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Original Research Article

Antimicrobial efficacy and chemical constituents of pseudo-stem essential oils from *Zingiber castaneum*

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ABSTRACT

In this paper, chemical constituents and antimicrobial activity of essential oil from the pseudo-stem of *Zingiber castaneum* Škorničk. & Q.B. (Zingiberaceae) Nguyễn growing in Vietnam havw been reported. Essential oils were obtained by hydrodistillation using the Clevenger-type apparatus. Chemical components of the essential oil were analyzed by gas chromatography (GC) and gas chromatography coupled with mass spectrometry (GC/MS). The minimum inhibitory concentrations (MIC) were evaluated by the method of microdilution broth susceptibility assay. The main constituents of the oil were bicyclogermacrene (**28**, 15.8%), *cis*-β-elemene (**18**, 9.8%) and germacrene D (**26**, 9.2%). The pseudo-stem oil of *Z. castaneum* displayed antimicrobial activity against *Pseudomonas aeruginosa* (ATCC 25923), *Aspergillus niger* (ATCC 9763) and *Fusarium oxysporum* (ATCC 48112) with MIC values of 12.5 ± 0.57 µg/mL, 50 ± 1.00 µg/mL and 50 ± 0.50 µg/mL, respectively. The results indicate the potential of *Z. castaneum* essential oil as a source of antimicrobial agent.

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1. Introduction

Plants are a part of our daily life and their essential oils have been extracted from over 3000 different species that have domestic, industrial and medicinal uses (Adorjan and Buchbauer, 2010). Essential oils have an important role in the protection of plants and pollination and their biological activities have been long related to the substances they contained. Essential oils have exhibited a large number of biological potentials including anti-inflammatory and anti-nociceptive (Ogunwande et al., 2019), antimicrobial (Ha et al., 2019; Huong et al., 2019a) and larvicidal (Ban et al., 2019) among others. Research has shown that the chemical contents of essential oils are valuable in the delineation of chemotypic forms of the oils. For example, high content of phytol was reported for the first time in the mosses *Rhodobrum ontariense* (Pejin et al., 2011). In addition, the large amount of methyl salicylate and potent antimicrobial activity of *Gaultheria procumbens* essential oil generates data for hitherto unknown essential oils or samples in which little information is not readily available (Nikolic et al., 2013).

The *Zingiber* species are noted for their economic importance mainly due to volatile and non-volatile constituents and the various biological activities they exhibited. *Zingiber castaneum* Škorničk. & Q.B. Nguyễn is easily recognized among other terminally flowering species by its upright inflorescence with reflex bracts. The plant is also a rhizomatous herb forming small clumps. The creeping aromatic rhizome which grows up to 1.5 cm in diameter is externally light brown and internally cream white (Hung et al., 2017a). The translucent light green leaves are glabrous. Flowering starts in July and extends to September. It has been found growing in Ninh Bình Province (Hung et al., 2017a).

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Previously, the chemical compounds and biological activities of essential oils of some other *Zingiber* plants grown in Vietnam were reported by us. Although terpene compounds were prominent in these essential oils, the identities of the compounds differ from one species to another. The significant compounds of *Z. nudicarpum* (Hung et al., 2019) leaf oil were α-cedrol (14.8%), β-eudesmol (13.8%) and β-pinene (11.7%), while β-pinene (27.6%) and α-pinene occurred in the root oil. The fruit oil was dominated by (*E*)-nerolidol (30.0%). The main constituents of the leaf oil of *Z. nitens* were δ-elemene (17.0%), β-pinene (12.8%) and β-elemene (8.8%), while the stem oil comprised mainly of δ-elemene (20.1%), germacrene D (8.6%) and bicyclogermacrene (8.1%) with β-pinene (21.0%), δ-elemene (12.8%) and bornyl acetate (11.8%) making up the compositions of the root oil (Hung et al., 2017b). Also, β-pinene (24.7% and 26.1%) and β-caryophyllene (12.3% and 13.9%) were the major compounds in the leaf and stem oils of *Z. vuquangensis* (Huong et al., 2018). The main compounds in the leaf oil of *Z. montanum* were β-pinene (13.8%), β-phellandrene (11.3%) and α-pinene (7.3%), while the rhizome oil was dominated by sabinene (41.1%), terpinen-4-ol (22.7%) and (*E*)-nerolidol (14.3%). The rhizome oil displayed larvicidal activity against *Aedes albopictus, Aedes aegypti* and *Culex. quinquefasciatus* (Huong et al., 2020a). In addition, the larvicidal action of *Z. collinsii* (Huong et al., 2020b) and *Z. zerumbet* (Huong et al., 2019b) as well as the antimicrobial activity of essential oil of *Z. zerumbet* (Huong et al., 2019b) have been reported.

The authors are not aware of any biological effects attributed to the volatile and non-volatile fractions of this species. In a recent report, β-pinene (30.6%), α-pinene (9.5%), β-caryophyllene (9.4%) and bicycloelemene (9.1%) were identified as the main constituents of *Z. castaneum* leaf oil, while β-caryophyllene (14.7%), δ-cadinene (9.8%), bicycloelemene (8.4%) and α-cubebene (7.8%) were the compounds occurring in higher quantity in the pseudo-stem oil from collected from Ha Tinh Province, Vietnam (Huong et al., 2018). However, large quantity of camphene (15.1%), 1,8-cineole (13.6%), linalool (11.3%) and δ-3-carene (8.5%) were present in the root oil, with (*E*)-nerolidol (23.2%), (*Z*)-9-octadecenamide (17.3%) and β-caryophyllene (10.8%) occurring in the fruit oil (Huong et al., 2018).

As part of our ongoing research aimed at the identification of the chemical constituents and biological activities of essential oils from plants grown in Vietnam (Dai et al., 2018; Huong et al., 2019a; Huong et al., 2019b; Huong et al., 2020a; Huong et al., 20120b) to source for potential chemicals for control of diseases, we obtained essential oil from pseudo-stem of *Z. castaneum,* analysed the compounds present therein and examined the antimicrobial activity for the first time.

2.1. Plant collection

Mature pseudo-stem of *Z. castaneum* (Fig. 1) was collected from individual plants growing in Pu Hoat Nature Reserve, Nghệ An Province (GPS 19°20′N 104°50′E), Vietnam (Fig. 2), in August 2018. Botanical identification was accomplished at Botany Museum, Nghệ An College of Economics, Vietnam, where a voucher specimen, LTH 741 was deposited for future references.

2.2. Preparation of samples

In the course of preparation for hydrodistillation process, the pseudo-stems were air-dried (22 ºC) under laboratory shade for two weeks to reduce the moisture contents. Moreover, unwanted materials were also removed by handpicking. Afterwards, samples were pulverized to a coarse powder using a locally made grinder.

2.3. Hydrodistillation procedure

A total of 1000 g of the pulverized sample was used for the experiment. A known weight of sample was separately and carefully introduced into a 5 L flask and distilled water was added until it covered the sample completely. Essential oil was obtained by hydrodistillation which was carried out in an all glass Clevenger-type distillation unit designed according to an established protocol (Vietnamese Pharmacopoeia, 2009) as described previously (Dai et al., 2018). The distillation time was 3 h and conducted at normal pressure. The volatile oil which distilled over water was collected by running through the tap in the receiver arm of the apparatus into clean and previously weighed sample bottles. The oil was kept under refrigeration (4 ºC) until the moment of analyses as described previously (Dai et al., 2018). All experiments were conducted in triplicate.

2.4. Gas chromatography (GC) analysis

Gas chromatography (GC) analysis was performed on an Agilent Technologies HP 7890 Plus Gas chromatograph equipped with a flame ionization detector (FID) and fitted with HP-5MS column (30 m x 0.25 mm, film thickness 0.25 μm, Agilent Technology). The analytical conditions were as follows. He: carrier gas (1 mL/min), injector temperature at 250 ºC, detector temperature 260 ºC, column temperature programmed from 40 ºC (2 min hold) to 220 ºC (10 min hold) at 4 ºC/min. Samples were injected by splitting and the split ratio was 10:1. The volume of diluted oil in hexane (1:10) injected was 1.0 μL. Inlet pressure was 6.1 kPa. Each analysis was performed in triplicate. The relative amounts of individual components were calculated based on the GC peak area (FID response) without using correction factors.

2.5. Gas chromatography-mass spectrometry (GC/MS) experiment

An Agilent Technologies HP 6890N Plus Chromatograph fitted with capillary HP-5 MS column (30 m x 0.25 mm, film thickness 0.25 μm) and interfaced with a mass spectrometer HP 5973 MSD was used for this experiment, under the same conditions as those used for gas chromatography analysis as described previously (Dai et al., 2018). The GC conditions were the same as described above with He (1 mL/min) as the carrier gas. The MS conditions were as fol-

Fig. 1. The photograph of *Zingiber castaneum* Škorničk. & Q.B. (Zingiberaceae) Nguyễn growing in Vietnam.

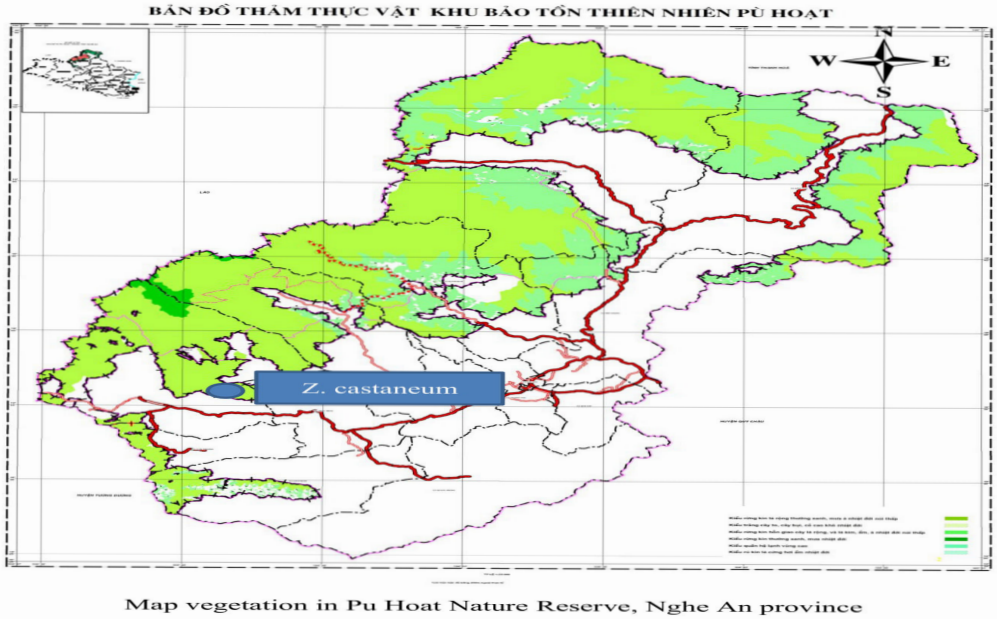

Fig. 2. The geographical map of the sampling area.

lows: ionization voltage 70 eV; emission current 40 mA; acquisitions scan at mass range of 35-350 amu and at a sampling rate of 1.0 scan/s.

2.5.1. Identification of the components of the oils

The identification of constituents from the GC/MS spectra of *Z. castaneum* was performed on the basis of retention indices (RI) determined with reference to a homologous series of *n*-alkanes (C_4-C_{40}), under identical experimental conditions. In some cases, co-injection with known compounds or standards under the same GC conditions was employed. The mass spectral (MS) fragmentation patterns were checked with those of other essential oils of known composition (NIST, 2011) and with those in the literature as described previously (Dai et al., 2018).

2.6. Antimicrobial activity assay

2.6.1. Microbes

Eight standardized ATCC strains from laboratory stock cultures were used in the evaluation of the antimicrobial activity of the oil samples. The Gram-negative strains were *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 25923). The Gram-positive strains were *Bacillus subtilis* (ATCC 11774), *Staphylococcus aureus* subsp. *aureus* (ATCC 11632), while mycetes include *Aspergillus niger* (ATCC 9763) and *Fusarium oxysporum* (ATCC 48112). Two strains of yeast, *Candida albicans* (ATCC 10231) and *Saccharomyces cerevisiae* (ATCC 16404) were also used for the experiment. Testing media included Mueller-Hinton Agar (MHA) used for bacteria and Sabouraud Agar (SA) used for fungi.

2.6.2. Microdilution broth susceptibility assay

The minimum inhibitory concentration (MIC) values were measured by the microdilution broth susceptibility assay (Vanden Bergher and Vlietinck, 1991; Vlietinck, 1999). Stock solutions of the oil were prepared in dimethylsulfoxide (DMSO). Dilution series 16384 to 2 µg/mL (214, 213, 212, 211, 210, 29, 27, 25, 23 and 21 µg/mL) were prepared in sterile distilled water inside the micro-test tubes from where they were transferred separately to 96-well microtiter plates with each of the microbial strains. The plate was then incubated overnight at 37 ºC. One hundred microlitre of the microbial culture of an approximate inoculums size of 1.0 x 106 CFU/mL was added to all well and incubated at 37 ºC for 24 h. The last row, containing only the serial dilutions of the sample without microorganisms, was used as a negative control, while streptomycin was used as positive control. The MIC values were determined as the lowest concentration of the oil that completely inhibits the growth of microorganisms.

3.1. Chemical composition of the essential oil

Table 1 presents the identities, retention indices and percent compositions of the oil.

The average yield of the essential oil of *Z. castaneum* was 0.18% (v/w, ± 0.01), calculated on a dry weight basis. The main classes of compounds in the oil were monoterpene hydrocarbons (10.1%), sesquiterpene hydrocarbons (66.2%) and oxygenated sesquiterpenes (16.5%). The major compounds include bicyclogermacrene (**28**, 15.8%), *cis*-β-elemene (**18**, 9.8%) and germacrene D (**26**, 9.2%). There were significant amounts of α-humulene (**22**, 7.5%), δ-elemene (**16**, 5.4%) and α-zingiberene (**27**, 4.6%). The results indicated both qualitative and quantitative variations between the samples of *Z. castaneum* collected from the Ha Tinh Province (Huong et al., 2018) and Nghean Province (this study) of Vietnam. For example, β-caryophyllene, δ-cadinene and bicycloelemene, the main constituents of oil sample Ha Tinh province occurred in much lower amounts in the present study. Likewise, α-cubebene, as seen in the previous analysis was also conspicuously absent in the present investigated oil sample from Nghean province. Likewise, compounds such as bicyclogermacrene, germacrene D and *cis*-β-elemene were detected in the present analyzed sample in amount higher than reported previously (Huong et al., 2018). Zerumbone present in the previous analyzed sample was not detected in the present study. Nevertheless, terpenes compounds constituted the bulk of the essential oils, in consistent with data reported for essential oils from other *Zingiber* plants grown in Vietnam (Huong et al., 2018; Hung et al., 2019; Huong et al., 2019a,b; Huong et al., 2020a,b). However, the amount and the composition of the bioactive substances may vary among different *Zingiber* species, and according to different factors such as the extraction methods, the geographic and the growing conditions, the harvest time etc. (Sharifi-Rad et al., 2017).

3.2. Antimicrobial test

The results of the antimicrobial study are presented in Table 2. The essential oil from the pseudo-stem of *Z. castaneum* showed a stronger inhibitory effect on *P. aeruginosa* with MIC of 12.5 µg/mL. The oil sample also displayed potent antimicrobial activity towards *A. niger* and *F. oxysporum* with MIC value of 50 µg/mL. The present data represent the first report on the antimicrobial action of essential oils from *Z. castaneum*. The antimicrobial activity of essential oil of *Z. castaneum* competes favorably with other *Zingiber* oil samples screened for their activities.

The antimicrobial activities of essential oils of some *Zingiber* species were reported previously. The leaf and rhizome oils of *Z. zerumbet* from Vietnam displayed antimicrobial activity against *A. niger* with MIC values of 25.0 and 50. 0 µg/mL, respectively (Huong et al., 2019b). The essential oil of *Z. zerumbet* was shown to inhibit the growth of *A. flavus* and *A. ochraceus* with MIC of 160 and 175 ppm respectively (Madegowda et al., 2016). *Z. officinale* and *Z. zerumbet* essential oils were considered potential therapeutic agents against bacterial several infections such as *S. aureus, B. cereus, P. aeruginosa* and *E. coli* (Azelan et al., 2015). *Zingiber officinale*

Table 1
Compounds identified in the pseudo-stem essential oil of *Z. castaneum*.

Sr. No	Rt (min)	Compounds[a]	Ri (Cal.)	RI (Lit.)	M	Percentages[b]	LTH
1	10.13	α-Pinene	939	932	136	2.6	0.8
2	10.63	Camphene	955	952	136	1	-
3	11.34	Sabinene	979	972	136	1.2	-
4	11.51	β-Pinene	985	978	136	3.3	4.9
5	11.73	Myrcene	992	988	136	0.2	0.6
6	12.74	α-Terpinene	1022	1024	136	0.2	-
7	13.01	*o*-Cymene	1030	1030	136	0.3	-
8	13.16	Limonene	1034	1034	136	0.4	0.9
9	14.16	γ-Terpinene	1064	1062	136	0.4	0.9
10	15.21	Terpinolene	1095	1094	136	0.5	0.5
11	15.77	1-Octen-3-yl acetate	1110	1112	170	0.3	-
12	19.87	Fenchyl acetate	1228	1229	196	0.3	-
13	22.08	2-(*E*)-Decanal	1265	1264	156	0.2	-
14	22.16	Bornyl acetate	1294	1295	196	0.5	-
15	23.87	Bicycloelemene	1345	1343	204	0.5	8.4
16	23.97	δ-Elemene [c]	1348	1350	204	5.4	-
17	25.32	α-Copaene	1390	1391	204	0.4	0.3
18	25.68	*cis*-b-Elemene [c]	1405	1407	204	9.8	-
19	25.81	β-Caryophyllene	1437	1437	204	1.7	14.7
20	27.1	γ-Elemene [c]	1445	1445	204	0.8	6.1
21	27.47	*allo*-Aromadendrene	1457	1457	204	0.4	-
22	27.95	α-Humulene	1472	1475	204	7.5	3.2
23	28.17	9-*epi*-(*E*)-Caryophyllene [c]	1479	1480	204	2	-
24	28.52	β-Chamigrene	1490	1489	204	0.6	-
25	28.59	*ar*-Curcumene	1493	1494	204	1.6	-
26	28.79	Germacrene D [c]	1499	1500	204	9.2	0.7
27	28.96	α-Zingiberene [c]	1505	1506	204	4.6	-
28	29.36	Bicyclogermacrene	1516	1517	204	15.8	4.3
29	29.38	β-Bisabolene	1518	1520	204	1.3	0.7
30	29.74	γ-Cadinene [c]	1531	1530	204	0.3	-
31	29.87	β-Sesquiphellandrene [c]	1536	1535	204	1.1	-
32	29.94	δ-Cadinene	1538	1540	204	1.3	9.8
33	30.77	Elemol	1565	1563	222	0.2	-
34	30.94	(*E*)-Nerolidol	1571	1571	222	0.5	1.2
35	31.15	Germacrene B	1578	1580	222	1.6	0.7
36	31.67	Germacrene D-4-ol [c]	1595	1594	222	1.8	-
37	31.78	Spathulenol	1599	1600	222	2	1.1
38	31.97	Caryophyllene oxide	1605	1606	222	0.6	0.9
39	32.24	Guaiol	1615	1618	222	0.4	-
40	32.56	Zingiberenol [c]	1626	1626	222	1	-
41	32.73	Humulene epoxide II [c]	1632	1632	222	0.6	-
42	33.05	α-Acorenol [c]	1644	1644	222	0.3	-
43	33.19	1-*epi*-Cubenol [c]	1649	1652	222	3.2	-

Table 1 Continued

Sr. No	Rt (min)	Compounds[a]	Ri (Cal.)	RI (Lit.)	M	Percentages[b]	LTH
44	33.56	*epi*-α-Muurolol [c]	1662	1664	222	1.1	-
45	33.93	α-Cadinol	1675	1676	222	1.6	0.8
46	34.03	*ar*-Turmerone [c]	1682	1680	216	1.5	-
47	35.08	Curlone [c]	1716	1720	218	1	-
Total						**94.1**	
Monoterpene hydrocarbons (Sr. No. 1-10)						**10.1**	
Oxygenated monoterpenes (Sr. No. 12, 14)						**0.8**	
Sesquiterpene hydrocarbons (Sr. No. 15-32,35)						**66.2**	
Oxygenated sesquiterpenes (Sr. No. 33, 34, 36-47)						**16.5**	
Non-terpenes (Sr. No. 11, 13)						**0.5**	

[a] Elution order on HP-5MS column; RI (Cal.) Retention indices on HP-5MS column; RI (Lit.) Literature retention indices (NIST, 2018); Sr. No, Serial Number; [b] Standard deviation (SD ±) were insignificant and excluded from the Table to avoid congestion; [c] Further identification by co-injection with authentic compounds; M Molecular mass; LTH Values from Huong et al., 2018 (see Reference).

Table 2
Antimicrobial activity of *Z. castaneum* essential oil.

Organism	MIC (mg/mL)	Str (mg/mL)
E. coli	-	NT
P. aeruginosa	12.5 ± 0.57	0.56
B. subtillis	-	NT
S. aureus	-	NT
A. niger	50 ± 1.00	1.28
F. oxysporum	50 ± 0.50	1.8
S. cerevisiae	-	NT
C. albicans	-	NT

-: No activity; Str: Streptomycin; NT: Not tested

essential oil presented antimicrobial activity against *P. fluoresecence* and *S. epidermis* with MIC values of 3.125 and 6.25 µg/mL, respectively and displayed weak antimicrobial activity against *C. albicans, E. faecalis, P. aeruginosa, S. aureus*, with MIC value of 100 µg/mL (Sener et al., 2017). The nano-emulsion form of essential oil of *Z. officinale* displayed antimicrobial activity against *Streptococcus mutans* with MIC value of 62.5 µL/mL (Mostafa, 2018). Essential oil of *Z. officinale* was efficient against three positive strains of bacteria (*S. aureus, B. cereus* and *L. monocytogenes*), with a minimum inhibitory concentration of 6.25 mg/mL (Norajit et al., 2007).

Generally, the biological properties of the essential oils are determined by their major components, or synergy between the major and some minor compounds. Two groups of distinct bio-synthetical origins are deeply involved in this activity. Terpenes and terpenoids comprise the main groups whereas aromatic and aliphatic constituents comprise the other group, all characterized by low molecular weight. The observed antimicrobial activity of *Z. castaneum* essential oil can be related to the compounds present in it (Swamy et al., 2016). For example, the essential oil constituents such as α-pinene, β-pinene, sabinene, 1,8-cineole, terpinen-4-ol, β-caryophyllene, bicyclogermacrene and germacrene were previously reported to inhibit significantly the growth and cell viability of potential infectious of broad-spectrum microorganisms (Swamy et al., 2016). For instance, the antibacterial activity of β-caryophyllene against *S. aureus* was reported recently (Dahham et al., 2015). Essential oil with high contents of bicyclogermacrene and germacrene D have displayed antimicrobial activity against *P. aeruginosa, C. albicans* and *S. aureus* with MIC 125 mg/mL (Tabanca et al., 2001) as well as *C. albicans, C. krusei, S. aureus, B. cereus* and *E. coli* all MIC value of 64 µg/mL (Fabiola et al., 2012). The antimicrobial activity of germacrene D has been documented (Dorman and Deans, 2000; Ali et al., 2014).

4. Concluding remarks

In the present paper, the chemical composition of the essential oil from the pseudo-stem of *Z. castanuem* has been reported. The main constituents of the oil were bicyclogermacrene, *cis*-β-elemene and germacrene D. The antimicrobial activity of the oil was being reported for the first time. The oil displayed antimicrobial activity against *Pseudomonas aeruginosa* (ATCC 25923), *Aspergillus niger* (ATCC 9763) and *Fusarium oxysporum* (ATCC 48112) at reasonable MIC values. In conclusion, the results indicate the potential of *Z. castaneum* essential oil as a source of antimicrobial agent.

Conflict of interest

The authors declare that there is no conflict of interest.

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