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CHEMICAL COMPOSITION, ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF *ZINGIBER OFFICINALE* AND *ZINGIBER ZERUMBET* ESSENTIAL OILS

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Summary

Chemical Composition, Antibacterial and Antifungal Activities of *Zingiber officinale* and *Zingiber zerumbet* Essential Oils

Gingers have been widely used for a long time to treat many diseases. In this study, the essential oils of two ginger species *Zingiber zerumbet* (ZZ-EO), and *Zingiber officinale* (ZO-EO) were tested *in vitro* for their antibacterial activity using the micro-broth dilution method while the antifungal effect was evaluated using the agar well diffusion technique. The results showed that ZO-EO significantly inhibited five tested bacterial strains with a maximum percentage of inhibition (%I) of 98.95%, while ZZ-EO only expressed a weak action (%I from 17.69 to 69.40%). Similarly, ZO-EO was remarkably more effective than ZZ-EO in inhibiting most of the ten tested fungal strains. Particularly, ZO-EO inhibited completely the growth of two rice blast fungi (strains VNN-1247 and VNN-0737), and one human fungus, *Talaromyces marneffei* strain, for up to 14 days after infection. On the contrary, ZZ-EO produced mycelium growth inhibition in some strains for up to only 5 days. GC-MS revealed that oxygenated monoterpenes (36.37%) and monoterpene (33.44%) were the main components in ZO-EO while the oxygenated sesquiterpenes (52.44%) dominated the composition of ZZ-EO. In detail, the primary constituents in ZO-EO included camphene (14.33%), geranial (13.09%), neral (9.82%), and β-phellandrene (9.24%). On the other hand, the most abundant components in ZZ-EO were zerumbone (45.02%), followed by α-humulene (12.94%) and camphene (12.68%). The difference in composition of two essential oils might explain the significant difference in their anti-microorganism activity. In comparison to ZZ-EO, ZO-EO exhibited a greater potential in treating many diseases caused by several plant and human-infected microorganism strains.

Keywords: *Antibacterial activity, Antifungal activity, Essential oils, Zingiber zerumbet, Zingiber officinale.*

1. Introduction

Zingiber officinale (belonging to the family Zingiberaceae), or ginger, is among the most used spices. The essential oils of ginger have been extensively tested for their antibacterial activity against many bacterial strains such as *Escherichia coli* and *Staphylococcus aureus* [1], *Pseudomonas aeroginosa*, *Salmonella typhimurium* and *Shigella flexneri* [2], *Xanthomonas oryzae*, *Ralstonia solanacearum*, *Bacillus* sp., *Klebsiella* sp. [3], *Listeria monocytogenes* [4], *Campylobacter jejuni* and *Campylobacter coli* [5]. In 2006, Albena Stoyanova and colleagues showed a weak antibacterial effect of the ginger essential oil from Vietnam against several gram-negative bacteria such as *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella abony* or gram-positive strains such as *Bacillus pumilus*, *Bacillus subtilis*, *Staphylococcus epidermidis* and *Staphylococcus aureus* [6]. In addition, the essential oil from *Z. zerumbet*, also known as bitter ginger, has attracted less attention than *Z. officinale*. A comparative study between these two ginger species indicated a stronger antibacterial activity of the essential oil from *Z. officinale* than from *Z. zerumbet* against common bacteria including *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Escherichia coli* [7]. In contrast, an Indian investigation in 2018 demonstrated that *Z. zerumbet*'s essential oil and its major component zerumbone expressed considerable inhibitory effect against *Staphylococcus aureus*, *Streptococcus mutans*, and *Escherichia coli* with minimum inhibitory concentration (MIC) ranging from 52.0 to 208.0 µg/mL [8]. However, the effect of essential oils from both ginger species against *Klebsiella pneumoniae* and *Enterococcus faecalis* has not been much investigated.

Fungal diseases affecting animals, humans, and plants have become a pressing issue that demands immediate attention due to their detrimental impact on health and the environment. *Fusarium* is a prevalent fungus that can cause severe diseases in plants and animals, including humans [9],[10],[11]. *Fusarium oxysporum*, a commonly found fungus in the environment, is known to produce white, light green, or light orange mycelium on infected plants. This fungus primarily affects tropical fruits like bananas and some plants like coffee,

causing widespread wilt disease [9]. Besides, humans or animals consuming *Fusarium*-contaminated plants can also become infected with *Fusarium* toxins. Although the effects of *Fusarium* toxic exposure on humans are poorly understood, it can cause symptoms such as vomiting, abdominal pain, and diarrhea or exacerbate existing infections caused by other pathogens [12]. Besides the antibacterial activity, *Z. officinale*'s essential oil was also reported as a potential antifungal agent that inhibited the growth of many fungal pathogens such as *F. oxysporum*, *F. graminearum*, and *F. moniliform* [13], *F. verticillioides* [14], *Aspergillus flavus* [15], etc. According to Stoyanova et al. [6], *Z. officinale*'s essential oil from Vietnam possessed a significant fungicidal effect against *Penicillium* sp., *A. niger*, *Botrytis cinerea* and *Rhizopus nigricans*. In addition, Kader et al. indicated that the essential oil of *Z. zerumbet* was able to inhibit two fungal pathogens, including *C. albicans* and *A. niger* [16]. However, the investigation on *Talaromyces marneffei* and *Aspergillus fumigatus* fungi is still limited.

This study aimed to investigate the chemical composition of two common ginger species *Z. officinale* and *Z. zerumbet* using the GC-MS method. In addition, the antibacterial activity of these samples was carried out against 2 gram-negative (*Escherichia coli* and *Klebsiella pneumoniae*) and 3 gram-positive bacterial strains (*Enterococcus faecalis*, *Listeria monocytogenes*, and *Staphylococcus aureus*). Meanwhile, the antifungal effect was determined against 7 plant strains (*F. oxysporum*, Foc-TG, Foc-VP, VNC-0031, VNN-0737, VNC-0076, and VNN-1247) and 3 human fungal strains (*Talaromyces marneffei*, *Fusarium* sp. ATCC60289, and *Aspergillus fumigatus*).

2. Materials and Methods

2.1. Materials

2.1.1. Plant material:

The rhizome of two ginger species, including *Zingiber officinale* Rosc. (ZO) and *Zingiber zerumbet* (L.) Sm. (ZZ) were respectively collected from Son La and Tien Giang province in February 2023. The plants were identified by Dr. Nguyen Quoc Binh, Vietnam National Museum of Nature, Vietnam Academy of Science and Technology (VAST).

Fresh ginger rhizomes were carefully washed with tap water. Then, the samples were drained before the extraction of essential oils.

2.1.2. Microorganism material:

In this study, two gram-negative bacteria including *E. coli* and *K. pneumoniae* and three gram-positive bacterial strains including *E. faecalis*, *L. monocytogenes*, and *S. aureus* were provided by the International Mixed Laboratory on Drug Resistance in South East Asia, at University of Science and Technology of Hanoi.

Fusarium strains were isolated from infected plants in different provinces in Vietnam. The *F. oxysporum* strain was isolated from infected coffee roots in Dak Lak (Fo-DL), *F. oxysporum* F. sp. *cubense* (Foc-TG and Foc-VP) strains were isolated from infected banana leaves in Tien Giang and Vinh Phuc, four rice blast fungal strains VNC-0031, VNN-0737, VNC-0076, and VNN-1247 isolated from rice infected leaves in Quang Binh, Yen Bai, Thua Thien Hue, and Ninh Binh, respectively. Three strains harm humans and animals, including *T. marneffei*, *Fusarium* sp. ATCC60289, and *A. fumigatus* were provided by the Oxford University Clinical Research Unit Ho Chi Minh City (OUCRU - HCMC).

2.2. Methods

2.2.1. Essential oils extraction:

Essential oils (EOs) from fresh rhizomes of ZO and ZZ were extracted by hydrodistillation in the Clevenger-type apparatus for 5 hours. The collected EOs were dried with anhydrous sodium sulfate and then stored at 4°C until analysis. The samples were labeled as ZO-EO for essential oil from *Z. officinale* and ZZ-EO for essential oil from *Z. zerumbet*. The extraction yield of ZO-EO and ZZ-EO was 0.20 % and 0.22% (w/w), respectively.

2.2.2. GC-MS and GC-FID analysis:

The volatile components of EOs were determined by an Agilent 7890A gas chromatography (GC) system equipped with a mass spectrometry detector (MSD) Agilent 5975C detector and an HP-5MS column (60 m × 0.25 mm, 0.25 µm film thickness) (Agilent Technologies, CA, USA). One µL of EOs was introduced by an injector setting at 250°C, with helium as the carrier gas at a 1mL/min flow rate. The running temperature was programmed from 60°C to 240°C at 4°C/min. The split ratio was 100:1. The MSD full-scan mode ranged from 35 to 450 amu.

The composition of EOs was analyzed by using the NIST mass spectral library (NIST27, WILEY7) and the NIST Chemistry WebBook (http://webbook.nist.gov/chemistry/) database. The retention indices (RIs) of EOs constituents were calculated by MassFinder 4.0 software

based on homologous *n*-alkanes with the same conditions. The percentage of the amount of EOs compounds was estimated based on their GC-FID peak areas.

2.2.3. Antifungal activity:

The antifungal activity of EOs was evaluated by an agar well diffusion test [17] against tested fungal strains. Briefly, on the plastic petri dish (60 × 15 mm) containing 1/6 PTSA (one-sixth strength of Potato Tryptic Soy Agar medium, HiMedia Laboratories, India), one 6-mm-diameter hole was punched aseptically using a sterile cork borer at 10 mm from the plate's edge. Subsequently, 20 µL of the EOs was introduced into the well. Afterward, a 5 × 5-mm agar piece carrying 5-day-old appropriate mycelium was plated on the opposite side of the well. The sterile distilled water was used as the control. All tested plates were incubated at 28 ± 2°C (for plant fungi) and at 37 ± 2°C (for animal and human fungi) for up to 5 - 14 days or until the fungal growth fully covers the control plate. The fungal growth was measured in millimeters. The percentage of inhibition was calculated by the following formula [18]:

$$I = \frac{C - T}{C} 100\ (\%)$$

where I - The percentage of inhibition (%); C - Radical growth of fungi in the control plate (mm); T - Radical growth of fungi in the test plate (mm).

The fungal growth was monitored daily, and the data was recorded at three-time points of 5-, 10-, and 14-days post incubation (dpi).

2.2.4. Antibacterial activity:

The antibacterial activity of two ginger essential oils was investigated using the micro-broth dilution method [19]. For each pathogen, a suspension culture was freshly prepared in Mueller Hilton broth (MHB) to obtain a final concentration of 10^8 CFU/mL. In each well of a 96-well plate, 20 µL of essential oil was incubated with 180 µL bacterial at 37°C for 18 hours. The absorbance of the samples was determined at 600 nm using a microplate reader (Bio-Rad, California, USA). The antibiotic mixture including tetracycline, chloramphenicol, and kanamycin (1:1:1, v/v/v) at 4 µg/mL was used as the positive control. The suspension culture without a tested compound solution served as the negative control. The inhibition percentage was calculated using the following formula:

% inhibition = [(Absorbance of negative control - Absorbance of sample)/Absorbance of negative control] × 100%.

All experiments were performed in triplicate.

2.2.5. Data analysis:

Statistical analysis of the data was made with a 5% level of significance using ANOVA in Microsoft Office Excel 2016 assuming a normal distribution.

3. Results and discussion

3.1. Chemical composition of two essential oils

A total of 42 compounds accounted for 91.42% were identified in ZO-EO (Table 1). The oxygenated monoterpenes represented the highest abundance with 36.37%, followed by monoterpenes (33.44%) and sesquiterpene (17.68%). The main volatile constituents in ZO-EO included camphene (14.33%), geranial (13.09%), neral (9.82%), *β*-phellandrene (9.24%), *α*-zingiberene (7.98%), and 1,8-cineole (6.67%). This result was significantly different in comparison with the study of Stoyanova et al. [6]. The authors found that *ar*-cucumene (11.7 %), *β*-bisabolene (4.1 %), 1,8-cineole (3.2%), and camphene (2.5%) were the primary constituents in the ginger essential oil in Vietnam. According to a review by Sharifi-Rad et al. in 2017 [20], the main phytoconstituents in ginger essential oils included *α*-zingiberene, geranial, *ar*-curcumene, *β*-bisabolene, *β*-sesquiphellandrene and neral with varying amount, depending on collecting locations, cultivating seasons or extracting methods.

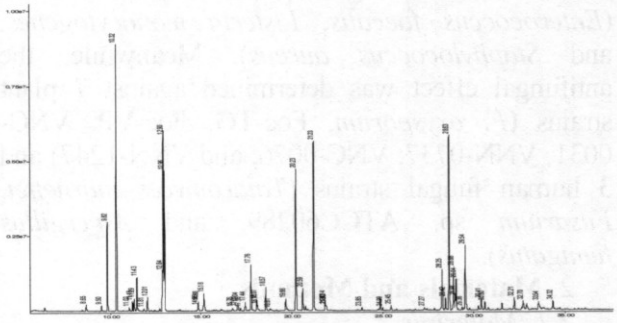

Fig. 1. GC chromatogram of ZO-EO

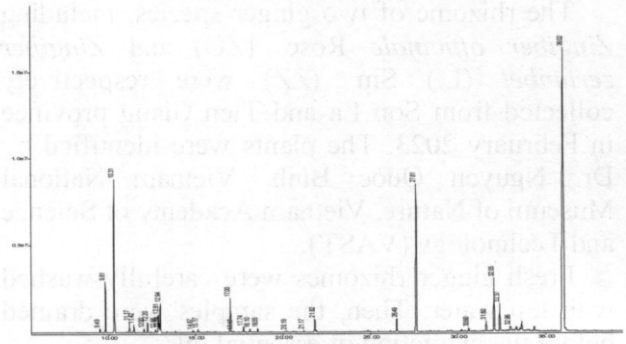

Fig. 2. GC chromatogram of ZZ-EO

Table 1. The chemical composition of ZO-EO and ZZ-EO

№	Retention time	RI	Name	ZO-EO		ZZ-EO	
				%	% hit	%	% hit
1	8.66	899	2-Heptanol	0.48	85	-	-
2	9.82	938	*α*-Pinene	4.21	93	3.68	93
3	10.32	955	**Camphene**	**14.33**	90	**12.68**	**90**
4	11.02	978	Sabinene	0.17	94	-	-
5	11.18	983	*β*-Pinene	0.56	89	0.88	90
6	11.29	987	6-Methylhept-5-en-2-one	0.75	86	-	-
7	11.44	992	Myrcene	2.02	89	0.73	94
8	12.01	1009	*α*-Phellandrene	0.63	84	0.24	81
9	12.20	1015	D-3-Carene	-	-	0.77	97
10	12.66	1028	o-Cymene	-	-	0.21	95
11	12.84	1034	Limonene	2.28	83	1.45	90
12	12.90	1035	***β*-Phellandrene**	**9.24**	65	0.85	69
13	12.96	1037	**1,8-Cineole**	**6.67**	72	2.05	63
14	14.86	1093	2-Nonanone	0.20	73	-	-
15	14.88	1093	Terpinolene	0.39	87	0.13	60
16	15.18	1102	Linalool	0.97	64	-	-
17	17.01	1154	Camphor	0.42	75	-	-
18	17.04	1155	Citronellal	0.20	77	-	-
19	17.18	1159	Camphene hydrate	0.14	68	-	-
20	18.07	1184	Isogeranial	0.64	79	-	-
21	18.11	1185	Terpinen-4-ol	0.33	81	0.23	62
22	18.57	1198	*α*-Terpineol	1.28	66	-	-
23	18.81	1205	Myrtenol	0.15	84	-	-
24	19.66	1230	Citronellol	0.80	88	-	-
25	20.23	1246	**Neral**	**9.82**	93	-	-
26	20.59	1257	Geraniol	1.53	81	-	-
27	21.23	1275	**Geranial**	**13.09**	86	0.19	77
28	21.87	1294	*trans*-Anethole	0.23	75	0.44	75
29	21.90	1295	2-Undecanone	0.25	72	-	-
30	23.85	1354	Citronellyl acetate	0.14	87	-	-
31	24.99	1388	*α*-Copaene	0.11	81	-	-
32	25.45	1402	*cis*-*β*-Elemene	0.26	64	-	-
33	26.49	1435	β -Caryophyllene	-	-	1.01	74
34	27.61	1471	**α -Humulene**	-	-	**12.94**	98
35	28.25	1491	*ar*-Curcumene	1.58	89	-	-
36	28.42	1496	Germacrene D	0.55	87	-	-
37	28.63	1503	***α*-Zingiberene**	**7.98**	73	-	-
38	28.88	1511	(*E*,*E*)-*α*-Farnesene	1.92	83	-	-
39	28.98	1515	*trans*-Muurola-4(14),5-diene	0.69	79	-	-
40	29.04	1517	*β*-Bisabolene	1.29	93	-	-
41	29.36	1528	*γ*-Cadinene	0.11	76	-	-
42	29.54	1533	*β*-Sesquiphellandrene	3.19	95	-	-
43	30.41	1563	Elemol	0.64	78	-	-
44	30.60	1569	(*E*)-Nerolidol	0.43	60	-	-
45	31.60	1603	Caryophyllene oxide	-	-	0.90	69
46	32.05	1618	**Humulene epoxide I**	-	-	**4.25**	88
47	32.37	1630	Humulene Epoxide II	-	-	2.27	82
48	32.70	1641	*α*-Acorenol	0.42	73	-	-
49	34.33	1699	*α*-Bisabolol	0.33	71	-	-
50	36.02	1761	**Zerumbone**	-	-	**45.02**	94
			Monoterpenes	33.44		21.49	
			Oxygenated monoterpenes	36.37		2.91	
			Sesquiterpenes	17.68		13.95	
			Oxygenated sesquiterpenes	1.82		52.44	
			Others	2.21		0	
			Total	91.42		90.79	

-: not detected; %: percentage of composition by mass of each compound; % hit: ratio of similarity between software predictions of retention index.

Unlike ZO-EO, the profile of ZZ-EO was predominantly composed of only 20 volatile compounds, in which, oxygenated sesquiterpenes (52.44%) dominated the chemical profile, followed by monoterpene hydrocarbons (21.49%) and sesquiterpene (13.95%). The main components included zerumbone (45.02%), *α*-humulene (12.94%), camphene (12.68%), and

humulene epoxide I (4.25%). In general, the chemical composition of the two samples is considerably different in terms of the total number of compounds and composition (Fig. 3). The difference in the amount and composition of these two essential oils might probably affect their bioactivities in the next part of this study.

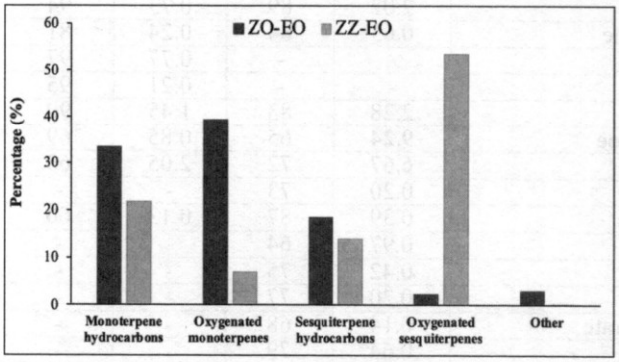

Fig. 3. Difference in chemical composition between ZO-EO and ZZ-EO

3.2. Antibacterial activity of ginger essential oils

The result showed that ZO-EO considerably impeded the growth of most tested bacteria with the percentage of inhibition ranging from 69.36 to 98.95% (Fig. 2). The inhibitory effect of this essential oil was weakest against *E. faecalis* and strongest against *S. aureus*. Moreover, ZO-EO expressed a comparable effect with the positive control against some bacteria such as *S. aureus* and *L. monocytogenes* ($p > 0.05$). Especially, in Vietnam, this is the first time the essential oil from *Z. officinale* was tested against *K. pneumoniae*, a gram-negative bacterium that might cause severe lung or blood infections and shows a great deal of antibiotic resistance. The promising result of ZO-EO in this study can be valuable for further treatment of bacterial-infected diseases. In contrast, ZZ-EO exhibited a significantly weaker impact than ZO-EO on all five bacterial strains. In detail, the maximum percentage of inhibition of ZZ-EO reached 69.40% against *S. aureus* but only 17.69% against *E. coli* (Fig.4).

According to Al-Dhahli and colleagues, the intense antibacterial activity of ZO-EO might be due to the presence of several main bioactive components such as α-zingiberene, *ar*-curcumene and β-sesquiphellandrene [21]. On the other hand, ZZ-EO contained a significant amount of zerumbone (45.02%), a promising antibacterial agent [8], and other components such as humulene, camphene, and α-pinene, also exhibiting good antibacterial properties [22],[23],[24], but the whole essential oil only demonstrated a weak inhibitory effect against all tested bacteria.

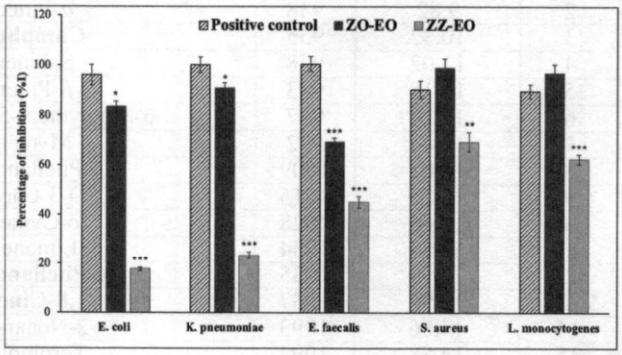

Fig. 4. Percentage of inhibition of ginger essential oils against various bacterial strains
*, **, and *** indicate a significant difference compared to the positive control with levels $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

3.3. Antifungal activity of ginger essential oils

In this experiment, the ginger essential oils were tested for inhibiting the growth of 7 plant-infected strains and 3 human-infected strains for the first time. The results showed that ZO-EO had high effects on all tested fungal strains at 5 dpi. The inhibition rate was observed over 50% except for the strain *F.* sp ATCC60289 (30.30 %). Especially, the growth of strains VNN-1247, VNN-0737, VNC-0031, and *T. marneffei* was completely inhibited at 5 dpi. The ZO-EO was found to completely inhibit the growth of the strains VNN-1247 and *T. marneffei* for up to 14 dpi. The strain VNN-0737 was also significantly inhibited, with nearly 86% of inhibition observed at 14 dpi (Table 2). The VNC-0031 strain was totally inhibited only in the first 5 dpi, then the inhibition decreased dramatically to 11% at 14 dpi. The ZO-EO showed an inhibitory effect with more than 60% inhibition for the other strains during the first five days of the experiment. However, the inhibition rate was drastically decreased at 10 and 14 dpi. The antifungal properties of ZO-EO can be attributed to its main chemical compounds including geranial and neral [25], 1,8-cineole [26], β-phellandrene [27], camphene [23] or α-zingiberene [3]. In the current study, the total percentage of these compounds reached 61.13% over the whole essential oil of *Z. officinale*, which is higher compared to other previous reports [3],[6]. This finding might explain the powerful antifungal action of this ginger. Moreover, the effect of ZO-EO could also be obtained due to the synergic

combination of minor components such as *ar*-curcumin [28], α-pinene, or β-pinene [29]. It is worth emphasizing that ZO-EO was not only able to impede the growth of plant pathogens effectively, but it was also extremely active against *T. marneffei*, a fungus that can cause deep-seated infections in humans. Therefore, the essential oil of *Z. officinale* deserves to be further explored for its potential use in the treatment of plant and human fungi-infected diseases.

Table 2. Antifungal activity of ZO-EO determined by well diffusion assay

Type	Fungal strains	Inhibition rate (%)*		
		5 dpi	10 dpi	14 dpi
Plant strains	**VNN-1247**	**100.00** ± 0.00	**100.00** ± 0.00	**100.00** ± 0.00
	VNN-0737	**100.00** ± 0.00	**100.00** ± 0.00	85.96 ± 0.12
	VNC-0076	85.19 ± 0.26	22.22 ± 0.04	25.00 ± 0.05
	VNC-0031	**100.00** ± 0.00	30.56 ± 0.05	11.11 ± 0.04
	Fo-DL	62.67 ±0.08	0.00 ±0.00	0.00 ±0.00
	Foc TG	66.67 ± 0.05	29.49 ± 0.1	0.00 ± 0.00
	Foc VP	79.49 ± 0.18	0.00 ± 0.00	0.00 ± 0.00
Human strains	*F.* sp ATCC 60289	30.30 ± 0.05	0.00 ± 0.00	0.00 ± 0.00
	T. marneffei	**100.00** ± 0.00	**100.00** ± 0.00	**100.00** ± 0.00
	A. fumigatus	42.31 ± 0.19	5.13 ± 0.16	0.00 ± 0.00

*: the data represents mean ± standard deviation of triplicates; ZO-EO: Essential oils extracted from *Zingiber officinale*; VNC-0031, VNN-0737, VNC-0076, and VNN-1247: rice blast fungal strains isolated rice infected leaves in Quang Binh, Yen Bai, Thua Thien Hue, and Ninh Binh, respectively; Foc TG and Foc VP: *Fusarium oxysporum* f. sp. *cubense* isolated from banana infected leave in Tien Giang and Vinh Phuc, respectively; *T. marneffei: Talaromyces marneffei; F.* sp. ATCC 60289: *Fusarium* sp. ATCC 60289; *A. fumigatus: Aspergillus fumigatus;* dpi: day post incubation

Contrary to ZO-EO, the ZZ-EO sample did not display an effective activity against all the tested fungal strains. Three out of 7 strains (VNN-0737, VNC-0076, and *T. marneffei)* were slowly inhibited up to 14 dpi, with the inhibition ranging from 22% to 30% (Table 3). Still, the maximum inhibition percentage was tiny; the highest was 30%, belonging to the VNC-0076 strain. The others included the plant pathogens and human infection strains, which only showed inhibition up to 5 days after cultivation with a slight percentage inhibition (lower than 50%) and showed a considerable amount of fungal mycelium appearing as in Fig. 2. The VNN-1247 strain had no inhibition effect, and the mycelium covered almost all the petri dishes at the first 5 days of the experiment, respectively.

Like the antibacterial activity, although ZZ-EO also contained potential compounds such as zerumbone, camphene, α-humulene or α-pinene, it only displayed a negligible fungicide effect until day 5 of the experiment. The explanation for this phenomenon is still unclear, therefore, the mechanism of action for these activities of both essential oils needs further investigation.

Table 3. Antifungal activity of ZZ-EO determined by well diffusion assay

Type	Fungal strains	Inhibition rate (%)*		
		5 dpi	10 dpi	14 dpi
Plant strains	VNN-1247	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	VNN-0737	22.92 ± 0.04	19.61 ± 0.03	22.81 ± 0.03
	VNC-0076	**25.93 ± 0.06**	**26.67 ± 0.07**	**30.00 ± 0.03**
	VNC-0031	14.29 ± 0.02	16.67 ± 0.08	6.67 ± 0.12
	F. oxysporum	16.00 ± 0.08	0.00 ± 0.00	0.00 ± 0.00
	Foc TG	32.00 ± 0.28	0.00 ± 0.00	0.00 ± 0.00
	Foc VP	28.21 ± 0.04	0.00 ± 0.00	0.00 ± 0.00
Human strains	*F.* sp ATCC 60289	7.58 ± 0.03	0.00 ± 0.00	0.00 ± 0.00

Type	Fungal strains	Inhibition rate (%)*		
		5 dpi	10 dpi	14 dpi
	T. marneffei	20.00 ± 0.02	27.45 ± 0.03	26.32 ± 0.11
	A. fumigatus	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

*: the data represents mean ± standard deviation of triplicates; ZZ-EO: Essential oils extracted from *Zingiber zerumbet*; VNC-0031, VNN-0737, VNC-0076, and VNN-1247: rice blast fungal strains isolated rice infected leaves in Quang Binh, Yen Bai, Thua Thien Hue, and Ninh Binh, respectively; Foc TG and Foc VP: *Fusarium oxysporum* f. sp. *cubense* isolated from banana infected leave in Tien Giang and Vinh Phuc, respectively; *T. marneffei: Talaromyces marneffei; F.* sp. ATCC 60289: *Fusarium* sp. ATCC 60289; *A. fumigatus: Aspergillus fumigatus;* dpi: day post incubation.

4. Conclusion

In the current study, ZO-EO was found to have a strong inhibitory effect against most of the five tested bacterial strains with an average inhibition of 87.84%. In addition, this essential oil also exerted a remarkable activity on inhibiting completely two plant pathogens, VNN-1247 and VNN-0737, and one human strain *Talaromyces marneffei* up to 14 days after inoculation. On the contrary, ZZ-EO only displayed a weak impact on both assays. In detail, its bacterial percentage of inhibition ranged from 17.69 to 69.40%. Meanwhile, the maximum inhibition of this sample against all fungal strains reached at best 30.00% on the last day of the experiment. GC-MS analysis revealed that monoterpenes and oxygenated monoterpenes in ZO-EO were much more abundant than in ZZ-EO but the content of oxygenated sesquiterpenes was significantly higher in ZZ-EO than in ZO-EO. This finding leads to a suggestion that monoterpenes and/or monoterpenoids might be responsible for the powerful antibacterial and fungicidal effects of ZO-EO. Further investigation should be carried out to elucidate the mechanism of action of this sample's activities.

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QUALITY EVALUATION OF *PIPER BETLE* L. CULTIVATED IN VIETNAM BY HPLC-DAD METHOD

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Summary

Quality Evaluation of *Piper betle* L. Cultivated in Vietnam by HPLC-DAD Method

Piper betle L. is a popular traditional herbal medicine. This plant has several potential health benefits, including antioxidant, anti-inflammatory, and antimicrobial properties. The main active ingredient in *Piper betle* is hydroxychavicol. In this study, the HPLC-DAD method was applied to quantify hydroxychavicol in leaves and branches of *Piper betle*. This method uses a C_{18} column (250 x 4.6 mm, 5 µm), mobile phase includes acetonitrile and 0.05% phosphoric acid (30:70, v/v), flow rate of 0.5 mL/min, and sample injection volume of 10 µL. The HPLC-DAD method was validated according to ICH guidelines, the results showed that this method has high specificity, achieves linearity, achieves repeatability with RSD = 1.29%, and achieves accuracy with recovery rates of 97-103%. The method was applied to evaluate the hydroxychavicol content in *Piper betle* samples collected in several regions in Vietnam. The results showed that the hydroxychavicol content in *Piper betle* leaves was higher than in *Piper betle* branches. *Piper betle* leaves samples contain approximately 4.55-6.88% hydroxychavicol: highest in the sample collected in Thanh Hoa (6.88%) and lowest in the sample collected in Nam Dinh (4.55%). *Piper betle* branches contain only 0.57-1.68% hydroxychavicol. The results obtained are intended to contribute to standardizing the quality of *Piper betle* in Vietnam.

Keywords: *Piper betle* L., *Hydroxychavicol, HPLC-DAD, Quality control.*

1. Introduction

Piper betle is a well-known medicinal plant cultivated primarily in Southeast Asia. This plant comprises many bioactive compounds such as tannins, flavonoids, eugenol, hydroxychavicol, and chavibetol, representing the major components of the plant. This plant has been extensively studied for its pharmacological properties such as antibacterial, anticancer, antioxidant, antidiabetic, and anticancer [1],[2]. In particular, the antifungal and antibacterial effects of *Piper betle* have been published by many studies [3],[4],[5]. Ethanol extract from *Piper betle* leaves (with the main ingredient hydroxychavicol) have antifungal effects against *Malassezia furfur* and *Candida spp*. In addition, the hot water extract from *Piper betle* leaves has antibacterial effects against *C. albican*, *E. coli*, and *S. aureus*. Many studies confirm that the biologically active ingredient in *Piper betle* is hydroxychavicol [6]. In Vietnam, *Piper betle* is currently often used in decoction or extract form.