

# Chemical constituents, antioxidant and antifungal activities of the essential oil from Vietnamese *Ocimum tenuiflorum* L. leaves

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## Abstract:

In this study, the authors aim to determine the chemical compositions of the essential oil from *Ocimum tenuiflorum* leaves and to evaluate its antioxidant and antifungal activities. The essential oil used in this research, extracted by hydrodistillation, was chemically profiled using gas chromatography/mass spectrometry (GC/MS) analysis. Methyl eugenol (46.07%), caryophyllene (15.65%), eugenol (11.83%),  $\beta$ -elemene (4.33%),  $\beta$ -copaene (4.18%), caryophyllene oxide (3.18%), and  $\alpha$ -copaene (1.95%) were identified as major components of the essential oil. The results indicated that the essential oil of *O. tenuiflorum* leaves possessed relatively high 2,2-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging activities with IC<sub>50</sub> values of 53.5 and 55.1  $\mu$ g/ml, respectively, compared with 67.2 and 63.2  $\mu$ g/ml, respectively, for the positive control. The reducing powers of *O. tenuiflorum* essential oil and butylated hydroxytoluene (BHT) at a concentration of 200  $\mu$ g/ml were 0.95 and 0.73, respectively. Additionally, the essential oil of *O. tenuiflorum* at a concentration of 200  $\mu$ g/ml strongly inhibited the mycelial growth of *Rhizoctonia solani*.

**Keywords:** eugenol, *Ocimum sanctum*, *Rhizoctonia solani*, 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid), 2,2-diphenyl-2-picrylhydrazyl.

**Classification number:** 3.1

## 1. Introduction

*Ocimum tenuiflorum* (synonym: *Ocimum sanctum*) is a fragrant plant of the Lamiaceae family native to tropical and subtropical regions in Southern Asia. It is used in the cosmetics and food industries and as a traditional medicine to treat various conditions, including influenza, coughs, and headaches. It is readily differentiated from other *Ocimum* species by its internally glabrous calyces and spreading pedicels. Notable components in the essential oil of *O. tenuiflorum* composition include eugenol and methyl eugenol [1, 2]. It thrives in the plains, midlands, and low mountains, growing slowly at altitudes above 1.000 m, above sea level. The height of the plant varies from 0.5 to 1.5 m, and it has the ability to regenerate shoots. Inflorescences are often situated in the interstitial leaves or branches of the plant. The leaves, arranged in two successive pairs perpendicular to each other, are simple, petioled, and purple with an ovate blade up to 5 cm long and a slightly toothed margin. The purplish flowers are placed in close whorls on elongated racemes [2].

The biologically active components of the essential oil of *O. tenuiflorum*, which possess antimicrobial, anti-stress, anti-inflammatory, immunomodulatory, antipyretic, antiasthmatic, hypoglycaemic, hypotensive, and analgesic effects, have made the plant one of nature's "mother medicines" [1, 3]. Its application is known to retain skin moisture, reduce skin roughness and scaliness, prevent wrinkles, and make the skin smooth. It may also play a major role in treating inflammatory diseases [4]. Many studies related to the chemical composition and biological activity of extracts from *Ocimum* species, especially their antibacterial and antioxidant properties, have been conducted. The health benefits of *Ocimum* species could invariably be attributed to their abundant plant-derived phytochemicals [4-9].

To the best of our knowledge, only a few reports regarding the antifungal activity of the essential oil from Vietnamese *O. tenuiflorum* leaves have been published. However, a study by T.T.Q. Cung, et al. (2018) [10] shows

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the *O. sanctum* essential oil inhibited three important pathogenic fungi, namely *Rhizoctonia solani* and *Fusarium oxysporum*, with the inhibition zone ranging from 20 to 30 mm. Furthermore, the T.T.T. Vo, et al. (2019) [11] results in Binh Dinh province showed that *O. sanctum* essential oils have strong inhibitory activity against the growth of *Lactobacillus fermentum* and *Staphylococcus aureus*. It also inhibits the development of *Bacillus subtilis* and *Pseudomonasa eruginosa*. The essential oil of *O. sanctum* grown in Hanoi shows strong activity against three fungal strains of human skin pathogen (*Malassezia furfur* VNF01, *M. furfur* ATCC14521 and *M. globosa* VNG02) with the minimum concentration inhibiting the growth of fungal strains of 2.5 µl/ml. In addition, the cell number of the tested yeast strains was eliminated by 74.7-80.7% after 20 min of treatment at this concentration [12].

The purpose of the present research is to determine the chemical constituents of *O. tenuiflorum* leaves grown in Hanoi via GC/MS analysis and to elucidate their antioxidant and antifungal activities against plant pathogenic fungi (*F. oxysporum*, *F. solani*, *Phytophthora capsici*, *R. solani*).

## 2. Materials and methods

### 2.1. Materials and chemicals

Fresh leaves of *O. tenuiflorum* (1 kg, 30-35% moisture) were collected from the National Institute of Medicinal Materials (Hanoi, Vietnam) in October 2022. They were then hydrodistilled separately for 4 h, with the process performed in triplicate. The obtained essential oil was separated, dehydrated, and stored at 4°C until analysis.

Pathogenic fungi (*Fusarium oxysporum* KACC 40032, *Fusarium solani* KACC 40484, *Phytophthora capsici* KACC 40473, *Rhizoctonia solani* KACC 40111) were provided by the Korean Agricultural Culture Collection (KACC). DPPH, potassium persulphate, potassium ferricyanide, and dimethyl sulfoxide were purchased from Sigma-Aldrich. ABTS and BHT were purchased from Merck. All solvents were of analytical grade.

### 2.2. Gas chromatography/mass spectrometry (GC/MS) analysis

The chemical composition of the essential oil of *O. tenuiflorum* was analysed by GC/MS. A GC/MS (QP2020) system from Shimadzu Company (Japan) with a capillary column SH-Rxi-5Sil MS (30 m x 0.32 mm x 0.25 µm, non-polar phase: 100% dimethyl polysiloxane) was used. The essential oil of *O. tenuiflorum* was diluted in n-hexane and mixed using a vortex mixer. For the MS analysis, the

electron ionisation source was set at 200°C and the interface source was set at 250°C to isolate and fragment the ions. The GC/MS (QP2020) scan mode was used with an MS range from 50 to 900 m/z. Peaks were observed in the total ion chromatogram. Interpretation of the mass spectrum of GC/MS was done using the NIST mass spectral search program. The mass spectrum was compared with the spectrum of the components stored in the NIST library. The name, chemical structure, and molecular weight of the major components were determined [13, 14].

### 2.3. Antioxidant activity determination

The DPPH free radical scavenging ability was conducted according to the spectrophotometric method described in G. Miliauskas, et al. (2004) [15] with slight modifications [16]. Briefly, various concentrations of essential oil (0.1 ml) were mixed with 0.9 ml of 0.2 mM DPPH dissolved in methanol. The samples were then vortexed and incubated at 25±1°C for 15 min in the dark. The absorbance was measured at 517 nm using a BioTek MicroFill Dispenser (Agilent, Santa Clara, CA, USA). The ability to scavenge DPPH radicals was calculated as

$$\text{DPPH radical-scavenging activity (\%)} = [(C - T)/C] \times 100 \quad (1)$$

where, C and T are the absorbances of the DPPH radicals in methanol and essential oil, respectively. Meanwhile, BHT was used for comparison. The assays were performed twice, with three replicates for each treatment.

The ABTS free radical scavenging ability was conducted according to the method of R. Re, et al. (1999) [17] with some modifications [16]. For the ABTS assay, various concentrations of essential oil (0.2 ml) were mixed with 1.8 ml of ABTS solution consisting of a mixture of ABTS (7.4 mM) and potassium persulphate (2.6 mM). The reaction mixture was incubated at 25±1°C for 2 h in the dark. The sample was absorbed at 734 nm using a BioTek MicroFill dispenser. The ABTS radical-scavenging activity was calculated as

$$\text{ABTS radical-scavenging activity (\%)} = [(C - T)/C] \times 100 \quad (2)$$

where, C and T are the absorbances of the ABTS radicals in methanol and essential oil, respectively. BHT was used as a positive control. The experiments were performed twice, with three replicates for each treatment.

The reducing power of *O. tenuiflorum* was determined according to the method of M. Oyaizu (1986) [18] as described by T.H. Luong, et al. (2022) [19]. Various concentrations of the essential oil were mixed with 2.5 ml of 0.2 M phosphate buffer and 2.5 ml of 1% (w/v) potassium

ferricyanide, followed by incubation at 50°C for 20 min. Thereafter, 2.5 ml of 10% (w/v) trichloroacetic acid was added to the sample, followed by centrifugation at 3000×g for 20 min. Finally, 2.5 ml of the supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% (w/v) ferric chloride, and the absorbance was measured at 700 nm using a BioTek MicroFill dispenser. Increased absorbance by the sample indicates an increase in reducing power. BHT, known for its antioxidant properties, was also used for comparison.

The concentration of the *O. tenuiflorum* essential oil that was used to evaluate the antioxidant activity ranged from 12.5, 25, 50, 100 to 200 µg/ml.

#### 2.4. Antifungal activity determination

The agar diffusion technique was used to determine the antifungal activity of the essential oil on various plant pathogens [20]. Various concentrations of the essential oil from stock solutions were added to 20 ml of sterilised potato dextrose agar in Petri dishes (90×15 mm) to provide final concentrations of 0, 12.5, 25, 50, 100, or 200 µg/ml. Test fungi (*R. solani*, *F. oxysporum*, *F. solani*, and *P. capsici*) were inoculated using 6 mm-diameter mycelial plugs and fungal growth was recorded after 3-9 days of incubation at 25°C until full fungal growth had occurred in the control plates. The percentage of growth inhibition was calculated from mean values as

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

where, C and T are the colony diameters in the methanol control and the essential oil-treated sample, respectively. The assay was performed twice, with three replicates for each treatment.

The minimum inhibitory concentration (MIC) that inhibited any visible fungal growth was analysed by using the twofold serial dilution method [21]. The essential oil of *O. tenuiflorum* was dissolved in ethanol to obtain a stock solution of 4000 µg/ml, then further diluted with the same solvent to obtain twofold serial dilutions of 400, 200, 100, 50, 25, and 12.5 µg/ml. These were added to Potato Dextrose Broth (PDB) in the wells of a 24-well Microtest™ tissue culture plate to obtain the desired essential oil concentration. A 6 mm-diameter plug cut from the actively growing region of a 1-week-old colony of *R. solani* was added to the culture. A control culture treated with methanol only was also prepared. The cultures were incubated at 25°C for 72 h. Four replicates for each were run simultaneously. The MIC value was defined as the lowest essential oil concentration from *O. tenuiflorum* without any visible fungal growth of *R. solani*.

#### 2.5. Statistical analysis

Treatment effects were determined by analysis of variance conducted according to the general linear model of the Statistical Analysis System 9.4 [22]. Means were separated with Tukey's honestly significant difference (HSD) test, with a significance of  $p \leq 0.05$ .  $IC_{50}$  values were calculated based on linear regression of DPPH and ABTS radical scavenging activity at different concentrations of essential oil. The experiments were performed twice with three replicates for each treatment (n=6). All data are presented as mean values ± standard deviation, based on the number of observations.

### 3. Results and discussion

#### 3.1. The composition of the essential oil from *Ocimum tenuiflorum* leaves

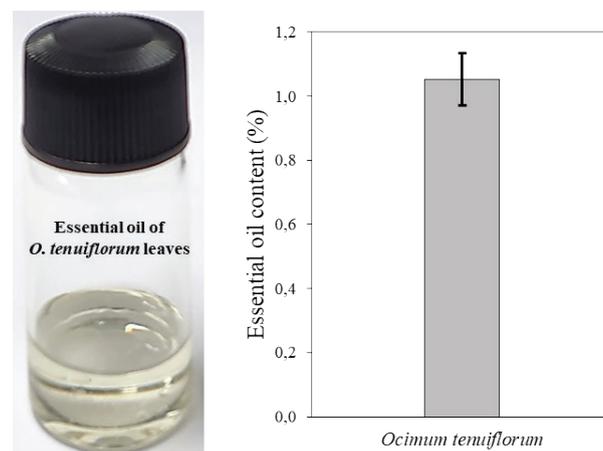


Fig. 1. Extracted essential oil of *Ocimum tenuiflorum* leaves (Hanoi, Vietnam).

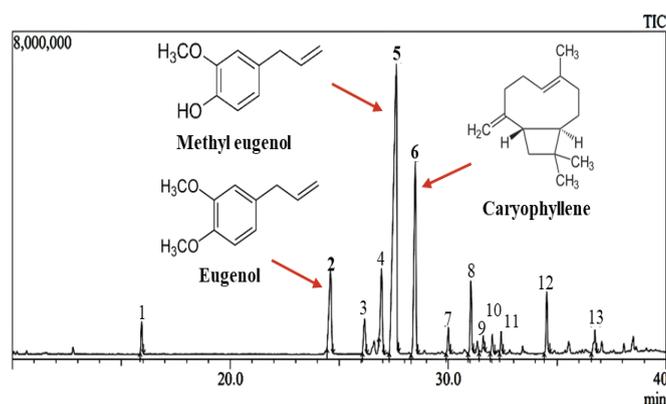


Fig. 2. A GC-MS profile showing the compounds in the essential oil obtained from the leaves of *Ocimum tenuiflorum*.

**Table 1. Chemical composition of the essential oil from the leaves of *Ocimum tenuiflorum* identified via GC-MS.**

Peak#	Compound	RT (min)	Retention Index		Content (%)
			RI <sub>Cal</sub>	RI <sub>Db</sub>	
1	Endo-borneol	15.931	1165±2	1165	1.92
2	Eugenol	24.596	1358±3	1356	11.83
3	α-Copaene	26.163	1376±3	1377	1.95
4	β-Elemene	26.937	1384±5	1384	4.33
5	Methyl eugenol	27.625	1410±4	1412	46.07
6	Caryophyllene	28.491	1426±2	1426	15.65
7	Humulene	30.009	1452±3	1455	1.63
8	β-Copaene	31.045	1483±6	1482	4.18
9	α-Selinene	31.613	1508±2	1508	1.02
10	Guaia-1(10),11-dien	32.027	1511±1	1510	1.14
11	β-Cadinene	32.431	1522±5	1524	1.32
12	Caryophyllene oxide	34.527	1583±1	1583	3.18
13	Neointermedeol	36.740	1632±2	1633	1.84
Monoterpene hydrocarbon (peak Nos.)					0
Oxygenated monoterpenes (peak Nos. 1, 2, 5)					58.82
Sesquiterpene hydrocarbon (peak Nos. 3, 4, 6-9, 11)					30.08
Oxygenated sesquiterpenes (peak Nos. 10, 12, 13)					6.16
Total identified					96.06

RT: retention time (min); RI<sub>Cal</sub>: retention index calculated using a standard non-polar GC column and library data from NIST: 17 (Software NIST MS Search v2.3); RI<sub>Db</sub> (Column Rxi-1 MS); retention index from the database.

The obtained essential oil from *O. tenuiflorum* leaves is transparent, with a recovery yield of 1.05±0.081% (Fig. 1). A total of 13 compounds were detected and identified by cross-referencing their mass spectra and retention indexes with those in GC/MS libraries (NIST) (Fig. 2, Table 1). As reported in Table 1, the essential oil contained three oxygenated monoterpenes (1, 2, and 5), seven sesquiterpene hydrocarbons (3, 4, 6-9, and 11), and three oxygenated sesquiterpenes (10, 12, and 13), accounting for 58.82, 30.08, and 6.16% of the total, respectively. The oxygenated monoterpene profile of the essential oil was characterised

by the presence of methyl eugenol (46.07%), caryophyllene (15.65%), and eugenol (11.83%); the sesquiterpene hydrocarbon profile featured β-elemene (4.33%) and β-copaene (4.18%); and the sesquiterpene profile comprised caryophyllene oxide (3.18%).

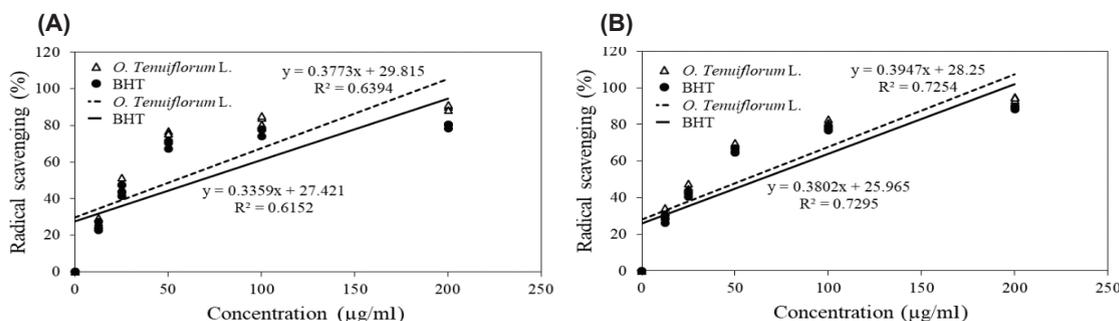
Comparatively, the essential oil of *O. tenuiflorum* contains methyl eugenol, β-caryophyllene [2], methyl eugenol, (E)-caryophyllene, eugenol, and β-elemene [23, 24]. According to İ. Gülçin (2011) [25], eugenol has powerful antioxidant and radical-scavenging abilities and is largely responsible for the therapeutic potential of the essential oil of *O. sanctum* [4]. The essential oil of *O. sanctum* identified 19 main substances, accounting for 99.88% of the total substances in the essential oil sample grown in Hanoi, with eugenol being the most predominant accounting for 42.4% [12]. Additionally, T.T.T. Vo, et al. (2019) [11] showed that the essential oil of *O. sanctum* contains 71.21% eugenol when grown in Binh Dinh province. Also, the eugenol content in *O. sanctum* essential oil grown in Iran was 15.7-37.5% [26]. Meanwhile, in this study, the main component in essential oils of *O. tenuiflorum* was methyl eugenol.

**3.2. Antioxidant activity of the essential oil from *Ocimum tenuiflorum* leaves**

**Table 2. Radical-scavenging activity of the essential oil obtained from *Ocimum tenuiflorum*.**

Concentration (µg/ml)	DPPH		ABTS		Reducing power	
	OT	BHT	OT	BHT	OT	BHT
12.5	27.5±2.0 <sup>e</sup>	24.5±2.5 <sup>c</sup>	32.2±1.9 <sup>f</sup>	28.3±2.2 <sup>f</sup>	0.08±0.01 <sup>d</sup>	0.05±0.01 <sup>d</sup>
25	48.5±2.9 <sup>d</sup>	44.2±3.2 <sup>d</sup>	45.5±1.8 <sup>e</sup>	42.2±3.2 <sup>e</sup>	0.19±0.02 <sup>d</sup>	0.13±0.04 <sup>d</sup>
50	76.0±0.8 <sup>bc</sup>	69.6±3.3 <sup>c</sup>	69.1±0.9 <sup>d</sup>	65.6±3.3 <sup>d</sup>	0.40±0.03 <sup>c</sup>	0.31±0.02 <sup>cd</sup>
100	83.3±2.4 <sup>b</sup>	76.6±3.6 <sup>bc</sup>	81.4±1.2 <sup>e</sup>	77.9±3.6 <sup>c</sup>	0.82±0.03 <sup>b</sup>	0.60±0.02 <sup>c</sup>
200	89.9±1.3 <sup>a</sup>	79.9±4.1 <sup>b</sup>	94.2±0.8 <sup>a</sup>	89.2±4.1 <sup>b</sup>	0.95±0.02 <sup>a</sup>	0.73±0.03 <sup>b</sup>
IC <sub>50</sub> (µg/ml)	53,5±2.1	67.2±1.8	55.1±1.3	63.2±2.7	-	-

The results are expressed as the mean ± SD from triplicate measurements. Different letters in the column indicate a significant difference at each observation time based on a Tukey's HSD test with significance p≤0.05; OT: *Ocimum tenuiflorum*.



**Fig. 3. Percentage radical scavenging vs. concentration of *Ocimum tenuiflorum* essential oil along with the linear regression equations used to calculate the values for 2,2-diphenyl-2-picrylhydrazyl (A) and 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) (B). Different letters above the standard error bars indicate a significant difference at each observation time based on Tukey's HSD test, with a significance of p≤0.05.**

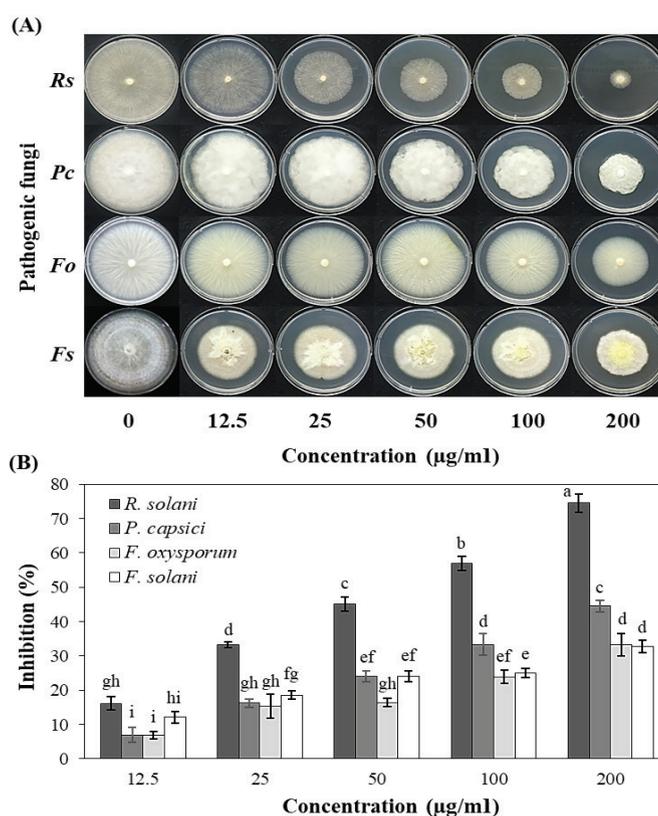
According to the studies conducted by [27] and [28], methyl eugenol is one of the derivatives of eugenol. Methyl eugenol is a phenylpropanoid chemical that is found in the essential oil of *O. sanctum* and has been reported to have antioxidant activity [28, 29]. It has also been reported that methyl eugenol has antioxidant activity [29, 30]. Eugenol is a similar compound to methyl eugenol and has demonstrated relevant biological potential with well-known antimicrobial and antioxidant action. However, eugenol has a dual effect on oxidative stress, which can act as an antioxidant or prooxidant agent [30]. Antioxidants play an important role in protecting the body against damage from free radicals by stopping the formation of new free radical species, converting existing ones to less toxic molecules that can be easily eliminated, and preventing radical chain reactions [31]. Various *Ocimum* species and their extracts or essential oils have shown antioxidant activity [32]. In our study, 0, 12.5, 25, 50, 100, and 200  $\mu\text{g/ml}$  of the *O. tenuiflorum* essential oil provided DPPH radical-scavenging activity of 0.0, 27.5, 48.5, 76.0, 83.3, and 89.9%, and BHT radical-scavenging activity of 0.0, 24.5, 44.2, 69.6, 76.6, and 79.9%, respectively (Table 2). Additionally, the  $\text{IC}_{50}$  values for the DPPH radical-scavenging activity of the *O. tenuiflorum* essential oil and BHT were 53.5 and 67.2  $\mu\text{g/ml}$ , respectively, which were significantly different (Table 2; Figs. 3A, 3B). In the ABTS radical-scavenging assay with 200  $\mu\text{g/ml}$  of the essential oil, the anti-radical activity of *O. tenuiflorum* was 94.2%, while that of BHT was 89.2% (Table 2). The  $\text{IC}_{50}$  values of the ABTS radical-scavenging activity by the *O. tenuiflorum* essential oil was 55.1  $\mu\text{g/ml}$ , which was significantly different from that of BHT (63.2, Table 2). The reducing power values ( $\Delta\text{OD}$ ) of 200  $\mu\text{g/ml}$  of *O. tenuiflorum* essential oil and BHT were 0.95 and 0.73, respectively (Table 2).

The study conducted by İ. Gülçin (2011) [25] suggested that eugenol inhibited 96.7% of lipid peroxidation in a linoleic acid emulsion at a concentration of 15  $\mu\text{g/ml}$ . At the same concentration, butylated hydroxyanisole, butylated hydroxytoluene,  $\alpha$ -tocopherol, and Trolox displayed 95.4, 99.7, 84.6, and 95.6% inhibition of peroxidation, respectively. Furthermore, E. Nagababu, et al. (2010) [33] demonstrated that eugenol significantly inhibits the rise in glutamic-oxaloacetic transaminase activity and cell necrosis without protecting the endoplasmic reticulum damage as assessed by its failure to prevent a decrease in glucose-6-phosphatase activity. The protective action of eugenol has been found to be due to interception of secondary radicals derived from endoplasmic reticulum lipids rather than interfering with primary radicals of  $\text{CCl}_4$ .

F.L. Hakkim, et al. (2008) [32] demonstrated that all *Ocimum* species extracts were capable of scavenging DPPH free radicals at a dose of 1  $\mu\text{g/ml}$ . However, none of the *Ocimum* species extracts were as effective as the positive control BHA (85.2%) in scavenging DPPH $\cdot$  radicals. The cultivar also had a significant impact on both FRAP (ferric reducing antioxidant power) and DPPH antioxidant capacities. Study of A. Chaudhary, et al. (2020) [29] conducted that *O. sanctum* extracted by butanol (OsB) was

rich in both total polyphenolic content (TPC) ( $212.26 \pm 6.3$  mg GAE/g extract) and total flavonoid contents (TFC) ( $54.51 \pm 3.5$  mg QE/g extract). Similarly, *O. sanctum* extracted by ethylacetate (OsE) also had significantly high TPC ( $202.71 \pm 5.5$  mg GAE/g extract). The  $\text{EC}_{50}$  values based on DPPH ( $3.91 \pm 0.3$   $\mu\text{g/ml}$ ), ABTS ( $1.6 \pm 0.1$   $\mu\text{g/ml}$ ), and phosphomolybdate ( $2.31 \pm 0.1$   $\mu\text{g/ml}$ ) for OsB, hydroxyl ( $5.3 \pm 0.4$   $\mu\text{g/ml}$ ), superoxide ( $7.32 \pm 0.9$   $\mu\text{g/ml}$ ) radicals for *O. sanctum* extracted by methanol (OsM), and DPPH ( $8.61 \pm 0.6$   $\mu\text{g/ml}$ ), phosphomolybdate ( $2.43 \pm 0.1$   $\mu\text{g/ml}$ ), and ABTS ( $5.3 \pm 0.4$   $\mu\text{g/ml}$ ) for OsE, were lower than that of ascorbic acid, indicating potential antioxidant properties [29]. The essential oils of *Ocimum* sp. also exhibited antioxidant activities through the DPPH radical scavenging assay, with an  $\text{EC}_{50}$  value of 9.43  $\mu\text{g/ml}$  [34]. In this study, the essential oil of *O. tenuiflorum* leaves could reduce the pink-coloured free radical to yellow-coloured diphenyl picrylhydrazine, confirming its DPPH radical scavenging activity and indicating that the essential oil possessed relatively high antioxidant activity.

### 3.3. The inhibitory effect of *Ocimum tenuiflorum* essential oil on the growth of fungal mycelia



**Fig. 4. The antifungal activity of essential oil of *Ocimum tenuiflorum* against four pathogenic fungi: (A) photos and (B) a bar chart of the mycelial growth inhibition.** The error bars represent the standard error of the mean. The calculated mean values are from three replicates. Mean values with the same letter are not significantly different at  $p \leq 0.05$  when compared using the least significant difference. Rs: *R. solani*; Pc: *P. capsici*; Fo: *F. oxysporum*; Fs: *F. solani*.

In the present study, the essential oil from *O. tenuiflorum* leaves displayed potent antifungal activity and inhibited the mycelial growth of *R. solani* in a dose-dependent manner, with a significant increase as the essential oil concentration was increased from 0 to 200 µg/ml (Fig. 4). Meanwhile, it showed weak-to-medium strong mycelial growth inhibition of *P. capsici*, *F. oxysporum*, and *F. solani*. In summary, 200 µg/ml of the essential oil from *O. tenuiflorum* markedly inhibited the mycelial growth of *R. solani*, *P. capsici*, *F. oxysporum*, and *F. solani* by 74.6±2.6%, 44.5±1.6%, 33.3±3.2%, and 32.7±1.8%, respectively (Fig. 4). Similar to our study, T.T.Q. Cung, et al. (2018) [10] suggested that essential oil of *O. sanctum* inhibited growth of *R. solani* and *F. oxysporum*. In particular, the effect of eugenol of different concentrations on the growth of mycelium was tested on a PDA medium against *R. solani*. The EC<sub>50</sub> and EC<sub>90</sub> values for eugenol were 52.77 and 166.37 µg/ml, respectively [35]. The fungicidal activity of eugenol (IC<sub>50</sub> = 58.9 and 52.9 µg/ml, respectively) against *R. solani* and *F. oxysporum* was also evaluated [36]. Also, no mycelial growth at concentrations of 200, 400 and 800 µg/ml was observed. According to the definition of MIC, 200 µg/ml was the MIC value for eugenol on *R. solani* [35]. Moreover, after treatment with eugenol, the mycelia lost water and shrank, accompanied by surface depression, roughness and unevenness.

Destruction of the mycelia was more serious at a eugenol concentration of 166.37 µg/ml than at 52.77 µg/ml. Meanwhile, mycelial morphology of *R. solani* showed that the mycelia in the control were regular, plump and uniform [35]. X. Zhou, et al.'s study on the antifungal activity of eugenol against *F. oxysporum* showed that eugenol treatment at half MIC decreased the spore germination of *F. oxysporum* by 90.22%, whereas the spore germination of *F. oxysporum* was almost completely inhibited at the MIC of eugenol, and there was no significant difference between 0.5 g/l of eugenol and 8 g/l of chlorothalonil. Especially, the mycelial morphology of *F. oxysporum* was wrinkled and rough after 3 day of eugenol treatment. Also, the structural integrity of the cells was damaged to varying degrees [37]. The antibacterial activity analysis demonstrated that β-caryophyllene presented MIC values equivalent to 32 µg/ml against *Staphylococcus aureus* [38].

#### 4. Conclusions

In this work, we investigated the chemical constituents, antioxidant and antifungal activity of the essential oils of *O. tenuiflorum*. The major compounds of *O. tenuiflorum* were methyl eugenol, caryophyllene, eugenol, β-elemene, β-copaene, and caryophyllene oxide, with the methyl eugenol being the most predominant compound, accounting for a very high share of 46.07%. The essential oil of *O. tenuiflorum* demonstrated a great potential as an alternative to synthetic fungicides for the control of plant pathogens. These results suggest that the essential oil of *O. tenuiflorum* could be a promising natural antioxidant and fungicide agents. However, the antioxidative activities of eugenol, methyl eugenol and

other major compounds from the essential oil of *O. tenuiflorum* identified in this study should be subjected to more thorough evaluation in future in-depth studies.

#### CRedit author statement

Thi-Hoan Luong: Conceptualisation, Methodology, Data analysis, Software, Data curation, Investigation, Original draft preparation, Writing; Huu-Khanh-Tan Tran: Methodology and Data analysis; Woo-Jin Jung: Methodology, Data analysis, and Editing; Van-Viet Nguyen: Methodology and Data analysis; Dang-Minh-Chanh Nguyen: Conceptualisation, Methodology, Data analysis, Writing - Reviewing and Editing.

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#### COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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