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# Volatile Composition and Bioactivity Assessment of *Wurfbainia schmidtii* Essential Oils from Thailand

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ABSTRACT

This research examined the volatile composition and biological activities of essential oils obtained from *Wurfbainia schmidtii*, collected from Western Thailand. Essential oils were extracted from the aerial parts (leaves and stems) and rhizomes, and analyzed using headspace solid-phase microextraction (HS-SPME) and gas chromatography-mass spectrometry (GC/MS). The analysis revealed 25 volatile compounds in the aerial oil and 33 in the rhizome oil. Both oils demonstrated potent bioactivity in inhibiting tyrosinase, with $IC_{50}$ values of 0.55 µg/mL and 1.24 µg/mL for aerial and rhizome oils, and significant antioxidant potential ($IC_{50}$ = 12.94 mg/mL and 27.07 mg/mL, respectively). The oils also exhibited moderate to low antibacterial activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, and methicillin-resistant *Staphylococcus aureus* (MRSA), with MIC and MBC values between 50 and 100 mg/mL. These findings highlighted the potential of *W. schmidtii* essential oils in pharmaceutical and cosmetic applications, particularly for their antioxidative and skin-enhancing properties.

***Keywords*:** Antibacterial activities, Antioxidant potential, Essential oil, Tyrosinase inhibition, Volatile composition, *Wurfbainia schmidtii*

## Introduction

The Zingiberaceae family, comprising a diverse range of species, is widespread across tropical regions, especially in Southeast Asia. In Thailand, with its favorable climatic and geographical diversity, this family includes over 300 species from 26 genera as a significant contributor to the country's rich botanical landscape. *Wurfbainia schmidtii* (K.Schum.) Škorničk. & A.D. Poulsen, previously classified under the genus *Amomum*, has been recently reclassified based on molecular phylogenetic studies.[1] Commonly known as Wan Sao Long in Thailand, this species predominantly inhabits the northern, northeastern, central, and western regions, thriving especially well in the high-altitude areas of Ratchaburi Province. Plants in these regions exhibit more substantial growth compared to their counterparts elsewhere. This perennial herb is characterized by smooth fruits, densely pubescent pseudostems, and a distinctive aroma that permeates all parts of the plant. *W. schmidtii* has long been valued in traditional medicine and its essential oils have recently garnered attention for their potential applications. However, the chemical composition and pharmacological activities of these oils, particularly those from the high-altitude western regions, remain largely unexplored.Essential oils are complex mixtures of volatile compounds such as terpenes, alcohols, aldehydes, and ketones, synthesized primarily through secondary metabolism in aromatic plants.[2] These compounds play crucial roles in plant ecological interactions, acting as defenses against herbivores and pathogens.

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Common extraction techniques for these oils include hydrodistillation, steam distillation, and supercritical fluid extraction.[3] Apart from their ecological functions, essential oils have significant industrial value, with applications in pharmaceuticals, cosmetics, agriculture, and the food industry, driven by their antimicrobial, antioxidant, and anti-inflammatory properties.[4,5] Recent research on Zingiberaceae essential oils from Thailand has focused on their chemical profiles and biological activities, highlighting their potential in antimicrobial, antioxidant, and enzyme inhibitory applications.[6-9] These studies underscore the need for more detailed chemical profiling to comprehend the variations in bioactivity among species and across different regions.

Headspace solid-phase microextraction (HS-SPME) is a highly efficient technique for isolating volatile compounds, favored for its solvent-free approach, simplicity, and capacity to capture a broad range of aromatic molecules.[10] When combined with gas chromatography-mass spectrometry (GC/MS), HS-SPME facilitates precise analysis of essential oil compositions, making it a powerful tool for investigating a variety of plant species.[11-24] Despite extensive research on essential oils from various Zingiberaceae species, studies focusing on *W. schmidtii*, particularly those from high-altitude regions in western Thailand, remain limited. This study addresses these gaps by systematically characterizing the volatile components and bioactivities of essential oils derived from the aerial parts (leaves and stems) and rhizomes of *W. schmidtii*. HS-SPME coupled with GC/MS was employed to identify and quantify the key volatile constituents of the essential oils. The antioxidant, tyrosinase inhibitory, and antibacterial properties of these oils were also systematically evaluated. The results contribute to a deeper understanding of *W. schmidtii* essential oils and provide a foundation for their sustainable utilization and development into value-added products that benefit both industry and local communities.

## Materials and Methods

*Plant material and microbial strains*

Three-year-old fresh aerial parts and rhizomes of *Wurfbainia schmidtii* (K.Schum.) Škorničk. & A.D.Poulsen (Figure 1) were collected in May

2023 from a plantation in Suan Phueng, Ratchaburi Province, Thailand (13°35'31.3"N, 99°12'45.5"E). The plant specimens were authenticated at the Herbarium of Mahidol University, Thailand and assigned voucher specimen numbers PBM 006357-006358. This study utilized three bacterial strains: *Staphylococcus aureus* DMST 8840, *Staphylococcus epidermidis* DMST 15505, and methicillin-resistant *Staphylococcus aureus* (MRSA) DMST 20651, all sourced from the Department of Medical Sciences, Ministry of Public Health, Thailand.

*Extraction of essential oils and analysis of physical properties*

The plant material was thoroughly washed to eliminate any soil particles and then divided into two components: aerial portions and rhizomes, both finely chopped. Approximately 1 kg of the sample underwent hydrodistillation using a Clevenger apparatus for 6 hours or until no further essential oil was obtainable. The essential oil was collected, and the yield was calculated as a percentage of the dry weight (w/w), with each extraction conducted in triplicate to ensure reproducibility. Following extraction, the oil was dried over anhydrous sodium sulfate to remove any residual moisture and subsequently stored in amber glass bottles in a refrigerator at 4°C until further analysis. The refractive index and optical rotation of the essential oil were determined using a handheld refractometer (Atago PAL-1, Tokyo, Japan) and a Bellingham Stanley ADP 220 polarimeter, respectively.

*Volatile composition analysis*

The volatile compositions of the essential oils were extracted using HS-SPME with divinylbenzene-carboxen-polydimethylsiloxane (DVB-CAR-PDMS) fibers. Ten microliters of each essential oil sample were transferred into a 20 mL headspace vial and enriched at 60°C for 30 minutes. Following enrichment, the fiber was placed in a GC injector and desorbed for 10 minutes at 220°C. The extracted volatiles were analyzed using GC/MS on a 7890A gas chromatograph equipped with a 5975C quadrupole mass spectrometer (Agilent Technologies, USA) and an HP-5MS capillary column (0.25 mm i.d. × 30 m, 0.25 µm film thickness). The GC injector operated in split mode with a 1:20 v/v split ratio, and 1 µL of the sample was injected. Both the injector and detector were maintained at 240°C. The column temperature was increased from 60°C to 240°C at a rate of 3°C/min and held at 240°C for 5 minutes. Helium was used as the carrier gas at a flow rate of 1 mL/min. Mass spectra were obtained in electron impact mode at 70 eV, scanning a mass range of 40-550 m/z. A mixture of $C_8$-$C_{20}$ n-alkanes (Sigma-Aldrich, USA) served as a standard, and retention indices (RIs) were calculated based on the chromatographic results according to the method of Vandendool and Kratz.[25] Compound identification was accomplished by comparing retention times and mass spectra with authentic standards, as well as using the "W11N17" (Wiley11-Nist17, Wiley, USA) mass spectral libraries.

*Determination of antioxidant properties*

*DPPH radical scavenging assay*

The free radical scavenging activity of the essential oils was assessed by measuring the extent of bleaching of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical solution, based on the modified method of Brand-Williams *et al.*[26] Different concentrations of essential oil samples (50 µL each) were mixed with 200 µL of DPPH solution (0.1 mM) in a 96-well plate. The mixtures were incubated in the dark at room temperature for 30 minutes, after which the absorbance was measured at 517 nm using a spectrophotometer (Genesys 10S, Thermo Scientific, Waltham, MA, USA). A DPPH solution without essential oil was used as a control, with trolox (≥97% purity, Sigma-Aldrich, USA) as the positive control. The scavenging activity was calculated using linear regression analysis according to the following equation 1:

$$\text{Radical scavenging (\%)} = \left(\frac{C - S}{C}\right) \times 100 \quad (1)$$

where *C* is the absorbance of the control and *S* is the absorbance of the sample.

The $IC_{50}$ value was determined from concentration-response curves through interpolation.

*ABTS radical scavenging assay*

The ability of essential oils to scavenge the 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{\bullet+}$) radical was evaluated following the modified protocol of Cai *et al.*[27] An $ABTS^{\bullet+}$ solution was generated by reacting a 7 mM stock solution of ABTS with 2.45 mM potassium persulfate and allowing it to stand in the dark at room temperature for 16 hours before analysis. This $ABTS^{\bullet+}$ solution was subsequently diluted with ethanol to an absorbance of 0.7 ± 0.05 at 734 nm.

Essential oil samples (50 µL each) were then combined with 150 µL of the $ABTS^{\bullet+}$ solution in a 96-well plate, and the absorbance was measured spectrophotometrically at 734 nm after a 6-minute incubation. Trolox was used as the positive control. The scavenging activity against $ABTS^{\bullet+}$ and the $IC_{50}$ values were calculated using the same methodology as for the DPPH assay.

*Determination of tyrosinase inhibitory activity*

A modified version of the tyrosinase inhibition assay based on the protocol by Chen *et al.*[28] was employed. Each essential oil sample (10 µL) was combined with 40 µL of sodium phosphate buffer (50 mM, pH 6.8) and 70 µL of tyrosinase solution (100 Units/mL in phosphate buffer) in a 96-well plate. The reaction mixture was incubated at 25°C for 10 minutes. Then, 80 µL of substrate (2.5 mM L-tyrosine or L-DOPA) was added to each well. The incubation was continued for 30 minutes at 25°C for L-tyrosine and 10 minutes for L-DOPA. Absorbance at 475 nm was measured using a UV-Vis spectrophotometer, with kojic acid (≥98.5% purity, Sigma-Aldrich, USA) as the positive control. The percentage inhibition of tyrosinase was calculated using the following formula (equation 2):

$$\text{Inhibition (\%)} = \left[1 - \left(\frac{S}{C}\right)\right] \times 100 \quad (2)$$

where *C* and *S* denote the absorbance of the control and the sample, respectively.

The $IC_{50}$ values, representing the concentration of the sample that inhibited 50% of tyrosinase activity were also determined.

*Assessment of antibacterial activities*

The essential oil samples were first dissolved in dimethyl sulfoxide (DMSO) and then sterilized through a 0.45 µm syringe filter. The sterile solutions were diluted via a two-fold serial dilution process to reach the desired concentrations. Bacterial strains were cultured on nutrient agar slants and maintained with an inoculum size of $10^8$ CFU/mL for each strain under examination. The MIC of the essential oil solutions was determined using a modified resazurin-based microdilution method, as outlined by Sarker *et al.*[29] Serial two-fold dilutions of the essential oils were prepared in a 96-well microtiter plate with nutrient broth, and bacterial suspensions were then added. Negative control wells contained only bacterial suspension, while positive controls contained nutrient broth and essential oil solutions. After 24 hours of incubation at 37°C, resazurin solution was added to each well as an indicator of bacterial viability, with a color change from blue to pink (or colorless) indicating growth. The MIC was recorded as the lowest concentration of essential oil that inhibited this color change. The MBC was determined following the method of Basri and Fan.[30] Wells showing no visible growth were subcultured onto sterile agar plates. The MBC was defined as the lowest concentration at which no bacterial growth was observed. Erythromycin was used as the standard antibiotic control.

*Statistical analysis*

The experimental data were analyzed using IBM SPSS Statistics software, version 22.0 (IBM Corp., Armonk, NY, USA; released 2013). Results are presented as mean ± standard deviation (SD).

## Results and Discussion

*Isolation of essential oils, physical properties, and volatile composition analysis*

The oil yield and physical properties of the essential oils extracted from *W. schmidtii* are summarized in Table 1. The essential oils displayed a clear yellow to light green color, with yields ranging from 0.37% to 0.76% based on dry weight. Notably, the oil extracted from the rhizomes yielded approximately twice as much as the oil obtained from the aerial parts. The refractive indices of the essential oils from both plant parts were almost identical, and both displayed negative optical rotation. The chemical profiles of the essential oils were further

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**Figure 1:** *Wurfbainia schmidtii* trees from a plantation in Ratchaburi Province, Thailand

**Table 1:** Yield (%), refractive index and specific rotation of *W. schmidtii* essential oils

| Plant part | Yield (%)[a] | Refractive index (RI) | Specific rotation |
|---|---|---|---|
| Aerial parts | 0.37 ± 0.05 | 1.368 | 10.5 |
| Rhizomes | 0.76 ± 0.05 | 1.372 | -12.4 |

[a]Results are expressed as the mean ± standard deviation (SD) of triplicate measurements (n = 3).

investigated through HS-SPME-GC/MS analysis. As shown in Figures 2 and 3, the total ion chromatograms illustrate the separation of volatile components, and the identified compounds with their corresponding relative contents are detailed in Table 2.

Twenty-five volatile compounds were identified in the essential oil derived from the aerial parts, while 33 compounds were detected in the rhizome oil. The major compounds in both oils were 1-[(1E)-1-butenyl]-4-methoxybenzene (Figure 4a), a eugenol derivative, which constituted 60.48% of the aerial oil and 55.74% of the rhizome oil, and camphor (Figure 4b), which accounted for 9.34% and 7.92% of the aerial and rhizome oils, respectively. These results concurred with Uthairung *et al.*[31], who sampled local markets in Eastern Thailand and identified 44 compounds in the aerial oil and 37 in the rhizome oil. They also found 1-[(1E)-1-butenyl]-4-methoxybenzene (86.42%-92.63%) and camphor (1.39%-1.75%) as the dominant constituents. By contrast, Singtothong *et al.*[32] reported camphor (17.60%) and α-bisabolol (16.0%) as the most prevalent components in the whole plant essential oil from Central Thailand. Dung *et al.*[33] documented a high concentration of 1-[(1E)-1-butenyl]-4-methoxybenzene (90-95%) in essential oils extracted from the leaves, stems, and roots of this plant species in Vietnam.

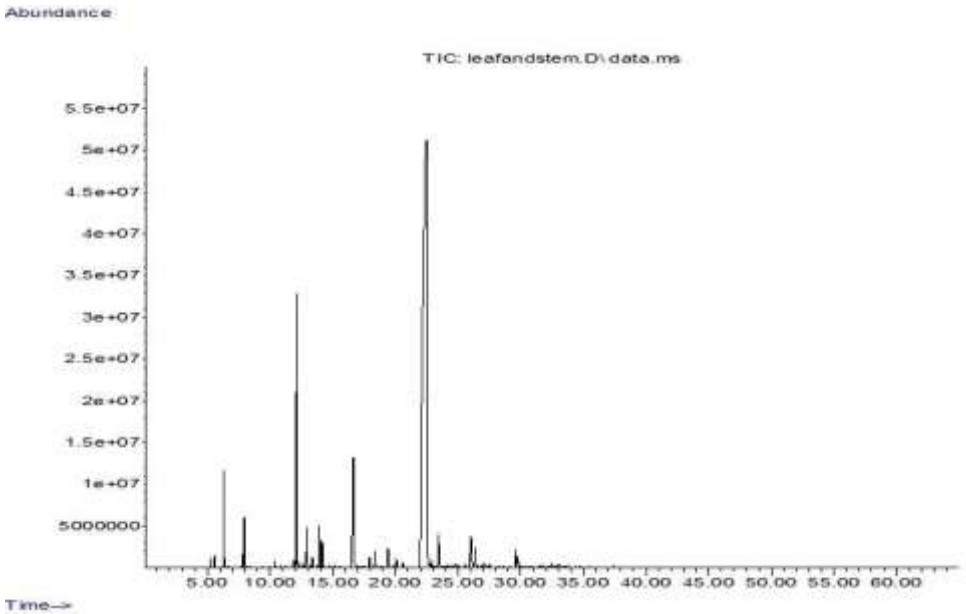


**Figure 2:** Total ion chromatogram of *W. schmidtii* essential oil from aerial parts

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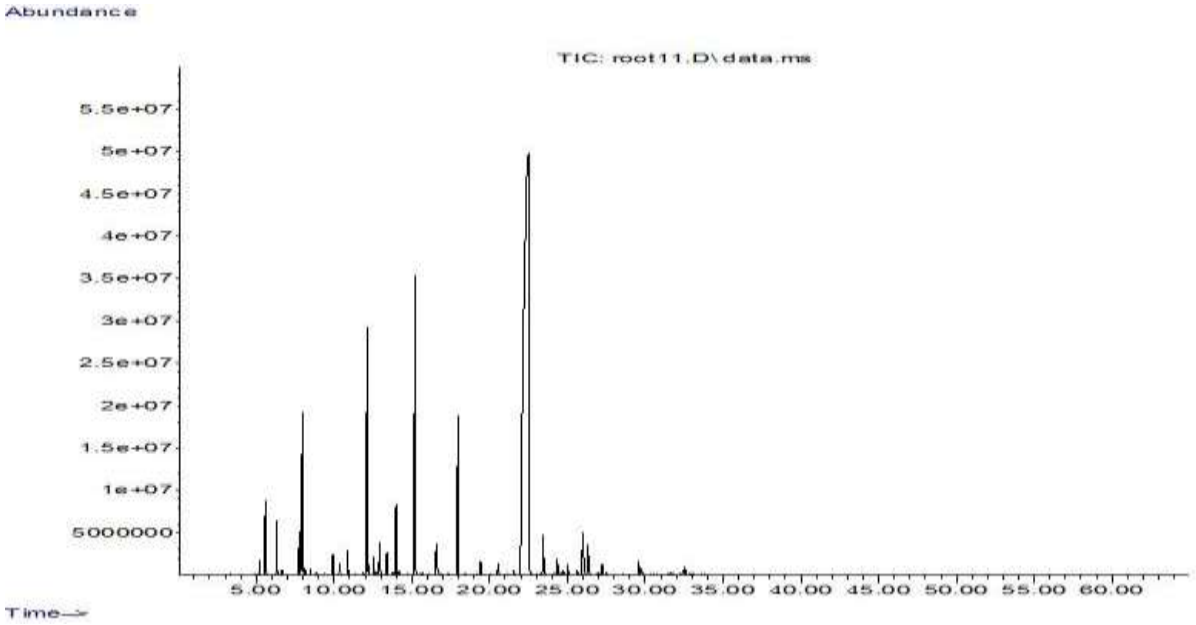


**Figure 3:** Total ion chromatogram of *W. schmidtii* essential oil from rhizomes

**Table 2:** Relative contents, retention indices and volatile compositions of *W. schmidtii* essential oils

| No | Volatile compound | RI[a] | Relative content (%) | |
|---|---|---|---|---|
| | | | Aerial | Rhizomes |
| 1 | *α*-Pinene | 911 | 0.15 ± 0.07 | 0.28 ± 0.09 |
| 2 | Camphene | 916 | 0.22 ± 0.11 | 1.32 ± 0.05 |
| 3 | *β*-Pinene | 927 | 1.52 ± 0.02 | 0.99 ± 0.07 |
| 4 | *β*-Myrcene | 932 | 0.06 ± 0.02 | 0.12 ± 0.04 |
| 5 | 1,8-cineole | 1009 | 1.14 ± 0.05 | 4.65 ± 0.35 |
| 6 | *γ*-Terpinene | 1018 | 0.03 ± 0.01 | 0.07 ± 0.01 |
| 7 | Fenchone | 1028 | 0.04 ± 0.02 | 0.53 ± 0.17 |
| 8 | Linalool | 1033 | 0.17 ± 0.07 | 0.30 ± 0.02 |
| 9 | Fenchol | 1103 | 0.08 ± 0.03 | 0.65 ± 0.04 |
| 10 | **Camphor** | 1112 | 9.34 ± 1.60 | 7.92 ± 0.08 |
| 11 | Isoborneol | 1115 | - | 0.49 ± 0.19 |
| 12 | endo-Borneol | 1117 | 1.03 ± 0.31 | 1.11 ± 0.02 |
| 13 | Terpinen-4-ol | 1121 | 0.30 ± 0.09 | 0.57 ± 0.02 |
| 14 | *α*-Terpineol | 1125 | 1.65 ± 0.15 | 1.08 ± 0.30 |
| 15 | Fenchyl acetate | 1204 | 0.09 ± 0.05 | 10.00 ± 1.32 |
| 16 | D-Carvone | 1209 | 0.06 ± 0.01 | 0.09 ± 0.01 |
| 17 | Anisaldehyde | 1212 | 3.94 ± 0.05 | 1.21 ± 0.07 |
| 18 | Isobornyl acetate | 1219 | 0.35 ± 0.09 | 4.59 ± 0.37 |
| 19 | Methyl myrtenate | 1221 | 0.49 ± 0.10 | - |
| 20 | *α*-Terpinyl propionate | 1309 | - | 0.34 ± 0.06 |
| 21 | Copaene | 1313 | - | 0.13 ± 0.02 |
| 22 | 1-[(1E)-1-Butenyl]-4-methoxybenzene | 1317 | 60.48 ± 4.29 | 55.74 ± 2.65 |
| 23 | Caryophyllene | 1403 | 1.13 ± 0.12 | 1.32 ± 0.23 |
| 24 | *α*-Humulene | 1407 | - | 0.15 ± 0.06 |
| 25 | Aromandendrene | 1405 | 0.28 ± 0.02 | - |
| 26 | Alloaromadendrene | 1409 | 0.13 ± 0.02 | 0.32 ± 0.09 |
| 27 | Aristolochene | 1412 | 1.26 ± 0.14 | 1.33 ± 0.18 |

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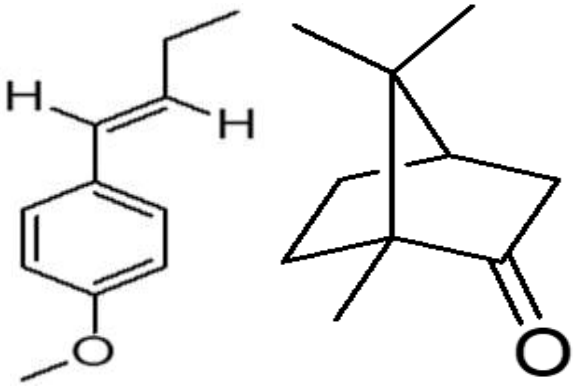
| | | | | |
|---|---|---|---|---|
| 28 | $\alpha$-Selinene | 1414 | 0.59 ± 0.04 | 1.14 ± 0.25 |
| 29 | $\beta$-Bisabolene | 1501 | 0.14 ± 0.03 | 0.11 ± 0.03 |
| 30 | 7-epi- $\alpha$-Selinene | 1502 | - | 0.36 ± 0.16 |
| 31 | $\delta$-Cadinene | 1503 | - | 0.11 ± 0.06 |
| 32 | $\alpha$-Calacorene | 1505 | - | 0.06 ± 0.02 |
| 33 | $\beta$-Elemene | 1509 | - | 0.35 ± 0.07 |
| 34 | $\alpha$-Eudesmol | 1603 | - | 0.34 ± 0.13 |
| 35 | Maaliol | 1606 | - | 1.02 ± 0.27 |
| | Total identified | | 84.67 | 98.79 |



(a) 1-[(1E)-1-Butenyl]-4-methoxybenzene (b) Camphor

**Figure 4:** Chemical structures of major components in *W. schmidtii* essential oils

Research has shown that essential oils with camphor as a predominant component demonstrate antimicrobial, anticancer, cough-suppressant, and enhanced transdermal absorption properties.[34] However, only a few studies have investigated the bioactivity of 1-[(1E)-1-butenyl]-4-methoxybenzene. Our study underscores the need for a reliable quantification method for 1-[(1E)-1-butenyl]-4-methoxybenzene as a quality marker in *W. schmidtii* essential oils in Thailand's herbal medicine market.

**Table 3:** Antioxidant activities ($IC_{50}$, mg/mL) of *W. schmidtii* essential oils and trolox determined by DPPH and ABTS assays

| Sample | $IC_{50}$ by DPPH assay | $IC_{50}$ by ABTS assay |
|---|---|---|
| Aerial parts | 12.94 ± 0.10 | 18.94 ± 0.17 |
| Rhizomes | 27.07 ± 0.08 | 20.75 ± 0.14 |
| Trolox | 20.16 ± 0.05 | 80.32 ± 0.09 |

Results are expressed as the mean ± standard deviation (SD) of triplicate measurements (n = 3).

*Determination of antioxidant properties*

The radical scavenging activities of *W. schmidtii* essential oils were evaluated through the DPPH and ABTS assays, which are widely recognized for their reliability, rapid execution, and reproducibility in antioxidant research. The $IC_{50}$ values, which indicate the concentration required to scavenge 50% of DPPH and ABTS radicals, are presented in Table 3 with the corresponding values for trolox, a standard antioxidant reference. Results revealed that both essential oils from *W. schmidtii* exhibited notable antioxidant activity.

The essential oil extracted from the aerial parts demonstrated significantly higher antioxidant effectiveness compared to the rhizome oil and trolox in both assays. Interestingly, while the rhizome-derived oil surpassed trolox in the ABTS assay, it was less effective than trolox in the DPPH assay.

*Determination of tyrosinase inhibitory activity*

The inhibitory activity of *W. schmidtii* essential oils against tyrosinase was assessed, using kojic acid as a positive control, with the $IC_{50}$ values summarized in Table 4. Both essential oils exhibited significantly stronger tyrosinase inhibition compared to the positive control, with the essential oil extracted from the aerial parts displaying the highest inhibitory activity.

**Table 4:** Inhibition of tyrosinase ($IC_{50,}$ µg/mL) by *W. schmidtii* essential oils and kojic acid

| Sample | $IC_{50}$ |
|---|---|
| Aerial parts | 0.55 ± 0.01 |
| Rhizomes | 1.24 ± 0.03 |
| Kojic acid | 144.53 ± 2.59 |

Results are expressed as the mean ± standard deviation (SD) of triplicate measurements (n = 3).

These findings underscored the significant potential of *W. schmidtii* essential oils as active ingredients in cosmetic formulations targeting skin depigmentation. In light of the growing consumer preference for natural over synthetic ingredients in cosmetic products,[35] *W. schmidtii* essential oils offer a particularly compelling and sustainable choice as depigmenting agents in skincare applications.

*Antibacterial activity evaluation*

The antibacterial activities of the essential oils were assessed against three human pathogenic strains: *S. aureus* DMST 8840, *S. epidermidis* DMST 15505, and methicillin-resistant *S. aureus* (MRSA) DMST 20651. These test strains consist of Gram-positive bacteria associated with dermal infections. MRSA, a strain of *S. aureus*, has developed resistance to methicillin and beta-lactam antibiotics,[36] as well as to other antibiotic classes including erythromycin, gentamicin, and tetracyclines.[37] Both essential oils exhibited antibacterial activity against all the tested strains, with MIC and MBC values ranging from 50 to 100 mg/mL (Table 5).

**Table 5:** Minimal inhibitory concentration (MIC, mg/mL) and minimal bactericidal concentration (MBC, mg/mL) for *W. schmidtii* essential oils and erythromycin

| Sample | *S. aureus* | | *S. aureus* (MRSA) | | *S. epidermidis* | |
|---|---|---|---|---|---|---|
| | MIC | MBC | MIC | MBC | MIC | MBC |
| Aerial parts | 100 | 100 | 50 | 100 | 100 | 100 |
| Rhizomes | 50 | 100 | 50 | 100 | 50 | 100 |
| Erythromycin | 0.01 | 0.01 | 0.01 | 0.01 | 3.13 | 3.13 |

These findings suggested that *W. schmidtii* essential oils exhibited moderate to low antibacterial activity against the tested strains compared to standard erythromycin. Essential oils derived from plants

could serve as promising candidates for developing effective, natural, and safe antimicrobial agents. Enhancing the efficacy of these oils, Particularly through nano-encapsulation, could increase their effectiveness against drug-resistant pathogens.[38] Future research should prioritize the application of advanced technologies and investigate the synergistic interactions between antimicrobial agents to maximize their therapeutic potential in pharmaceutical applications.

## Conclusion

Twenty-five volatile compounds were identified in the aerial oil and 33 in the rhizome oil of *W. schmidtii* collected from Western Thailand, with 1-[(1E)-1-butenyl]-4-methoxybenzene and camphor as the major constituents. The essential oils exhibited potent bioactivity, particularly in tyrosinase inhibition ($IC_{50}$: 0.55 µg/mL for aerial oil and 1.24 µg/mL for rhizome oil), underscoring their potential as promising candidates for cosmetic applications targeting skin depigmentation. Their antioxidant properties ($IC_{50}$: 12.94 mg/mL for aerial oil and 27.07 mg/mL for rhizome oil) highlighted the capacity to combat oxidative stress, which is crucial in anti-aging and skin protection formulations. The essential oils demonstrated moderate to low antibacterial effects against *S. aureus*, *S. epidermidis*, and MRSA (MIC and MBC values of 50-100 mg/mL). Further enhancement through nano-encapsulation offers a promising avenue for improving efficacy, especially against drug-resistant pathogens.

## Conflict of Interest

The authors declare no conflict of interest.

## Authors' Declaration

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

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