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Original Article

# *Kaempferia attapeuensis* Picheans. & Koonterm Growing Wild in Vietnam: Chemical Composition of Fresh Rhizomes Essential Oil and Its *In vitro* Antimicrobial Activity

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**Abstract:** *Kaempferia* is a genus of the Zingiberaceae family containing a rich resource of essential oils (EOs) with various important biological activities. In the present study, the chemical compositions and *in vitro* antimicrobial activity of the EO of *Kaempferia attapeuensis* Picheans. & Koonterm were reported for the first time. By the Gas Chromatography-Mass Spectrometry (GC/MS) analysis, a number of 46 compounds were identified in *K. attapeuensis* rhizomes EO (accounted for 90.61 % of the total content), among which germacrone (25.80%), camphene (18.63%) and *α*-pinene (10.57%) were the major components. In the *in vitro* antimicrobial assay, *K. attapeuensis* rhizomes EO exhibited promising activity against the growth of three

Gram-positive bacteria strains (*Enterococcus faecalis*, *Staphylococcus aureus*, and *Bacillus cereus*), three Gram-negative bacteria strains (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella enterica*) and the yeast strain (*Candida albicans*) with their MIC values all of 32 µg/mL. In general, the present investigation has provided the foundation for additional research on the chemical compositions and biological effects of *K. attapeuensis.*
**Keywords:** *Kaempferia attapeuensis*, essential oil, chemical composition, antimicrobial activity.

## Introduction

Genus *Kaempferia* L. belongs to the Zingiberaceae family, which is widely dispersed throughout Asian countries [1]. *Kaempferia* species are commonly used as traditional medicines for various diseases, including infectious diseases, wound infections, coughs, discomfort, and digestive issues [1]. Studies on the phytochemicals of *Kaempferia* plants have revealed that diterpenoids are the main components, besides other compounds such as flavonoids, phenolic glycosides, cyclohexane oxide derivatives, and diarylheptanoids [1,2]. Particularly, the EOs from *Kaempferia* species are significant sources of compounds with biological activity such as anti-inflammatory, anti-microbial, larvicidal, and antioxidant properties [1-6].

*Kaempferia attapeuensis* Picheans. & Koonterm, a medicinal plant of the *Kaempferia* L. genus (Zingiberaceae) is an endemic species to Laos, then this plant was first recorded in the flora of Vietnam in 2018 [7]. *K. attapeuensis* has a slender, long creeping rhizome that produces clustered roots. The leaves are sessile, oblong-elliptic to broadly ovate, with glabrous surfaces. The inflorescence is sessile to pedunculated, with 3-13 flowers. Locally, the leaves and roots of *K. attapeuensis* Picheans. & Koonterm is used for various purposes. The leaves are used to prepare fish salad and the roots are employed in treating stomach pain and stimulating digestion, particularly in the Gia Lai province of Vietnam [7]. Based on our knowledge, there are no reports on the chemical composition and antimicrobial activity of *K. attapeuensis* EO. Hence, this is the first study on the chemical constituents of *K. attapeuensis* and its corresponding antimicrobial assay.

## Material and methods

### *Chemicals*

Streptomycin and cycloheximide were purchased from Sigma-Aldrich. Sodium sulfate, dimethyl sulfoxide, and *n*-hexane were purchased from Merck. All other chemicals were of analytical grade.

### *Plant material*

Fresh rhizomes of *K. attapeuensis* were obtained in June 2022 from Thanh Tan, Tay Ninh, Vietnam (11°23'16.2"N 106°08'49.7"E) and were identified by Assoc. Prof. Ph.D. Nguyen Hoang Tuan. The voucher specimen of *K. attapeuensis* (No. NHTuan 26) was deposited in the Herbarium of Hanoi National University, Vietnam.

### *Hydrodistillation of the essential oil*

The fresh rhizomes of *K. attapeuensis* were cleansed, diced into small pieces, and hydrodistilled using a Clevenger-type apparatus for 4 h. The vapor mixture of distillate and EO was condensed into a separator. The EO was then separated from the water using a separator funnel. To further remove any remaining moisture, the resulting EO was dehydrated using anhydrous sodium sulfate and subsequently kept at 4°C in a dark vial before use. The experiments were repeated three times.

### *GC/MS analysis of the essential oil*

The chemical compositions of *K. attapeuensis* rhizomes EO were examined using GC/MS analysis [8-10.] The analysis was conducted on an Agilent Technologies 7890B GC connected to a 5977B MSD system operating in EI mode. A column with dimensions of 30 m × 0.25 mm id. × 0.25 µm film thickness (HP-5MS Ultra-Inert, Agilent Technologies) was employed. The experimental setup included Helium as the carrier gas (flow rate 1.0 mL/min), an amount of 1.0 µL injection volume with a split ratio of 25:1, and a temperature program ranging from 60°C (maintained for 1 min) to 240°C (maintained for 4 min) with a ramp rate of 2°C/min. The injector temperature was set at 300°C, MS Quad temperature at 150°C, transfer line temperature at 300°C, MS source at 230°C, ionization energy at 70 eV, and mass range from 50 to 550 amu with a scan rate of 2.0 scans/s.

Identification of the EO components was accomplished by comparing their mass spectra to those in the NIST17 library and further verified by comparing retention indices (RI) of the compounds determined under identical conditions using a mixture of *n*-alkanes ($C_7$-$C_{35}$). The RI value of each compound was calculated as previously reported [8,11]. Quantification was based on the percentage of relative peak area, according to Hieu Tran-Trung *et al*.[11].

### *Antimicrobial assay*

The investigation of the antibacterial bioactivity was conducted using Hadacek and Greger's method [12]. The antimicrobial assay of *K. attapeuensis* rhizomes EO was assessed against seven pathogenic bacterial strains, consisting of three Gram-positive bacterial strains (*Enterococcus faecalis* ATCC 299212, *Staphylococcus aureus* ATCC 25923, and *Bacillus cereus* ATCC 14579), three Gram-negative bacterial strains (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Salmonella enterica* ATCC 13076), and one yeast strain (*Candida albicans* ATCC 10231). These microorganisms were obtained from the Institute of Marine Biochemistry, VAST, Vietnam.

To evaluate the antimicrobial assay, the rhizomes EO of *K. attapeuensis* was dissolved in dimethyl sulfoxide (DMSO) at decreasing concentrations (µg/mL): $2^8$, $2^7$, $2^6$, $2^5$, $2^4$, $2^3$, $2^2$, and $2^1$. The test EO samples were standardized on a 96-well plate with a concentration of $2 \times 10^5$ colony-forming units (CFU)/mL, were incubated at 37°C and shaken at 120 revolutions per minute for 24 and 48 h, for bacteria and yeast, respectively. The Minimum Inhibitory Concentration (MIC) value was determined as the lowest concentration of each sample that completely inhibited the growth of microorganisms (97%-100%). Turbidity measurements were conducted using a BioTek Epoch spectrophotometer (USA) and RawData software (Belgium) to accurately determine the MIC value. Streptomycin and cycloheximide were utilized as antibacterial and antifungal standards for comparison purposes, respectively; DMSO was used as a negative control. The entire process was performed following standard protocols and guidelines.

## Results and discussion

### *GC/MS profiling of the essential oil*

*K. attapeuensis* rhizomes EO exhibited a pale-yellow color and was lighter than water. The hydrodistillation yield of this EO was approximately 0.21% (w/w) of fresh rhizomes mass.

The result of GC/MS analysis (Figure 1) reveals the presence of 46 different compounds (representing 90.61% of the content) in *K. attapeuensis* rhizomes EO, as shown in Table 1. Based on the obtained result, the major class was identified as oxygenated sesquiterpenes (36.27 %), followed by monoterpene hydrocarbons (33.74 %), and sesquiterpene hydrocarbons (14.12 %). Besides, eight oxygenated monoterpenes and two other compounds (*o*-cresol methyl ether and benzyl benzoate) were also found, but in lesser proportions of 6.28 % and 0.20 %, respectively. Remarkably, the prominent chemical constituents found in higher concentrations in *K. attapeuensis* rhizomes EO were identified as germacrone (25.8 %), camphene (18.63 %), and *α*-pinene (10.57 %).

In numerous previous studies, the chemical composition of *Kaempferia* species EOs has demonstrated variations in the content and presence of certain compounds [5,6,13-15]. This difference may be attributed to various factors such as ecological conditions, plant parts used, harvesting time, environmental conditions, climate, pH level, and others. Among the *Kaempferia* species, germacrone, an oxygenated sesquiterpene, was found in lower quantities, constituting only 9.85% of the EO of *K. champasakensis* leaves [14]. Additionally, α-pinene and camphene were present in minor amounts, accounting for approximately 2.02 % and 1.32 % respectively [14]. Conversely, the main component analysis of the rhizomes EOs of *K. daklakensis*

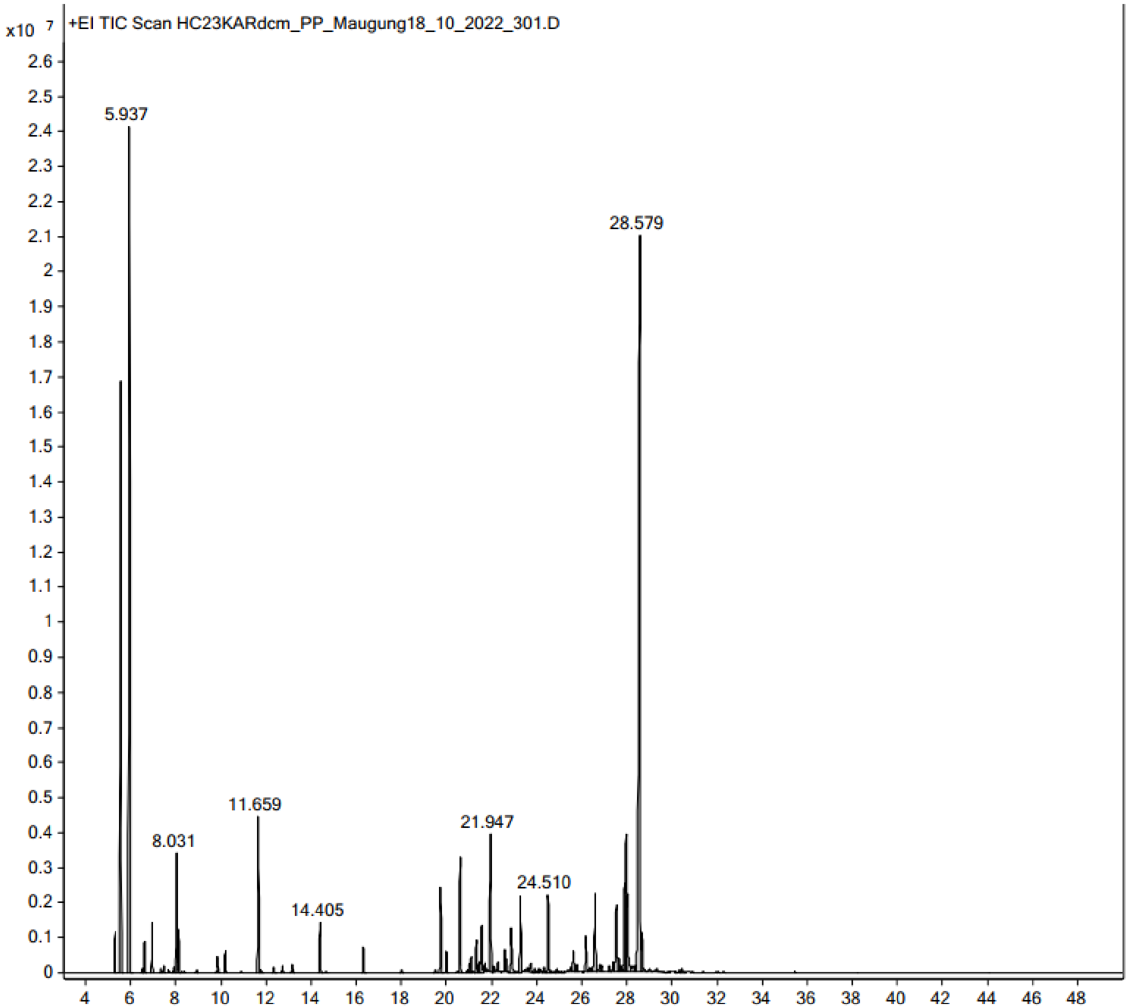


**Figure 1.** Chromatogram of *K. attapeuensis* rhizomes essential oil on HP-5MS Ultra-Inert

**Table 1. Chemical composition of Kaempferia attapeuensis rhizomes essential oil**

| Peak | RT | Compounds | Type | RI (Cal.) | RI (Lit.) | Content (%) |
|---|---|---|---|---|---|---|
| 1 | 5.296 | Tricyclene | MH | 928 | 925 | 0.64 |
| 2 | 5.565 | α-Pinene | MH | 940 | 937 | **10.57** |
| 3 | 5.937 | Camphene | MH | 956 | 952 | **18.63** |
| 4 | 6.520 | Sabinene | MH | 978 | 974 | 0.06 |
| 5 | 6.618 | β-Pinene | MH | 982 | 979 | 0.51 |
| 6 | 6.944 | β-Myrcene | MH | 993 | 991 | 0.78 |
| 7 | 7.338 | α-Phellandrene | MH | 1007 | 1005 | 0.07 |
| 8 | 7.487 | o-Cresol methyl ether | Other | 1013 | 1009 | 0.12 |
| 9 | 7.911 | p-Cymene | MH | 1029 | 1025 | 0.10 |
| 10 | 8.031 | Limonene | MH | 1033 | 1030 | 2.03 |
| 11 | 8.117 | Eucalyptol | OM | 1036 | 1032 | 0.76 |
| 12 | 8.924 | γ-Terpinene | MH | 1063 | 1060 | 0.05 |

table 1. (continued).

| Peak | RT | Compounds | Type | RI (Cal.) | RI (Lit.) | Content (%) |
|---|---|---|---|---|---|---|
| 13 | 9.845 | Terpinolene | MH | 1091 | 1088 | 0.30 |
| 14 | 10.182 | Linalool | OM | 1100 | 1099 | 0.39 |
| 15 | 11.659 | Camphor | OM | 1149 | 1145 | 3.15 |
| 16 | 12.345 | Borneol | OM | 1170 | 1166 | 0.13 |
| 17 | 12.729 | Terpinen-4-ol | OM | 1181 | 1177 | 0.13 |
| 18 | 13.164 | α-Terpineol | OM | 1193 | 1189 | 0.16 |
| 19 | 14.405 | Isobornyl formate | OM | 1232 | 1232 | 1.04 |
| 20 | 16.316 | Bornyl acetate | OM | 1289 | 1285 | 0.52 |
| 21 | 17.993 | δ-Elemene | SH | 1342 | 1338 | 0.07 |
| 22 | 19.744 | β-Elemene | SH | 1395 | 1391 | 1.78 |
| 23 | 19.990 | Cyperene | SH | 1402 | 1399 | 0.49 |
| 24 | 20.614 | α-Santalene | SH | 1424 | 1420 | 2.45 |
| 25 | 21.026 | γ-Elemene | SH | 1437 | 1433 | 0.19 |
| 26 | 21.089 | α-Bergamotene | SH | 1440 | 1435 | 0.36 |
| 27 | 21.340 | Selina-5,11-diene | SH | 1448 | 1447 | 0.73 |
| 28 | 21.569 | Aristolene | SH | 1455 | 1453 | 1.22 |
| 29 | 21.947 | Ishwarane | SH | 1467 | 1465 | 3.10 |
| 30 | 22.096 | Patchoulene | SH | 1472 | 1467 | 0.15 |
| 31 | 22.593 | Aristolochene | SH | 1488 | 1487 | 0.57 |
| 32 | 22.868 | Valencene | SH | 1496 | 1492 | 1.18 |
| 33 | 23.292 | γ-Cadinene | SH | 1511 | 1513 | 1.74 |
| 34 | 24.104 | Selina-3,7(11)-diene | SH | 1540 | 1542 | 0.09 |
| 35 | 24.510 | Elemol | OS | 1554 | 1549 | 1.68 |
| 36 | 24.899 | Nerolidol | OS | 1567 | 1564 | 0.12 |
| 37 | 25.517 | Caryophyllene oxide | OS | 1587 | 1582 | 0.12 |
| 38 | 25.632 | Viridiflorol | OS | 1591 | 1591 | 0.57 |
| 39 | 26.593 | Isolongifolen-9-one | OS | 1625 | 1628 | 2.00 |
| 40 | 26.885 | Isospathulenol | OS | 1636 | 1638 | 0.10 |
| 41 | 27.543 | Neointermedeol | OS | 1660 | 1660 | 1.95 |
| 42 | 27.955 | Allohimachalol | OS | 1674 | 1674 | 3.55 |
| 43 | 28.253 | Cedr-8-en-13-ol | OS | 1685 | 1688 | 0.27 |
| 44 | 28.373 | α-Bisabolol | OS | 1689 | 1684 | 0.11 |
| 45 | 28.579 | Germacrone | OS | 1696 | 1693 | **25.80** |
| 46 | 30.450 | Benzyl benzoate | Other | 1767 | 1762 | 0.08 |
| Monoterpene hydrocarbons (MH) | | | | | | 33.74 |
| Oxygenated monoterpenes (OM) | | | | | | 6.28 |
| Sesquiterpene hydrocarbons (SH) | | | | | | 14.12 |
| Oxygenated sesquiterpenes (OS) | | | | | | 36.27 |
| Others | | | | | | 0.20 |
| Total | | | | | | 90.61 |

RT: Retention time (min)
RI (Cal.): Retention Indices on HP-5MS Ultra-Inert
RI (Lit.): Retention Indices in literature

and *K. angustifolia* revealed a high content of camphene, reaching 23.63 % and 45.53 % respectively [5,16].

***Antimicrobial activity of the essential oil***

The obtained *K. attapeuensis* rhizomes EO was further evaluated for its activity against three Gram-positive bacteria (*E. faecalis* ATCC 299212, *S. aureus* ATCC 25923, and *B. cereus* ATCC 14579), three Gram-negative bacteria (*E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *S. enterica* ATCC 13076), and a yeast (*C. albicans* ATCC 10231). The obtained data on the antimicrobial activity of the studied EO are recorded in Table 2. Except for Gram-negative bacterium *P. aeruginosa* ATCC 27853 (the MIC value > 256 μg/mL), this EO sample was able to inhibit most bacteria and yeasts with the MIC value of 32 μg/mL. The streptomycin-positive control for bacteria showed activity with MIC values in the range of 32-256 μg/mL, while the control cycloheximide, an antifungal compound, showed activity with MIC values of 32 μg/mL. The EO of *K. attapeuensis* rhizomes showed activity four to eight times as strong as streptomycin against *S. aureus* ATCC 25923*, B. cereus* ATCC 14579*, S. enterica* ATCC 13076*,* and *E. faecalis* ATCC 299212. Regarding activity on yeast, results were shown to be equivalent to the positive control cycloheximide. The use of *K. attapeuensis* EO in antimicrobial testing has never been conducted before. In previous studies, the EOs of *Kaempferia* species were considered interesting topics of research. For instance, the rhizomes EO of *K. rotunda* was found to have good to moderate antibacterial activity against the pathogenic bacteria *E. faecalis*, *S. aureus*, *E. coli*, *C. albicans*, *A. baumannii*, and *A. niger* with MIC values ranging from 8.34 to 10.91 μg/mL.[6] The observed antibacterial activity of the EO of *K. attapeuensis* rhizomes may be related to the major compounds or the synergistic effects of several compounds are recorded. A monoterpene hydrocarbon compound, α-pinene is known for its broad-spectrum antibacterial activity [17].

Previously, the EO of *Cupressus sempervirens* L. (Cupressaceae family) with high content of *α*-pinene (42%) and some other compounds such as δ-3-carene and limonene was reported to have strong antibacterial activity against *S. aureus*, *E. faecalis* and *E. coli* [18]. Camphene is reported to act as an antibacterial factor against most pathogenic bacteria [19]. In the rhizomes EO of *Curcuma aeruginosa* (Zingiberaceae family), germacrone (15.76%) was one of the major compounds along with β-pinene (9.97%) and camphor (9.96%) found to contribute to the potential activity against *Staphylococcus aureus* (MIC value of 125 μg/mL), *Bacillus cereus* (MIC value of 125 μg/mL), and *Candida albicans* (MIC value of 250 μg/mL) [20].

**Conclusions**

The current investigation revealed the major constituents of the rhizomes EO of *K. attapeuensis* and its related antimicrobial activity. α-pinene (10.57%), camphene (18.63%), and germacrone (25.80%) were found to be the main components of the EO. In addition, the EO displayed promising antimicrobial activity against *E. faecalis, S. aureus, B. cereus, E. coli* and *S. enterica* with MIC values of 32 μg/mL, stronger than positive control streptomycin. The EO also showed anticandidal action against the yeast

**Table 2. Antimicrobial activity of *Kaempferia attapeuensis* rhizomes essential oil**

| Samples | Minium inhibitory concentration (MIC μg/mL) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Gram (+) | | | Gram (-) | | | Yeast |
| | ***E. faecalis*** | ***S. aureus*** | ***B. cereus*** | ***E. coli*** | ***P. aeruginosa*** | ***S. enterica*** | ***C. albicans*** |
| Essential oil | 32 | 32 | 32 | 32 | - | 32 | 32 |
| Steptomycin | 256 | 128 | 128 | 32 | 256 | 128 | - |
| Cycloheximide | - | - | - | - | - | - | 32 |

*C. albicans* as strong as cycloheximide. These findings provide important information for the potential future application of *K. attapeuensis* EO.

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**Competing interests**
The authors declare that no competing interest exists.

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