufu revealed that flavonoids from *A. officinarum* may be used for stomach cancer treatment, probably with minimal toxicity. It might be linked to the suppression of cancer-associated inflammation, tumor cell proliferation, and tumor angiogenesis [13]. The study of Panigrahi et al. (2023) showed that ethyl-p-methoxycinnamate in *A. officinarum* has antiproliferative and cytotoxic effects on human breast and cervical cancer cells, suggesting that it may have anticancer characteristics [14]. This study enriches the scientific data on anatomy, chemical compounds, antioxidant, antimicrobial, and anti-inflammatory activities of *A. officinarum* roots essential oil.

## 2. Materials and methods

### *2.1. Studies on Anatomy*

*A. officinarum* roots were harvested in Can Tho City, cleaned, and the damaged roots were further removed. According to morphological



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figures [15], plant identification was conducted at the Biological Laboratory of the School of Education, Can Tho University, Vietnam, and a sample code of RIENG-01 was encoded. The sample was then observed to contain vesicles of essential oil. Firstly, use a 5 % sodium hypochlorite solution to immerse the sliced roots for twenty minutes, and then remove the solution with deionized water. The samples were cleaned once again using distilled water after steeping in a 0.5 % acetic acid solution for fifteen minutes. Following cleaning, the samples were dyed using iodine green and alum carmine. It was eventually determined by microscopic inspection that the samples included essential oil (EO) vesicles [16-18].

*2.2. Essential oils distillation*

By employing the Clevenger apparatus and about a 1 mm sample size, a 1:2 (g/ml) ratio between the material and water, and 240 minutes of distillation, the essential oil (EO) of *A. officinarum* roots was extracted by steam distillation. Physicochemical indexes, chemical composition, and biological activity were assessed after the EO was removed from water with $Na_2SO_4$, and stored at 4 ˚C until use.

*2.3. Analysis of essential oil*

The EO chemical compositions were analyzed by Gas chromatography-mass spectrometry (GC-MS, Thermo Fisher Scientific), which ran at 70 eV in electron impact mode. Operating in SCAN mode (range 35–350 m/z), the mass spectrometer had a scan rate of 0.2 scans per second. The DB-5MS column was used to produce the reported results of the EOs analysis (30 m × 0.25 mm × 0.25 µm, Agilent Technologies, USA), using helium as the carrier gas, and 1 mL/min was employed. The injection system ran at 220 °C in split mode (40:1). The following was the program for the GC oven temperature: 60 °C as the starting temperature, maintained for five minutes, then a 3 °C/min ramp up to 250 °C, which was maintained for 10 minutes. 1 µl of the EO solution (diluted with $CH_2Cl_2$ in a ratio of 1:100 v/v) was injected for each analysis. The EO ingredients were identified by comparing their retention indices (RI), retention times (RT), and mass spectra with those of authentic samples and data already available in the Institute of Standards and Technology version 2.4 (2020).

*2.4. Antioxidant activities*

*2.4.1. DPPH assay*

Referred to Anh et al. [19], the DPPH (1,1-diphenyl-2-picrylhydrazine) free radical scavenging activity was performed as follows: 960 µL of various EO concentrations in methanol were mixed with 40 µl of a methanolic solution containing 1000 µg/mL of DPPH radicals. Additionally, a blank control was applied by combining 960 µl of methanol with 40 µl of DPPH solution. A popular antioxidant, ascorbic acid, was compared. The mixtures were measured spectrophotometrically at 517 nm following 30 minutes in the dark. The following formula (I %) was used to express the DPPH scavenging activity of the extract samples as the percentage of free radical inhibition. The experiment was repeated three times for each concentration of extract. The $IC_{50}$ value was determined by calculating the effective concentrations of the EOs required to scavenge 50 % of the DPPH in the test solution.

$$I(\%) = \frac{A_0 - A_c}{A_0} x100 \quad (1)$$

$A_c$: Samples absorbance; $A_0$: DPPH Absorbance (control)



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*2.4.2. ABTS assay*

The experiment of ABTS + was carried out according to Sun et al. [20] with small adjustments. The reaction was performed at room temperature in the dark for 12 to 16 hours after a 2.45 mM potassium persulfate and 7 mM ABTS solution were combined. This solution was then diluted with ethanol, yielding an absorbance of 0.70 ± 0.05 at 734 nm. A specified quantity of 100 µl of the standard and samples in different concentrations dissolved in 10 % DMSO was added to 900 µl of the diluted ABTS + solution. After six minutes of mixing, the solution was then measured at 734 nm using spectrophotometry. The ABTS + inhibition percentage was calculated in the same way as the DPPH inhibition percentage.

*2.4.3 FRAP assay*

The FRAP of EO was referenced to Berrezig et al. [21]. 0.5 ml of the sample, 0.5 ml of 0.8 M phosphate buffer at pH 7, and 0.5 ml of 1 % potassium ferricyanide were combined and incubated at 50 °C for 20 minutes. Then, 0.5 ml of 10 % trichloroacetic acid was added to the mixture, stirred, and centrifuged for 10 minutes at 3000 rpm. Then, 0.1 ml of 0.1 % $FeCl_3$ and 0.5 ml of distilled water were combined with 0.5 ml of the supernatant. At a wavelength of 700 nm, the concentration at which the Abs value = 0.5 is used to calculate the $EC_{50}$ value [21-22].

*2.5. Antimicrobial activity*

The antimicrobial activity was estimated using the paper plate method, as described by Maleki and Bakhsh (2023) with slight adjustments [23]. A clean tube was filled with four milliliters of Luria-Bertani liquid medium and six of the microorganisms, then mixed at 200 rpm overnight. Next, 200 µl of the mixture ($4\text{-}5 \times 10^8$ CFU/ml) was evenly distributed on a petri dish with Luria-Bertani solid medium. A sterile paper plate (6 mm, Whatman No.1) was then set on top of the Petri plates, and 10 µl of diluted EO ($10^o$–$10^{-1}$) was added to it. The control for the samples was the negative control (DMSO 1 % and Tween 0.1 %) and the positive control (tetracycline 320 µg/ml). After that, the disks were incubated for 18 hours at 37 °C. The diameter of the sterile zone is measured as D (mm), which includes the diameter of the paper disk.

*2.6. Anti-denaturation of Bovine serum albumin assay*

To evaluate the possible anti-arthritic effectiveness of *A. officinarum* EO, the in vitro protein denaturation assay was utilized, with slight modifications, as described in Ameena et al. (2023). A mixture of 0.5 mL of 0.5 % bovine serum albumin (BSA) and 0.5 mL of EO (0.16 mg/mL to 5.00 mg/ml). The mixture was allowed to stand at room temperature for 10 minutes before being heated for 25 minutes at 70 °C. Once the reaction mixture had cooled to room temperature, the absorbance at 660 nm was measured. As a positive control, diclofenac sodium was utilized. The following method was used to determine the % inhibition of protein denaturation:

$$Inhibition\ (\%) = \frac{blank - sample}{blank}\ x\ 100 \quad (2)$$

Where blank is the buffer solution and the control, and the sample is a diclofenac Sodium or tested sample [24, 25].

*2.7. Statistical analysis*

Using the Microsoft Excel application (Microsoft 365), the study's findings were expressed as the average of three separate tests.



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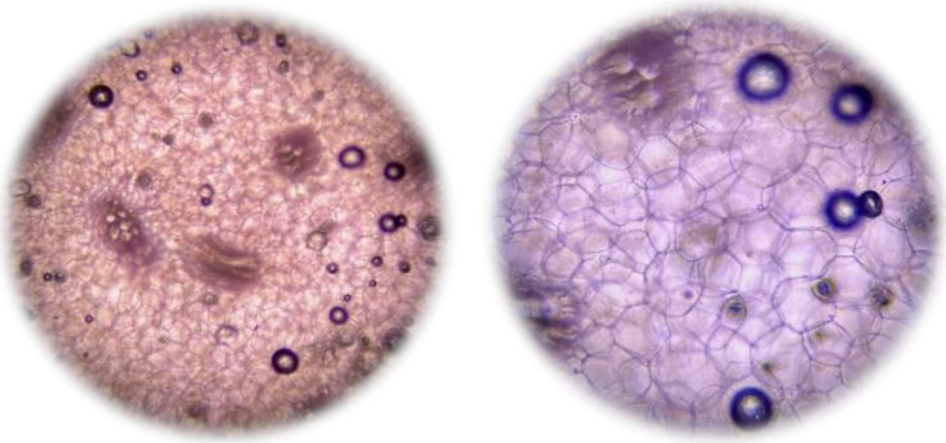
## 3. Results

### *3.1. Studies on anatomy*

The morphology of *A. officinarum* root was displayed in Figure 1. at magnifications of 4X (Fig. 1.a), and 10X (Fig. 1.b). As can be seen from the pictures, the EOs of *A. officinarum* are disseminated as secretory vesicles (circled in red), in large numbers and scattered throughout the root. This observation is similar to that of Trimanto et al., who revealed that the oils of *Alpinia galanga* roots scattered in the parenchyma cells [26].



(a) (b)

**Fig. 1.** Magnification of white *Alpinia officinarum* Hance root at 4X (a) and 10X (b)

### *3.2. Chemical compositions*

The chemical compositions of *A. officinarum* EO are listed in Table 1 and Figure 2, including 27 components, mainly sesquiterpene (50.0 %), followed by monoterpene (39.29 %) and other components (10.71 %). The highest contents are Eucalyptol (24.57 %), *β*-bisabolene (9.72 %), *β*-sesquiphellandrene (5.95 %), *β*-pinene (5.85 %), *α*-bergamotene (5.54 %), *α* -pinene (4.12 %), contributing to the characteristic smell of *A. officinarum*.

**Table 1.** The EO compositions

| No | Name | Area (%) | RT (min) | RI | Class |
|---|---|---|---|---|---|
| 1 | *α* -Pinene | 4.12 | 4.679 | 936 | Monoterpene |
| 2 | *β* -Phellandrene | 1.49 | 5.289 | 940 | Monoterpene |
| 3 | *β* -Pinene | 5.85 | 5.419 | 943 | Monoterpene |
| 4 | *β* -Myrcene | 1.30 | 5.480 | 988 | Monoterpene |
| 5 | 2-Carene | 0.56 | 6.041 | 1011 | Monoterpene |
| 6 | P-Cymene | 0.82 | 6.148 | 1026 | Monoterpene |
| 7 | D-Limonene | 3.66 | 6.278 | 1027 | Monoterpene |
| **8** | **Eucalyptol** | **24.57** | 6.388 | 1046 | Monoterpene |
| 9 | *δ*-Terpinene | 1.05 | 6.772 | 1062 | Monoterpene |
| 10 | Terpinen-4-ol | 3.39 | 9.136 | 1182 | Monoterpene |
| 11 | *α* -Terpineol | 2.04 | 9.390 | 1189 | Monoterpene |

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**Table 1.** The EO compositions (Continued)

| No | Name | Area (%) | RT (min) | RI | Class |
|---|---|---|---|---|---|
| 12 | *β* -Elemene | 1.58 | 12.965 | 1398 | Sesquiterpene |
| 13 | Eugenol Methyl Ether | 2.33 | 13.022 | 1403 | Sesquiterpene |
| 14 | *β* -Caryophyllene | 2.57 | 13.586 | 1418 | Sesquiterpene |
| 15 | *α* -Bergamotene | 5.54 | 13.704 | 1435 | Sesquiterpene |
| 16 | *β* -Farnesene | 1.21 | 13.936 | 1455 | Sesquiterpene |
| 17 | *α* -Caryophyllene | 1.44 | 14.201 | 1457 | Sesquiterpene |
| 18 | *α* -Curcumene | 0.92 | 14.497 | 1478 | Sesquiterpene |
| 19 | Germacrene D | 2.66 | 14.614 | 1481 | Sesquiterpene |
| 20 | Octadecane | 2.60 | 14.719 | 1800 | alkane |
| **21** | ***β* -Bisabolene** | **9.72** | 14.947 | 1509 | Sesquiterpene |
| **22** | ***β* -Sesquiphellandrene** | **5.95** | 15.228 | 1529 | Sesquiterpene |
| 23 | 1,3-Diisopropyl-1,3 Cyclopentadiene | 3.40 | 15.272 | 1012 | Cyclic alkenes |
| 24 | Ar-Tumerone | 2.59 | 17.415 | 1665 | Sesquiterpene |
| 25 | *α* -Tumerone | 1.77 | 17.494 | 1689 | Sesquiterpene |
| 26 | (9E)-9-Icosene | 1.85 | 17.676 | 2017 | Hydrocarbon |
| 27 | *α* -trans-Bergamotol | 1.81 | 17.907 | 1700 | Sesquiterpene |

RT (retention time), RI (retention indices), monoterpene (39.29%), sesquiterpene (50.0%), and others (10.71%).

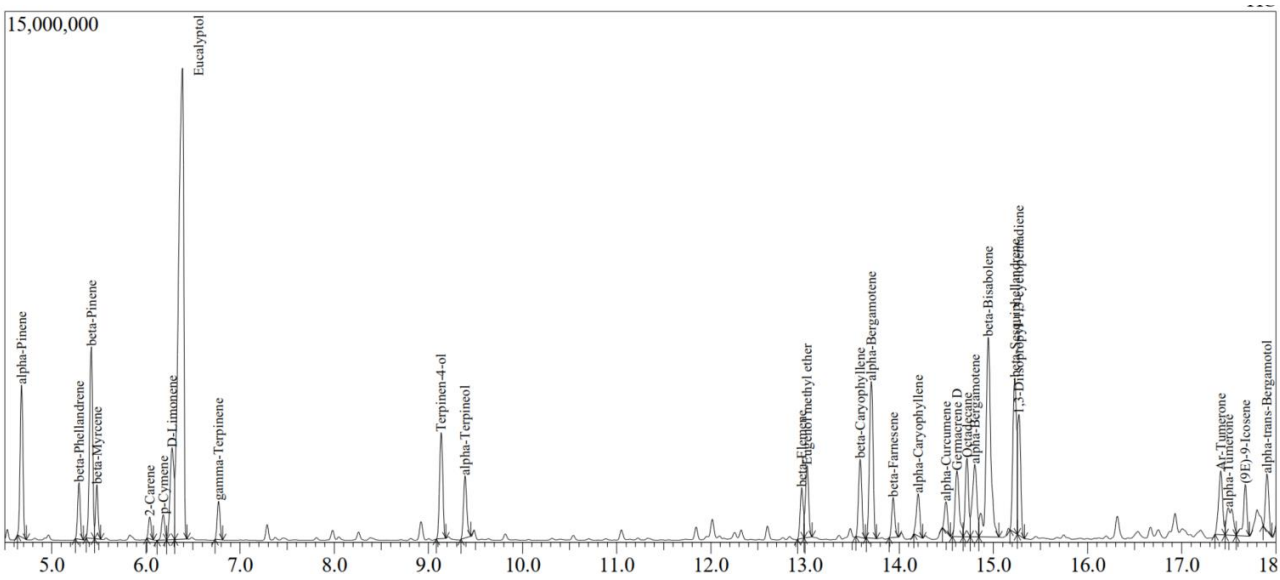


**Fig. 2.** GC-MS spectrum of *A. officinarum* EO

### *3.3. Biological activities*

#### *3.3.1. Antimicrobial activity*

The paper plate method for measuring the antibacterial activity of the six pathogens investigated is shown in Table 2 and Figure 3. The pure EO showed resistance against six different kinds of bacteria, including *Escherichia coli* ATCC 25922TM, *Pseudomonas aeruginosa* ATCC 27853TM, *Salmonella typhimurium* ATCC 13311, *Staphylococcus aureus* ATCC 25923TM, *Bacillus cereus* ATCC 10876TM, and *Listeria innocua* ATCC 33090TM.



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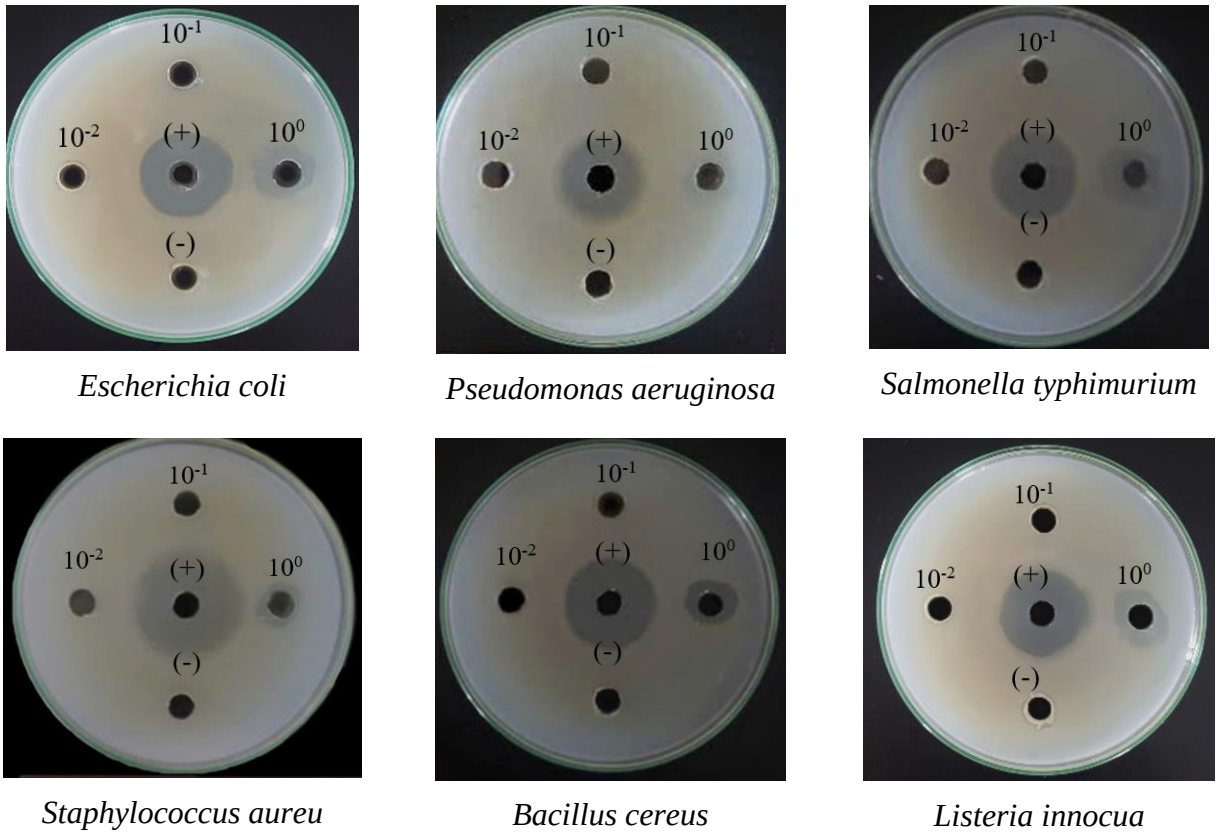
Strongest, the EO has good resistance to *Escherichia coli w*ith a sterile ring diameter of 17.33 ± 0.58 mm, followed by *Salmonella typhimurium* ≈ *Listeria innocua* (16.33 ± 1.50 mm) > *Pseudomonas aeruginosa* (16.00 ± 1.00 mm) > *Bacillus cereus* (15.33 ± 0.58 mm) > *Staphylococcs aureus* (13.33 ± 0.58 mm). When the EO concentration was diluted up to 10 times, the EO did not show antibacterial activity (D = 8.00 ± 0.00 mm), as seen in Table 2.

**Table 2.** Antibacterial efficacy based on sterile ring diameter (D)

| Strains | Sterile ring diameter D (mm) | | | |
|---|---|---|---|---|
| | Control concentration | | EO dilution | |
| | Negative control | Positive control | | |
| | DMSO (1 %) & Tween (0.1 %) | Tetracycline (320 µg/µl) | $10^0$ | $10^{-1}$ |
| *Escherichia coli* ATCC 25922TM | 8.00 ± 0.00 | 23.67 ± 1.15 | 17.33 ± 0.58 | 8.00 ± 0.00 |
| *Pseudomonas aeruginos* ATCC 27853TM | 8.00 ± 0.00 | 22.67 ± 1.15 | 16.00 ± 1.00 | 8.00 ± 0.00 |
| *Salmonella typhimurium* ATCC 13311 | 8.00 ± 0.00 | 23.33 ± 0.58 | 16.33 ± 1.50 | 8.00 ± 0.00 |
| *Staphylococcus aureus* ATCC 25923TM | 8.00 ± 0.00 | 25.66 ± 0.58 | 13.33 ± 0.58 | 8.00 ± 0.00 |
| *Bacillus cereus* ATCC 10876TM | 8.00 ± 0.00 | 25.33 ± 1.15 | 15.33 ± 0.58 | 8.00 ± 0.00 |
| *Listeria innocua* ATCC 33090TM | 8.00 ± 0.00 | 27.00 ± 1.00 | 16.33 ± 0.58 | 8.00 ± 0.00 |




*Escherichia coli* *Pseudomonas aeruginosa* *Salmonella typhimurium*

*Staphylococcus aureu* *Bacillus cereus* *Listeria innocua*

**Fig. 3.** The antibacterial activity of *A. officinarum* EO, (+): Tetracycline 320 µg/mL; (-): DMSO (1.0 %) & Tween (0.1 %)

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*3.3.2. Antioxidant activity*

*3.3.2.1. DPPH assay*

Ascorbic acid was used in this study as a positive control to find the DPPH free radical scavenging activity of the EO. The relationship between ascorbic acid concentration and DPPH free radical inhibition is shown in Fig. 4. When the ascorbic acid concentration increased from 6.0 to 40.0 µg/ml, the inhibition percentage increased from 20.95 % ± 1.1 to 92.99 % ± 0.99. Through the regression equation in Fig. 4, the $IC_{50}$ value of ascorbic acid was determined to be 20.41 µg/mL ± 1.30.

In parallel with the experiment of DPPH free radical inhibition of the ascorbic acid, the DPPH free radical inhibition of the EO was performed in Fig. 5. Through the equation y = 0.0019x - 1.2111, $R^2$ = 0.991, the $IC_{50}$ of the EO was found to be 26953.21 µg/mL ± 0.30. This result indicates that the EO has a weak ability to scavenge DPPH free radicals.

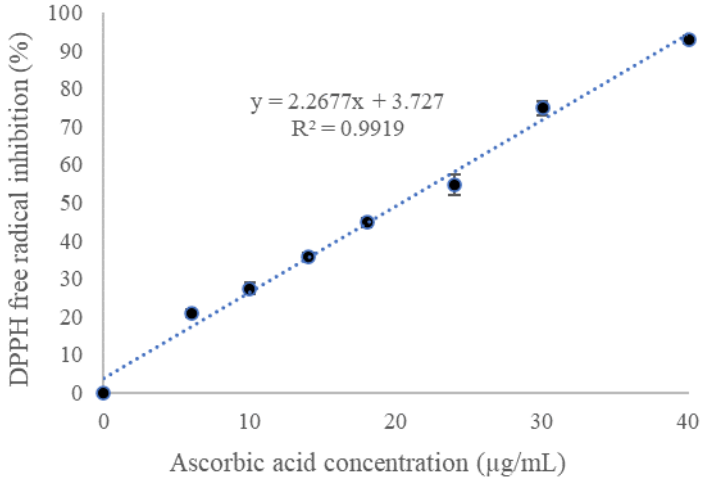


**Fig. 4.** DPPH free radical inhibition of ascorbic acid

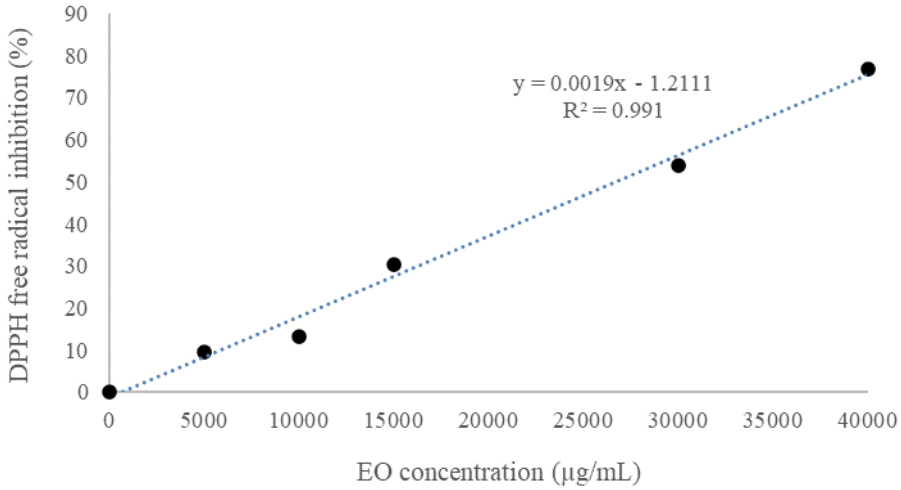


**Fig. 5.** DPPH free radical inhibition of the EO

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*3.3.2.2. ABTS and FRAP Assay*

In addition to the investigation of PDDH free radical activity, the ABTS free radical activity, as well as the ferric-reducing antioxidant power (FRAP) of the EO, were also studied and presented in Table 3, Figure 6, and Figure 7. The EO showed the best ferric-reducing antioxidant power with $IC_{50}$ = 810.00 µg/mL ± 0.01, followed by ABTS free radical scavenging activity ($IC_{50}$ =1343.71 µg/mL ± 0.01).

*3.3.3. Anti-inflammatory*

By protein denaturation assay, the possible anti-arthritic effectiveness of *A. officinarum* EO was performed in Figure 8 and compared with the positive control, diclofenac (Figure 9).

**Table 3.** The antioxidant capacity of *A. officinarum* EO

| Assays | DPPH | ABTS•+ | FRAP |
|---|---|---|---|
| | | ($IC_{50}$, µg/ml) | |
| **Standards (Ascorbic acid)** | 20.41 ± 1.30 (y = 2.2677x + 3.727 $R^2$ = 0.9919) | 3.05 ± 0.10 (y = 16.328x + 0.1301 $R^2$ = 0.9975) | 4.30 ± 0.03 (y = 0.0506x + 0.2823 $R^2$ = 0.9953) |
| **EO** | 26953.21 ± 0.30 (y = 0.0019x - 1.2111 $R^2$ = 0.991) | 1343.71 ± 0.01 (y = 0.0291x + 10.898 $R^2$ = 0.9526) | 810.00 ± 0.01 (y = 0.0003x + 0.257 $R^2$ = 0.9539) |

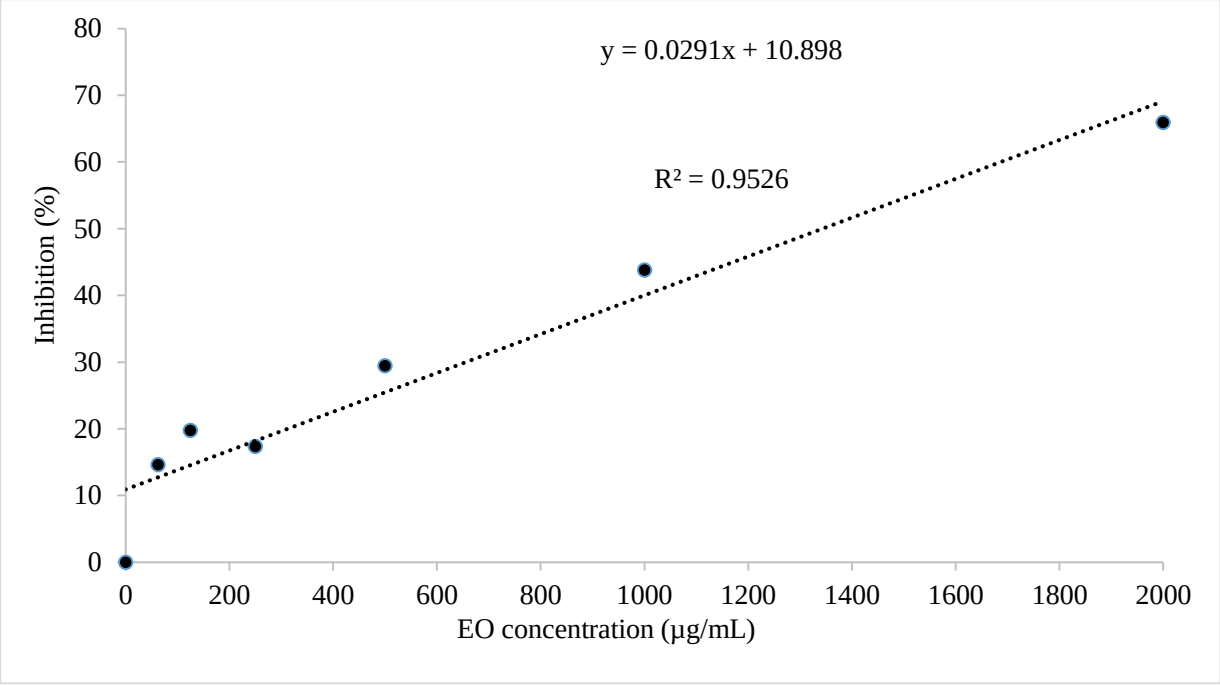


**Fig. 6.** The ABTS inhibition of the EO

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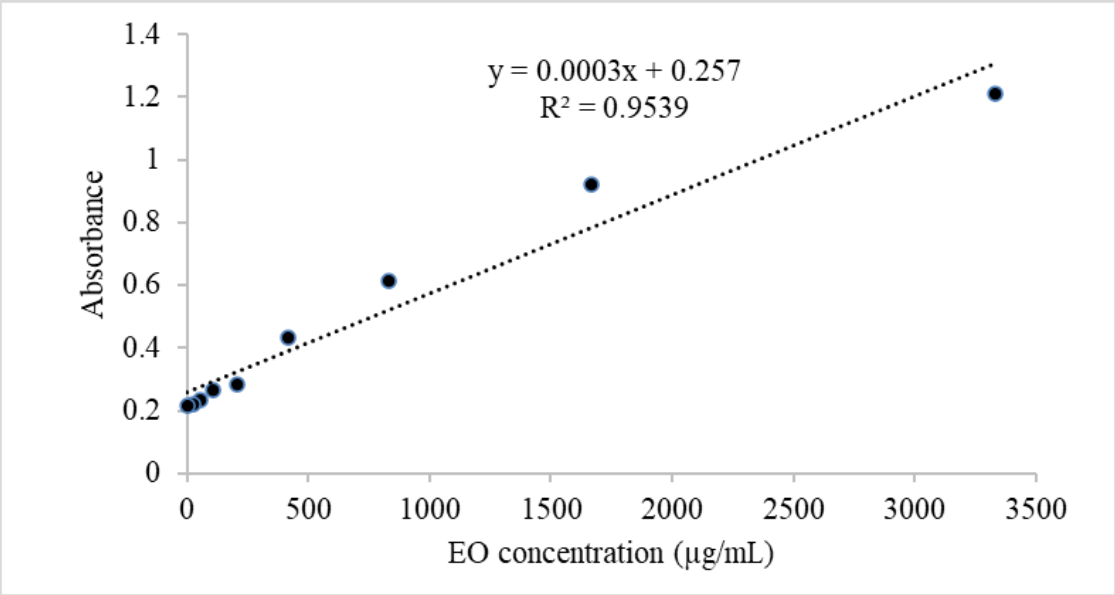


**Fig. 7.** The Ferric reducing antioxidant power (FRAP) of EO

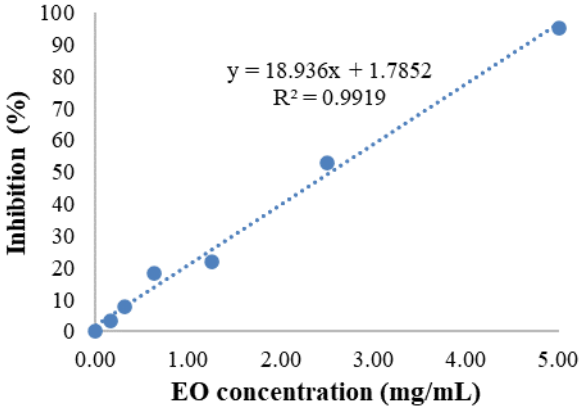


**Fig. 8.** Anti-inflammatory activity of EO

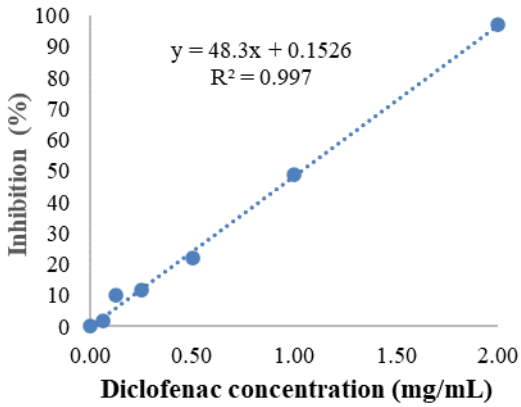


**Fig. 9.** Anti-inflammatory activity of diclofenac

Figure 8 shows that when the EO concentration increases from 0.16 to 5.00 mg/mL, the capacity to inhibit BSA gradually increases from 3.17 % ± 0.02 to 94.99 % ± 0.03. *A. officinarum*'s $IC_{50}$ value was found to be 2.55 mg/ml ± 0.02, which is greater than the standard Diclofenac's $IC_{50}$ value of 1.03 mg/ml ± 0.04 (Figure 9). This suggests that the EO has anti-inflammatory action that is 2.63 times lower than that of Diclofenac. The results demonstrate the anti-inflammatory properties of *A. officinarum* EO.

## 4. Discussion

Chemical profiling of *A. officinarum* EO in three regions is represented in Table 4. As seen in the table, 1,8-Cineole, *γ*-Cadinene, *α*-terpineol, *α*-fenchyl acetate and *β*-Selinene are the main constituents of the EO of *A. officinarum* grown in China and Imphal (India) [27-28]. Meanwhile, *α*-fenchyl acetate,

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1,8-cineole, borneol, bornyl acetate are the main compounds of *A. officinarum* EO in Southern India [29], and Eucalyptol (24.57%), *β*-bisabolene are the main constituents of the EO in Vietnam (this study). Thus, with the same plant, the chemical compositions and contents differ depending on various factors, and the different geographical conditions in which the tree lives are also a contributing factor [30-33].

**Table 4.** Comparison of *A. officinarum* EO's main chemical constituents in different regions

| No | Can Tho, Vietnam (This study) | China [27] | Imphal, India [28] | Southern India [29] |
|---|---|---|---|---|
| 01 | Eucalyptol (24.57 %) | 1,8-Cineole (9.06 %) | 1,8-cineole (28.3 %) | *α*-Fenchyl acetate (40.9 %) |
| 02 | *β*-Bisabolene (9.72 %) | γ-Cadinene (6.39 %) | *α*-fenchyl acetate (15.2 %) | 1,8-cineole (9.4 %) |
| 03 | *β*-Sesquiphellandrene (5.95 %) | α-Terpineol (3.73 %) | carotol (8.9 %) | borneol (6.3 %) |
| 04 | *β* -Pinene (5.85 %) | *β*-Selinene (2.70 %) | *α*-Terpineol (6.7 %) | bornyl acetate (5.4 %) |
| 05 | *α*-Bergamotene (5.54 %) | *α*-Bergamotene (2.46 %) | *β*-pinene (3.1 %) | Elemol (3.1 %) |
| 06 | *α* -Pinene (4.12 %) | *α*-Pinene (0.72 %) | *α*-pinene (1.2 %) | Camphene (2.43 %) |
| 07 | D-Limonene (3.66 %) | D-Limonene (1.24 %) | zingiberenol (1.6 %) | Myrcene (1.8 %) |
| 08 | Terpinen-4-ol (3.39 %) | *β* -Pinene (0.60 %) | terpinen-4-ol (1.2 %) | *p*-Cymene (1.1 %) |

The antimicrobial activity of the EO in this study was similar to that of the EO grown in India [34], as revealed by Indrayan et al. (2007), the zone diameter of inhibition of the EO against the microbial strains ranged from 12.3 – 17.1 mm for the tested strains including *Staphylococcs aureus*, *Bacillus subtilis*, and *Escherichia coli* [34]. Compared with the EOs grown elsewhere, for example, Zhang et al. (2020) studied 10 EO samples harvested in 10 provinces in China and found that the EOs were resistant to *Staphylococcus aureus* and *Bacillus subtilis* with diameters ranging from 2.10 to 10.9 mm. However, eight samples were not resistant to *Escherichia coli*, and nine samples were not resistant to *Pseudomonas aeruginosa* [27]. Thus, the different antibiotic activity of EOs from distinct locations may be due to the influence of their different chemical components.

The DPPH assay showed that the $IC_{50}$ of the EO was 26953.21 μg/ml ± 0.30. This result indicates that the EO has a weak ability to scavenge DPPH free radicals. The result is similar to the outcomes of Rahmi et al. (2024) and Bellarmine et al. (2024), who reported that the $EC_{50}$ values of *A. officinarum* rhizome essential oil were 20400 μg/ml and 37580 μg/ml, respectively, with no special activity towards DPPH [35, 36]. Thus, there appears to be a relationship between the primary chemical composition of oil and its ability to combat DPPH free radicals. Eucalyptol (24.57 %) is the main ingredient in *A. officinarum* EO in this study, as well as the main compound in *A. officinarum* EO (27.52 %) in Bellarmine's report [36] did not show anti-DPPH free radical activity. However, the activity can also be influenced by other ingredients and the interactions between them in the mixture [37, 38].

Regarding ABTS and FRAP Assay, the EO displayed the best ferric-reducing antioxidant power with $IC_{50}$ = 810.00 μg/ml ± 0.01, followed by ABTS free radical scavenging activity ($IC_{50}$ =1343.71 μg/ml ± 0.01). In the



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literature, only two studies about the $IC_{50}$ index of ABTS and the FRAP experiment of the EO were found. As Belamine et al. reported, the *A. officinarum* EO in Morocco has ABTS free radical scavenging ability with $IC_{50}$ = 45230 µg/mL [36], which is 33 times higher than the $IC_{50}$ in this study, or according to Ivanović et al., the EO of the *A. officinarum* showed weak activity toward both ferric reducing power and ABTS [3]. But *A. officinarum* showed strong antioxidant activity in its extracts; for example, Laksmitawati et al. said that the 70 % ethanol extract of *Alpinia galanga* had an $IC_{50}$ for ABTS of 121.20 µg/ml [39]. In addition, the water, ethanol, and water/ethanol extracts (50:50 v/v) of *Alpinia galanga* for ABTS had $IC_{50}$ of 33.007, 16.119, 12.377 µg/mL, and for FRAP of 1.332 ± 0.002, 1.976 ± 0.002 and 2.020 ± 0.003 µg/ml, respectively [40].

It is reported that 10 samples of *A. officinarum* EO exhibit a good inhibitory effect on 12-O-tetradecanoylphorbol-13-acetate-induced mice's ear edema with an inhibition percentage of 64.42 % - 91.62 % compared to the anti-inflammatory medication dexamethasone (86.95 %) [26]. From the in vitro results of Rajendiran et al., it is evident that *A. officinarum* EO efficiently reduced HT-29 cell growth and suppressed the production of inflammatory mediators [41].

## 5. Conclusion

*A. officinarum* essential oil is in the form of secretory vesicles throughout the roots. The essential oil consists mainly of sesquiterpene (50.0 %), then monoterpene (39.29 %). In relation to anti-bacterial properties, the essential oil inhibited six different kinds of bacteria. Regarding anti-inflammatory capabilities, *A. officinarum* roots' essential oil displayed a moderate anti-inflammatory effect with the $IC_{50}$, which is 2.63 times lower than the standard. In terms of antioxidant properties, *A. officinarum* showed good ferric-reducing antioxidant power (FRAP). These biological activity findings demonstrate that essential oils can be utilized in the production of anti-inflammatory agents as well as ferric-reducing antioxidant additives.

## Author contributions

NTBT conducted most of the experiments, including those on antioxidant, antimicrobial, and anti-inflammatory activities. TTM performed the antimicrobial experiment, while HLH carried out the anatomy, and chemical compounds were analyzed by CLNH. All authors read and approved the final manuscript.

## Conflicts of interest

No potential conflict of interest was reported by the authors.

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