



Chemical composition, antioxidant and antifungal activities of rhizome essential oil of *Kaempferia parviflora* Wall. ex Baker grown in Vietnam

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Abstract The aim of the present study was to evaluate the chemical composition and antioxidative activity of rhizome essential oil of *Kaempferia parviflora* Wall. ex Baker. The essential oil extracted by hydrodistillation was chemically profiled by GC/MS analysis. The antioxidative activity was determined and evaluated spectroscopically by the 2,2-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging assays. According to the results, the major essential oil components were camphene (18.03%), β -pinene (14.25%), α -pinene (12.38%), endo-borneol (10.23%), β -copaene (8.38%), and linalool (8.20%). *K. parviflora* rhizome oil possessed antioxidant potential, exhibiting DPPH and ABTS radical scavenging activities as high as 80.90 and 94.04%, respectively, at a concentration of 10 mg/mL. The corresponding IC₅₀ values were 0.451±0.051 and 0.527±0.022 mg/mL, respectively (IC₅₀ values for ascorbic acid, as the standard, were 0.209±0.016 and 0.245±0.022 mg/mL, respectively). The mycelium of *F. oxysporum* was distorted and collapsed when treated with 0.5 mg/mL of the EO of *K. parviflora* rhizome for 3 days treatment, which may provide an important information for exploring the metabolism of the fungicide *K. parviflora* rhizome and its derived

compounds against *F. oxysporum*. This study provides the chemical properties of the essential oil of *K. parviflora* rhizome grown in Vietnam and their potential antioxidant and antifungal activities.

Keywords *Fusarium oxysporum* · Gas chromatography/Mass spectrometry · *Kaempferia parviflora* · Radical scavenging

Introduction

Kaempferia parviflora Wall. ex Baker, or black ginger, is a plant from the family Zingiberaceae used for health promotion in traditional Thai medicine. The rhizomes of *K. parviflora* contain volatile oil [1], chalcones [2], phenolic glycosides [3], and many flavonoids [4,5] and can be eaten either fresh or dry before any physical performance to improve physical work capacity. Pharmacological studies indicate *K. parviflora* possesses antioxidant [6,7], antiplasmodial, antifungal, and antibacterial activities [4,8]. It has been reported that the numerous flavonoids in the alcoholic extract of *K. parviflora* rhizomes possess antioxidant activity and neuroprotective and cognitive-enhancing effects [5,9]. The chemical components identified in *K. parviflora* rhizomes include 5,7-dimethoxyflavone [4,5,10-12], *o*-cymene (14.89%), α -copaene (11.68%), dauca-5,8-diene (11.17%), camphene (8.73%), β -pinene (7.18%), borneol (7.05%) [10], limonene (10.04%), and saffrole (8.88%) [11]. 5,7-Dimethoxyflavone is a major active constituent of *K. parviflora* [5] that showed high potential inhibitory activity toward acetylcholinesterase and butyrylcholinesterase [12,13]. In particular, the vasodilator effects of 5,7-dimethoxyflavone provide experimental support for the potential use of *K. parviflora* as a medical plant in the treatment of cardiovascular diseases [14].

There have been many studies related to chemical composition and biological activities of *K. parviflora* such as antibacterial and antioxidant activities over the world [4,6-8,12,22,23]. To our

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knowledge, there have been few reports regarding the antifungal activity containing in essential oil of *K. parviflora* rhizome grown in Vietnam. The present study aims to determine the chemical profile of the EO of *K. parviflora* Wall. ex Baker and to evaluate their antioxidant and antifungal activities based on scavenging activities against 2,2-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radicals, and *Fusarium oxysporum*. The results showed that some components of the essential oil from *K. parviflora* in this study differ from those previously reported and show strong antifungal activity.

Materials and Methods

Plant materials

The rhizome of *K. parviflora* was collected from Lai Chau province, Vietnam, in October 2021. The samples were identified and deposited as voucher specimens at the Department of Medicinal Resources, National Institute of Medicinal Materials, Ha Noi, Vietnam. The plant are a short fleshy rhizome, with tuberous roots. The plant are a short fleshy rhizome, with tuberous roots. The leaves of the plant are curved ends and when the flower blooms it is white purple in color (Fig. 1A). The rhizome are a deep purple color (Fig. 1B). The phytopathogen (*Fusarium oxysporum* KACC 40032) was provided by the Korean Agricultural Culture Collection (KACC).

Chemicals and reagents

DPPH, potassium persulfate, and dimethylsulfoxide were purchased from Sigma-Aldrich (St. Louis, MO, USA). ABTS and ascorbic acid were purchased from Merck (Darmstadt, Germany). All other chemicals used, including solvents, were of analytical grade.

Hydrodistillation of EO

The fresh rhizome of *K. parviflora* (1 kg) was surface-sterilized with tap water, dried, pulverized into powder, and then hydrodistilled in a Clevenger-type apparatus for 4 h. The resulting EO was dried over anhydrous sodium sulfate and stored in a sealed vial at 10 °C in the dark before analysis.

Gas chromatography/Mass spectrometry analysis

For the GC/MS analysis, 10 μ L of the EO of *K. parviflora* was diluted in 1 mL of *n*-hexane and mixed using a vortex mixer. The homogeneous mixture was then analyzed using a Shimadzu GC/MS-QP2020 equipped with a Shimadzu SH Rxi-5SiLMS column (30 m \times 0.32 mm, 0.25 μ m film thickness; non-polar phase: 100% dimethyl polysiloxane). The sample was loaded into the injector at a split ratio of 20:1 at 180 °C. Helium was used as the carrier gas at a flow rate of 1.61 mL/min, and the column pressure was set at 21.6 kPa (3.13 psi). The temperature program was as follows: initial temperature of 60 °C for 3 min, increased to 120 °C at 4 °C/min and then to 140 °C at 5 °C/min for 4 min, and finally

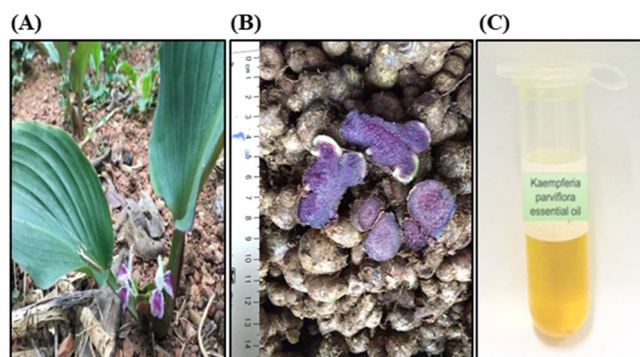


Fig. 1 Morphological characteristics of (A) plant of *Kaempferia parviflora* and (B) rhizome of *K. parviflora* (Lai Chau, Vietnam) and its essential oil extracted by hydrodistillation (C)

increased to 180 °C at 10 °C/min (held for 2 min). For the MS analysis, an electron ionization source set at 200 °C and an interface source set at 250 °C were used to isolate and fragment the ions. The GC program was set for 35 min, starting from 3 min and ending at 35 min (solvent cut time – the filament delay was 2 min). GCMS-QP2020 scan mode was used with MS range from 50 to 900 *m/z*. The peaks were observed in the total ion chromatogram.

Identification and quantification of EO constituents

Identification of the EO components was determined with reference to a homologous series of *n*-alkanes and by comparison of their mass spectral fragmentation patterns with those reported in the databases [15,16]. Quantification was done by the external standard method. Calibration curves of representative compounds from each class were drawn and used for quantification.

Determination of antioxidant activity

The DPPH free radical scavenging ability was conducted according to the spectrophotometric method described by Miliauskas et al. [17] with a slight modification as described by Nguyen et al. [18] The DPPH radical is reduced when it reacts with an antioxidant capable of donating hydrogen. Changes in color (from deep violet to light yellow) were measured at 517 nm using a BioTek MicroFill dispenser (Agilent, Santa Clara, CA, USA). In brief, 0.1 mL of each sample at various concentrations was mixed with 1 mL of 0.2 mM DPPH dissolved in 0.004% methanol. Absorbance at 517 nm was measured after the reaction mixture was incubated at 25 \pm 1 °C for 15 min in the dark. Another mixture containing all the reagents without the sample served as a blank. The ability to scavenge DPPH radicals was calculated by Eq. (1):

$$\text{DPPH radical scavenging activity (\%)} = \frac{[\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}] / \text{Abs}_{\text{control}} \times 100}{(1)}$$

where $\text{Abs}_{\text{control}}$ is the absorbance of DPPH radical in methanol, and $\text{Abs}_{\text{sample}}$ is the absorbance of DPPH radical solution mixed

with EO sample. Ascorbic acid was used as a positive control. The experiments were performed twice, with three replicates for each treatment.

The ABTS free radical scavenging ability was conducted according to the method of Nguyen et al. [18] with some modifications. ABTS solution consisting of a mixture of 7.4 mM ABTS and 2.6 mM potassium persulfate was stored for 12 h in a dark flask to obtain ABTS cation radicals with an absorbance of 1.1 ± 0.02 at 734 nm. A 1.8 mL aliquot of the ABTS cation radical solution was added to a test tube containing 0.2 mL of sample. The reaction mixture was incubated at 25 ± 1 °C for 2 h in the dark, and then the absorbance was measured at 734 nm, and the ABTS radical scavenging activity was calculated by Eq. (2). Ascorbic acid was used as a positive control. The experiments were performed twice, with three replicates for each treatment.

$$\begin{aligned} \text{ABTS radical scavenging activity (\%)} \\ = [(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}) / \text{Abs}_{\text{control}}] \times 100 \end{aligned} \quad (2)$$

where $\text{Abs}_{\text{control}}$ is the absorbance of ABTS radical in methanol, and $\text{Abs}_{\text{sample}}$ is the absorbance of ABTS radical solution mixed with EO sample.

The IC_{50} values of the DPPH and ABTS radical scavenging activities were also calculated.

Determination of antifungal activity

Antifungal assays were performed as previously described of Nguyen et al. [19] with slight modifications. Samples were dissolved with several different concentrations (0, 1, 5, 10 mg/mL). Thirty microliters of each concentration were loaded onto each paper disc and methanol was used as a control. The mycelial growth was measured at 3 and 6 days after treatment. The effective concentration for inhibition of *F. oxysporum* mycelial growth was estimated by Eq. (3) [19,20].

$$\text{Inhibition (\%)} = [(C - T)/C - 6] \times 100 \quad (3)$$

where C and T are colony diameter in the control sample and the treated sample respectively.

The IC_{50} values of the EO of *K. parviflora* for 50% inhibition of *F. oxysporum* mycelial growth (EC_{50}) was also calculated at 3 and 6 days after treatment.

Effect of EO on *Fusarium oxysporum* mycelium morphology

To determine the effect of EO on the hyphal morphology of *F. oxysporum*, the assays were performed as previously described of Nguyen et al. [19]. In brief, mycelial culture block (6 mm diameter) of the same age was placed into Petri dish containing 10 mL of PDA medium with a final concentration of 0, 0.1, 0.5, and 1.0 mg/mL of the EO of *K. parviflora*. The same volume of methanol only was considered as the control. Samples were incubated in the dark at 25 °C. At 3 days after treatment, the fungal blocks were cut into 3 mm pieces and stained with lactophenol blue. In order to remove the lactophenol blue solution,

the samples were washed away by sterile water for three time. *F. oxysporum* mycelium morphology in control and treatment with EO were observed under light microscope (40× magnification). Each treatment was replicated three times and the experiment was repeated twice.

Statistical analysis

Data were compared using Tukey's honestly significant difference (HSD) test, with a $p \leq 0.05$ indicating a significant difference among mean values. All data were analyzed using Statistical Analysis System 9.4 [21] and are presented as mean \pm SD.

Results and Discussion

EO composition of *K. parviflora* rhizome

The EO hydrodistilled from *K. parviflora* rhizome had a yield of 0.54% (fresh weight), yellow color (Fig. 1C), and a slightly strong odor. A total of 28 compounds were detected in *K. parviflora* EO (Table 1, Fig. 2). The 28 compounds were identified by a comparison of their mass spectra and retention indices with those in GC/MS libraries. As shown in Table 1, the EO contained six monoterpenes (1-4, 6, and 8), five monoterpenoids (7-12), 15 sesquiterpenes (5 and 13-26), and two sesquiterpenoids (27 and 28) with approximate amounts of 49.81, 22.32, 24.89, and 2.98% of the total oil, respectively. Wungintaweekul et al. [6] showed that the EO of *K. parviflora* from Thailand contained β -pinene (11.10%) as the main compound, followed by D-germacrene (9.55%), camphene (9.22%), and α -pinene (8.31%). By contrast, D-germacrene was not detected in the present study. In the present results, the major components of the EO were camphene (18.03%), β -pinene (14.25%), α -pinene (12.38%), endo-borneol (10.23%), $\beta\beta$ -copaene (8.38%), and linalool (8.20%). The contents of the remaining components were below 5%, most of them (22 compounds) even below 1%.

The monoterpene profile of the rhizome oil of *K. parviflora* was characterized by the presence of camphene (18.03%), β -pinene (14.25%), and α -pinene (12.38%), the monoterpenoids profile featured endo-borneol (10.23%) and linalool (8.20%), and the sesquiterpene profile presented β -copaene (8.38%), copaene (3.60%), and β -elemene (3.01%). These results contrast with those previously reported. Nguyen et al. [7] reported 16 compounds in the methanol extract of *K. parviflora* rhizomes purchased from Laos, with 5,7-dimethoxyflavone (75.5 mg/0.8 kg dried weight [DW]), 5-hydroxy-3,7-dimethoxyflavone (56.8 mg/0.8 kg DW), 3,5,7,3',4'-pentamethoxyflavone (55.6 mg/0.8 kg DW), 4'-hydroxy-5,7-dimethoxyflavone (52.8 mg/0.8 kg DW), and 5-hydroxy-3,7,3',4'-tetramethoxyflavone (52.5 mg/0.8 kg DW) as the main constituents. In addition, the adaptogenic-active fraction of the hexane extract of *K. parviflora* rhizomes obtained by maceration contained germacene D, β -elemene, α -copaene, and (*E*)-caryophyllene as the major components [22].

Table 1 Chemical composition of essential oil of *K. parviflora* Wall. ex Baker rhizome by GC-MS analysis

| Peak# | Compounds | RT (min) | Retention Index | | Content (%) |
|------------------------------------|-----------------------------------|----------|-------------------|------------------|--------------|
| | | | RI _{Cal} | RI _{Db} | |
| 1 | α-Pinene | 4.108 | 892±3 | 891 | 12.38 |
| 2 | Camphene | 4.410 | 917±4 | 918 | 18.03 |
| 3 | β-Pinene | 4.923 | 933±4 | 930 | 14.25 |
| 4 | β -Myrcene | 5.072 | 940±6 | 937 | 1.18 |
| 5 | <i>o</i> -Cymene | 5.841 | 966±2 | 968 | 0.33 |
| 6 | D-Limonene | 5.958 | 985±3 | 984 | 3.44 |
| 7 | Eucalyptol | 6.032 | 1012±5 | 1015 | 2.63 |
| 8 | (+)-4-Carene | 7.265 | 1017±3 | 1013 | 0.53 |
| 9 | Linalool | 7.585 | 1026±4 | 1024 | 8.20 |
| 10 | Endo-borneol | 9.463 | 1080±2 | 1083 | 10.23 |
| 11 | Terpinen-4-ol | 9.671 | 1084±1 | 1182 | 0.45 |
| 12 | L- α -Terpineol | 10.035 | 1103±7 | 1104 | 0.81 |
| 13 | Bornyl acetate | 12.385 | 1167±6 | 1266 | 0.46 |
| 14 | Copaene | 14.854 | 1209±4 | 1204 | 3.60 |
| 15 | β -Elemene | 15.256 | 1255±5 | 1253 | 3.01 |
| 16 | Caryophyllene | 16.239 | 1287±3 | 1285 | 2.19 |
| 17 | Humulene | 17.537 | 1302±6 | 1303 | 0.46 |
| 18 | <i>cis</i> -Muuroala-3-5-diene | 18.163 | 1371±4 | 1371 | 0.38 |
| 19 | γ -Muurolene | 18.293 | 1379±3 | 1372 | 0.68 |
| 20 | β -Copaene | 18.525 | 1381±5 | 1382 | 8.38 |
| 21 | β -Eudesmene | 18.801 | 1391±2 | 1387 | 0.40 |
| 22 | β -Gurjunene | 18.905 | 1410±5 | 1411 | 0.36 |
| 23 | Bicyclogermacrene | 19.067 | 1421±3 | 1421 | 1.81 |
| 24 | α -Muurolene | 19.195 | 1436±5 | 1435 | 0.32 |
| 25 | δ -Cadinene | 19.954 | 1439±4 | 1436 | 1.95 |
| 26 | (+)-Epi-bicyclosesquiphellandrene | 24.560 | 1488±2 | 1491 | 0.47 |
| 27 | δ -Cadinol | 24.720 | 1512±6 | 1510 | 0.39 |
| 28 | α -Cadinol | 25.055 | 1502±3 | 1501 | 2.59 |
| Monoterpene (Sr. Nos. 1–4, 6, 8) | | | | | 49.81 |
| Monoterpenoids (Sr. Nos. 7, 9–12) | | | | | 22.32 |
| Sesquiterpene (Sr. Nos. 5, 13–26) | | | | | 24.89 |
| Sesquiterpenoids (Sr. Nos. 27, 28) | | | | | 2.98 |
| Total identified | | | | | 100 |

RT = Retention Time (min)

RI_{Cal}: Retention Indices calculated with Standard Non-Polar GC Column in library spectra NIST 17 (Software NIST MS Search v2.3)

RI_{Db} (Column Rxi-1 MS): Retention Indices from the database

Furthermore, Pitakpawasutthi et al. [10] detected 20 compounds in volatile oils of *K. parviflora* rhizomes collected from various drug stores in Thailand. The main constituents were α -copaene (11.68%), dauca-5,8-diene (11.17%), camphene (8.73%), β -pinene (7.18%), borneol (7.05%), and linalool (6.58%), respectively. According to Begum et al. [22] linalool (26.89-43.35%) is the main compound in *K. parviflora*, followed by endo-borneol (19.6-22.73%) and camphene (13.43-16.13%). However, partial shade conditions provide a higher yield of EO than open field conditions of *K. parviflora* in India [22] In summary, the findings highlight

the impact of geographical location and extract conditions on the chemical composition of *K. parviflora* EO.

Antioxidant activity of EO of *K. parviflora* rhizome

The antioxidant activity of the EO from *K. parviflora* rhizome was assessed by the DPPH and ABTS assays. Both the EO and the positive control (ascorbic acid) reduced the pink-colored free DPPH radical to yellow-colored diphenyl picrylhydrazine, confirming their DPPH radical scavenging activity. The DPPH radical scavenging activity of the EO was 0.0, 23.2, 36.1, 44.2, 58.3, 68.5,

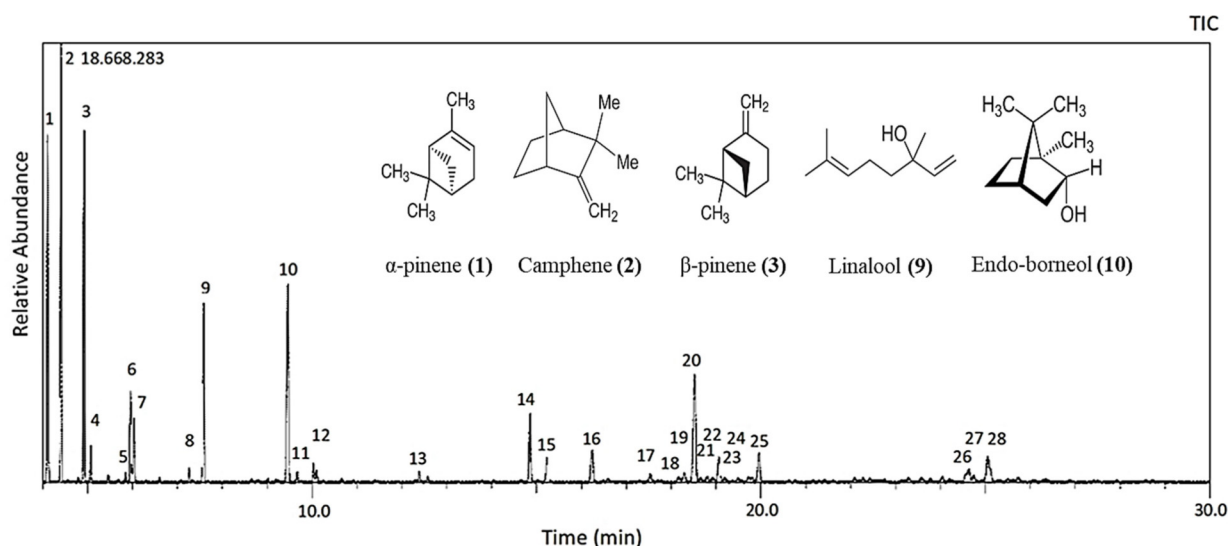


Fig. 2 Gas chromatography/mass spectrometry profile of the compounds in the essential oil obtained from *Kaempferia parviflora* rhizome. The chemical structures of the main compounds are shown in the figure

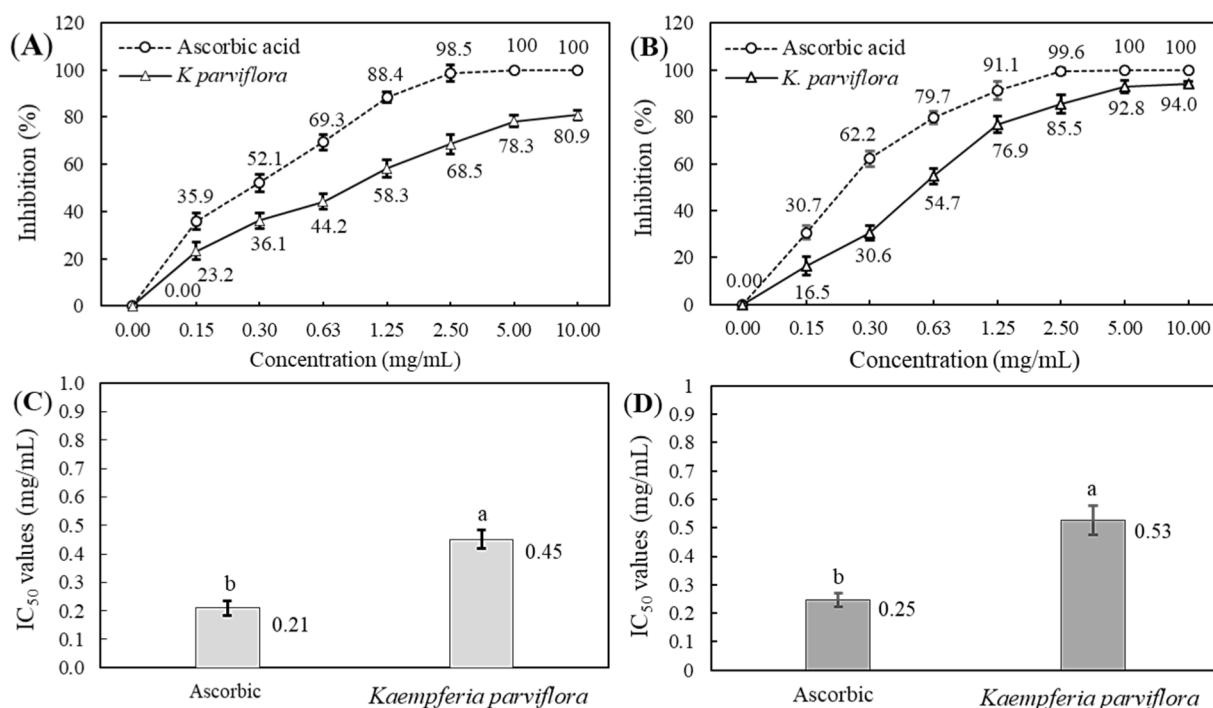


Fig. 3 Antioxidative activity of the essential oil obtained from *Kaempferia parviflora* rhizome. Ascorbic acid was used as the reference compound. (A) DPPH and (B) ABTS radical scavenging activity, IC₅₀ values of the (C) DPPH and (D) ABTS radical scavenging activity. Different letters above the standard error bars indicate a significant difference at each observation time based on Tukey’s HSD test ($p \leq 0.05$). Error bars indicate the SDs of the means ($n=3$)

78.3, and 80.9% at 0, 0.15, 0.30, 0.63, 1.25, 2.50, 5.0, and 10 mg/mL, respectively. The corresponding values for ascorbic acid were 0.0, 35.9, 52.1, 69.3, 88.4, 98.5, 100, and 100%, respectively (Fig. 3A). In addition, the IC₅₀ value of the DPPH radical scavenging activity was 0.451±0.015 mg/mL for the EO and 0.209±0.016 mg/mL for ascorbic acid (Fig. 3C). The IC₅₀ values in the DPPH

radical scavenging activity between of EO and ascorbic acid were significantly different (Figs. 3A, 3C). In the ABTS radical scavenging assay, the anti-radical activity of *K. parviflora* was 0.0, 16.5, 30.6, 54.7, 76.9, 85.5, 92.8, and 94.0% at 0, 0.15, 0.30, 0.63, 1.25, 2.50, 5.0, and 10 mg/mL, respectively. Ascorbic acid (positive control) displayed corresponding values of 0.0, 30.7,

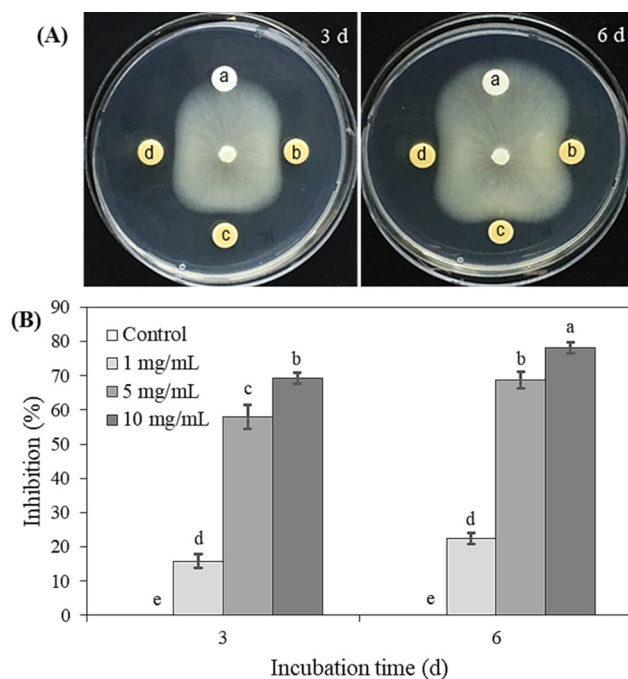


Fig. 4 Mycelial growth inhibition of *F. oxysporum* is affected by EO of *K. parviflora* rhizome. (A) *F. oxysporum* was incubated on PDA medium at 25 °C in dark for 3 and 6 days and the culture medium were complemented with EO to produce final concentrations (a) 0, (b) 1, (c) 5, and (d) 10 mg/mL, in which 0 represented the MeOH control. (B) Inhibition of mycelial growth by EO of *K. parviflora* rhizome at various concentrations 0, 1, 5, and 10 mg/mL for 3 and 6 days treatment. Mean values followed by different letters in each column represent statistically significant differences based on Tukey's honestly significant difference test ($p \leq 0.05$). Error bars indicate the SDs of the means ($n=3$)

62.2, 79.7, 91.1, 99.6, 100, and 100%, respectively (Fig. 3B). The IC_{50} value of the ABTS radical scavenging activity of the EO was 0.53 ± 0.05 mg/mL, which was significantly different from that of ascorbic acid, which had an IC_{50} of 0.25 ± 0.02 mg/mL (Figs. 3B, 3D).

It has been previously documented that the methanolic extract of *K. parviflora* had potent antioxidant activity by the DPPH radical scavenging method, with an IC_{50} of 61.5 ppm [6]. Thao et al. [7] emphasized the antioxidant activity, including reducing properties and peroxy radical scavenging capacities, of the polymethoxyflavonoids in the methanol extract of *K. parviflora* on pre-osteoclastic RAW 264.7 cells. Interestingly, a major bioactive 5,7-dimethoxyflavone (anti-inflammatory) in *K. parviflora* was identified in the ethanol extract by Pitakpawasutthi et al. [10] but was not found in the present study. Begum et al. [22] revealed that shade cultivation of *K. parviflora* led to higher DPPH antioxidant activity (58.1%) but lower ABTS activity (92.9%) compared to direct sunlight cultivation (56.9 and 94.6%, respectively) for the EO at the concentrations of 30 and 25 ppm, respectively; however, all activities were markedly higher than standard ascorbic acid (28.8 and 63.0%, respectively). Previous

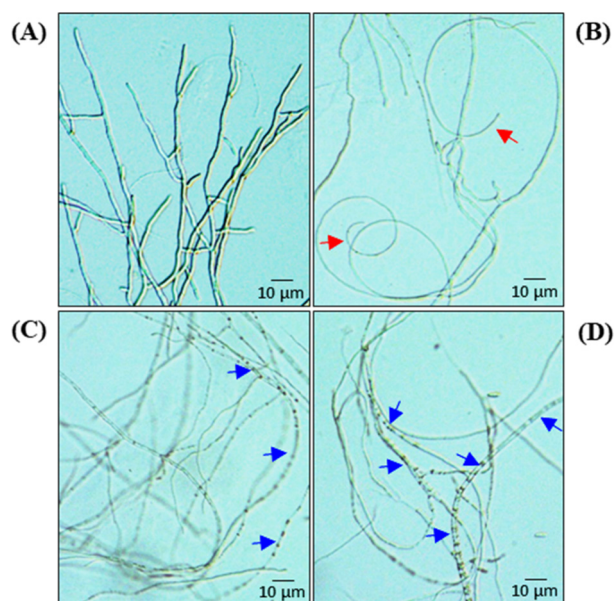


Fig. 5 Effects of EO of *Kaempferia parviflora* rhizome on the mycelium morphology of *Fusarium oxysporum* as observed microscopically (40× magnification). (A) Healthy hyphae growing on control plates. (B) Affected hyphae with reverse growth (arrow) in samples treated with 0.1 mg/mL *K. parviflora*. (C) Atrophied and broken mycelia (arrow) in samples treated with 0.5 mg/mL *K. parviflora* and (D) abnormal, atrophied and collapsed mycelia (arrow) in samples treated with 1.0 mg/mL *K. parviflora* at 3 days after treatment

reviews highlighted the antioxidant activity of camphene and β -pinene [23–25]. These results suggested that the antioxidant activity of *K. parviflora* EO was dependent on the chemical components. Both the DPPH and ABTS radical scavenging activity increased in a dose-dependent manner.

Inhibitory effect of EO of *K. parviflora* on mycelial growth and morphology of *F. oxysporum*

The results revealed that the mycelial inhibition exerted by the EO of *K. parviflora* occurred in a dose-dependent manner. EO of *K. parviflora* of higher concentration showed stronger inhibitory effect against *F. oxysporum*. Moreover, the development of hyphae was found to slow in response to treatments (Fig. 4). The mycelial growth of *F. oxysporum* gradually decreased as the dose of paste in methanol increased from 0 to 10 mg/mL (Fig. 4A). After 3 days treatment, the inhibition rates were 0, 15.8, 58.0, and 69.2% at 0, 1, 5, and 10 mg/mL, respectively. The corresponding values for 6 days treatment were 0, 22.4, 68.7, and 78.1%, respectively (Fig. 4B). The EC_{50} values of EO of *K. parviflora* against *F. oxysporum* were 0.43 and 0.36 mg/mL for 3 and 6 days after treatment, respectively.

The antifungal activity of medicinal plants and their derived compounds against pathogenic fungi has already been demonstrated. The mycelium morphology of *Fusarium solani*, *Rhizoctonia solani*, and *Phytophthora capsici* were to be affected by plant

extracts [19,26,27]. Microscopic observation of *F. oxysporum* mycelia exposed to EO of *K. parviflora* revealed degenerative changes in the mycelial morphology when compared with the mycelia of the controls (Fig. 5). The mycelia of the control was stretched, uniform and smooth mycelium surface (Fig. 5A). Meanwhile, mycelium began to grow abnormally and the top of the mycelium tends to reverse (Fig. 5B). The mycelia were degraded and contained cells with no cytoplasm or depleted levels of cytoplasm when *F. oxysporum* was treated with 0.5 mg/mL EO of *K. parviflora* (Fig. 5C). Specifically, the mycelium of *F. oxysporum* was distorted and collapsed (Fig. 5D). The analysis of Begum et al. [22] revealed that EO of *K. parviflora* was found to be highly ineffective in the control of *Bacillus cereus*, *Salmonella typhimurium*, *S. aureus*, *B. subtilis*, and *S. enterica*. The EO of *K. parviflora* was found to have shown activity against two fungus *Aspergillus oryzae* and *Fusarium keratoplasticum*. The highest inhibition was found in *A. oryzae* and *F. keratoplasticum* at the concentration of 500 ppm. Interestingly, camphene and β -pinene has two compounds that they demonstrated with biological activities included antifungal activity [24,25]. Moreover, the inhibitory activities of 1,8-cineole and α -pinene were described by Kadoglidou et al. [28], against *F. oxysporum* with a dose-dependent effect. In this study, camphene (18.03%), β -pinene (14.25%), and α -pinene (12.38%) are the major ingredients of the *K. parviflora* EO that collected from Lai Chau province, Vietnam. In addition, at 10 mg/mL EO of *K. parviflora* was showed antifungal activity against *F. oxysporum*.

Based on these results, the main compounds in the EO of *K. parviflora* rhizome were investigated. In addition, the potent antioxidant and antifungal activities of the EO was demonstrated. The major compounds identified from EO of *K. parviflora* rhizome were camphene, β -pinene, α -pinene, and Endo-borneol. Therefore, the major compounds from *K. parviflora* rhizome identified in this study should be more clearly evaluated in future in-depth studies.

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