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Research Article

Chemical composition and antimicrobial activity of essential oils from the leaves and rhizomes of *Hedychium yunnanense* Gagnep. (Zingiberaceae) collected in Vietnam

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Abstract
Hedychium yunnanense Gagnep., belonging to the Zingiberaceae family, has aroused much interest due to its non-volatile chemical constituents. Nevertheless, no data about essential oils (EOs) of this species is available. In this study, the chemical composition and antimicrobial activity of EOs from leaves and rhizomes of *H. yunnanense* collected in Vietnam were analyzed. The EOs of the rhizomes and leaves were obtained as yellow oils with extraction yields of 0.21 and 0.09%, respectively. Fifty-seven chemical components, accounting for 94.35-98.92%, were identified in the two EOs. The rhizomes EO mainly contains β-pinene (21.07%), 1,8-cineole (20.63%), α-pinene (8.80%) and camphene (6.35%), while the leaves EO was rich in β-pinene (45.92%), α-pinene (19.48%), and β-caryophyllene (18.26%). Both EOs exhibited more potent antimicrobial activities than the standard drug, streptomycin, as they demonstrated significantly lower minimum inhibitory concentrations against the tested bacteria and fungus. Additionally, the leaves EO may possess stronger antimicrobial activities than the rhizomes EO. The findings of the study will provide a better understanding of the chemical composition and bioactivities of *H. yunnanense*.

Keywords
Antimicrobial activity, Essential oil, GC/MS, *Hedychium yunnanense*, Sesquiterpenoid

Introduction

Hedychium species belongs to the Zingiberaceae family, with over 100 species found around the world. Among them, *Hedychium yunnanense* known as "Ngải tiên Vân Nam" in Vietnamese is only distributed in China and Vietnam. This species is characterized by stout pseudo-stems, leaf ovate-oblong to oblong blade, a base that attenuates into a short petiole, and a caudate apex. Its calyx measures 1.5-3 cm long, with an obtusely 3-toothed apex and its corolla tube is slender, ranging from 3.5-5 cm long, with linear lobes measuring 2-3 cm long. Its lateral staminodes are oblong-linear, shorter and wider than corolla lobes with a narrowed base while its labellum is obovate, 1.5-2 cm long, and apically 2-cleft. The fruits are 3-angled and glabrous [1].

H. yunnanense is well-known for its traditional applications, including food flavors and spices. Furthermore, it has gained attention for its potential medicinal properties due to the presence of several isolated compounds such as hedytriol,

hedyunchene A, yunnancoronarin D, and hedychenoids A and B[2-4]. These compounds have exhibited a wide range of biological activities, including anticancer, cytotoxicity, and inhibitory effects on Nitric oxide production[5,6].

Essential oils (EOs) from *Hedychium* species have gained much interest due to their potential applications in the food industry, dental care, or agriculture[7-10]. Previous studies reported that EOs from some species and herbs exert bactericidal, bacteriostatic, anticholinesterase, anti-tyrosinase and α-amylase inhibitory effects[11,12]. Additionally, *Hedychim* species have demonstrated antibacterial activities. For example, EOs extracted from rhizomes of five *Hedychium* species, including *H. coronarium, H. neocarneum, H. flavescens, H. speciosum* and *H. stenopetalum* displayed moderate to weak activity against Gram-positive bacteria such as *Staphylococcus aureus* and *Bacillus subtilis* (with minimum inhibitiory concentration MIC ranging from 0.3-8.3 mg/mL, and minimum bactericidal concentration MBC ranging from 0.6-8.3 mg/mL)[13]. However, there are no experimental data regarding the chemical compositions and antimicrobial activity of EOs from this species has been reported. Therefore, in this study, we aimed to determine the composition of EOs extracted from the leaves and rhizomes of *H. yunnanense* and to investigate their antimicrobial activity for the first time. The findings could be valuable for the pharmaceutical industry in developing new antimicrobial agents or natural antibiotics. Additionally, the knowledge of the chemical composition of EOs from *Hedychium yunnanense* could be applied to the food industry where these EOs might serve as natural preservatives or flavor enhancers in various food products.

Materials and methods

Plant materials

The fresh leaves and rhizomes of *H. yunnanense* were collected from Bidoup-Nui Ba National Park, Lam Dong Province, Vietnam, in June 2021 and the identification of the plant was conducted by Dr. Dang Van Son. A voucher specimen (No. HC007/21) was deposited in the Herbarium of the Department of Chemistry, Vinh University, Vietnam.

Isolation of the EOs

The fresh leaves and rhizomes of *H. yunnanense* (400 g for each) were hydro-distilled separately using Clevenger's apparatus until no further EO could be extracted (approximately 4 h). The experiment was repeated three times for each part. The resulting EOs were dried with anhydrous sodium sulfate and stored in the dark at 4°C for further analysis, as reported in previous studies[14-16].

Gas chromatography-mass spectrometry (GC/MS) analysis and identification of chemical constituents

GC/MS analysis of *H. yunnanense* EOs was performed on an Agilent Technologies 7890B GC System linked to a 5977B MSD model working in EI mode. An amount of 1 μL of the EO sample (diluted with *n*-hexane, 1:100 ratio) was injected into an HP-5MS Ultra Inert column (30 m × 0.25 mm i.d.; film thickness 0.25 μm). The flow rate of Helium carrier gas was 1.0 mL/min with a split ratio of 1:30. The oven temperature was set at 60°C for 1 min initially, increased to 240°C at the rate of 2°C/min, and held for 4 min. The injector temperature was 300°C, MS Quad temperature was 150°C, transfer line temperature and MS source were fixed at 300°C and 230°C, respectively. The MS values were recorded in 70 eV, and scan mass ranged from 50 to 550 amu (2.0 scan/s). Data were analyzed using MassHunter Workstation Software (Version B.08.00). The individual constituents were identified by comparison of the mass spectral fragmentation patterns of the components with those reported in the spectral library and confirmed by comparison of their retention indices (RI) on the HP-5 column in close agreement with those of the literature[17]. The formula used to calculate the RI value and the EO components were identified as previously reported[16,18,19]. The concentration of identified components is presented as a percentage and directly calculated from their respective peak areas[20,21].

Test organisms and media

The reference strains of bacteria and fungus were as follows: three Gram-negative bacteria (*Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853, *Salmonella enterica* ATCC13076), three Gram-negative bacteria (*Enterococcus faecalis* ATCC299212, *Staphylococcus aureus* ATCC25923, *Bacillus cereus* ATCC14579), and the pathogenic fungus (*Candida albicans* ATCC10231). These organisms were provided by the National Institute for Food Control (Ha Noi, Vietnam). The culture medium used for bacteria was Luria Bertani Agar (LBA), while Sabouraud Dextrose Agar (SDA) was used for growing fungus.

Antimicrobial assay

The minimal inhibitory concentrations (MIC) of EOs were determined using broth dilution assays in 96-well plates, as reported in previous studies[22]. Streptomycin and cycloheximide were used as positive controls for bacteria and fungus, respectively. Briefly, the appropriate amount of EOs was dissolved in DMSO, and 2-fold diluted by the broth medium, with concentrations ranging from 2-256 µg/mL. The samples were then inoculated with the culture of test organisms to a final concentration of 10^5 CFU/mL. Control broth containing DMSO or positive controls was also prepared in each case. Subsequently, the mixtures were incubated at 37°C for 24 h for bacteria or at 35-37°C for 36-48 h for fungus. After incubation, the MIC value was observed and preliminarily determined. The MIC values were determined on the well plate with the lowest reagent concentration that completely inhibited microbial growth after the incubation.

Results and discussion

The yields and chemical constituents of the EOs

The hydrodistillation of the rhizomes and leaves of *H. yunnanense* gave pale-yellow EOs with extraction yields of 0.21 and 0.09%, respectively (w/w, calculated on the fresh weight). The phytoconstituents present in these EOs were identified, and are presented in Table 1 according to their elution on an HP-5MS Ultra Inert column.

As shown in Table 1, a total of fifty-seven chemical components were identified in both EOs of *Hedychium yunnanense*. Among them, the results of GC/MS analysis (Fig. 1 and Fig. 2) revealed the presence of 51 (94.35%) compounds in the rhizomes and 33 (98.92%) compounds in the leaves EOs. Interestingly, both EOs had the highest concentration of monoterpene hydrocarbons (44.46% for rhizomes and 70.86% for leaves EOs). However, oxygenated monoterpenes (33.91%) were identified as the second major chemical class in the rhizomes EO, while sesquiterpene hydrocarbons (25.32%) were the next predominant chemical class identified in the leaves EO.

In both examined EOs, α-pinene (8.80-19.48%) and β-pinene (21.07-45.92%) were the major components. These compounds were also found as the main constituents in the EOs from *H. malayanum*[23], *H. coronarium*, *H. neocarneum*[13], *H. flavescens*, *H. speciosum*, *H. stenopetalum*, *H. villosum*[24], *H. greenii,* and *H. gracile*[25]. These two compounds displayed a variety of pharmacological effects, such as antibacterial, antiviral, antitumor, anti-inflammatory, and antioxidant activities[26]. Additionally, the present study also provided that 1,8-cineole (20.63%) and camphene (6.35%) were the major constituents in the rhizomes EO, while leaves EO was characterized by the presence of β-caryophyllene (18.26%). Many previous studies showed that 1,8-cineole, camphene, and β-caryophyllene showed extensive pharmacological properties, including anti-inflammatory, antioxidant, anti-fungal, anticancer, and antidiabetic[27-29]. Finally, the differences between the quantity and content of these two EOs might be affected by numerous factors[30,31].

The antimicrobial test on the EOs

Both EOs from the rhizomes and leaves of *H. yunnanense* exhibited strong antimicrobial profiles against six tested Gram-negative, and Gram-positive bacteria, and a pathogenic fungus. The MIC values of the EOs were 8-128 µg/mL against test bacteria and 32 µg/mL against *Candida albicans* ATCC10231 (Table 2). It should be noted that the EOs from *H. yunnanense* exerted higher antimicrobial activities than streptomycin,

Table 1. Constituents of the leaves and rhizomes EOs of *Hedychium yunnanense*

No.	RT	Compounds[a]	RI[b]	RI[c]	Concentration (%)	
					Rhizomes	Leaves
1	5.279	Tricyclene	927	925	0.23	-
2	5.370	α-Thujene	931	929	0.14	0.09
3	5.548	α-Pinene	939	937	**8.80**	**19.48**
4	5.903	Camphene	954	952	**6.35**	0.16
5	6.515	Sabinene	978	974	1.04	2.56
6	6.635	β-Pinene	982	979	**21.07**	**45.92**
7	6.927	β-Myrcene	992	991	0.69	0.54
8	7.322	α-Phellandrene	1006	1005	0.13	-
9	7.488	3-Carene	1013	1011	1.16	-
10	7.671	α-Terpinene	1020	1017	0.11	0.10
11	7.900	p-Cymene	1029	1025	0.52	-
12	8.025	Limonene	1033	1030	3.32	1.54
13	8.128	1,8-cineole (= eucalyptol)	1037	1032	**20.63**	0.15
14	8.265	*cis*-β-ocimene	1042	1038	-	0.07
15	8.580	*trans*-β-ocimene	1052	1049	-	0.08
16	8.912	γ-Terpinene	1063	1060	0.67	0.18
17	9.828	Terpinolene	1090	1088	0.23	0.14
18	9.919	2-Nonanone	1093	1092	0.18	-
19	10.183	Linalool	1100	1099	4.75	0.08
20	11.453	*trans*-Pinocarveol	1143	1139	0.07	-
21	11.647	Camphor	1149	1145	0.23	-
22	12.243	Pinocarvone	1167	1164	0.06	-
23	12.340	*endo*-Borneol	1170	1167	4.64	-
24	12.712	Terpinen-4-ol	1180	1177	0.33	0.06
25	13.141	α-Terpineol	1192	1189	0.67	-
26	13.347	Myrtenal	1198	1193	0.13	0.05
27	14.388	Isobornyl formate	1231	1232	0.05	-
28	14.600	Methyl thymyl ether	1238	1235	0.06	-
29	16.305	Bornyl acetate	1288	1285	2.29	-
30	16.534	2-Undecanone	1295	1294	0.05	-
31	16.580	Dihydroedulan	1296	1293	-	0.18
32	17.970	δ-Elemene	1341	1338	0.80	0.24
33	19.212	Copaene	1379	1376	0.09	0.05
34	19.727	β-Elemene	1394	1391	0.41	0.05
35	20.288	α-Gurjunene	1412	1409	0.09	-
36	20.597	β-Caryophyllene	1423	1419	2.33	**18.26**
37	21.644	Humulene	1458	1454	0.52	2.51
38	21.707	*trans*-β-farnesene	1460	1457	0.97	2.34

table 1. (continued).

No.	RT	Compounds[a]	RI[b]	RI[c]	Concentration (%)	
					Rhizomes	Leaves
39	21.867	Alloaromadendrene	1465	1461	0.98	0.71
40	22.347	γ-Muurolene	1480	1477	-	0.09
41	22.491	Germacrene D	1485	1481	0.79	0.45
42	22.645	β-Eudesmene	1489	1486	0.40	-
43	22.783	δ-Selinene	1494	1493	0.21	-
44	22.954	Bicyclogermacrene	1499	1495	1.27	0.42
45	23.475	γ-Cadinene	1517	1513	0.11	-
46	23.744	Cadina-1(10),4-diene	1527	1524	0.37	0.20
47	24.322	α-Calacorene	1547	1542	0.15	-
48	24.499	Elemol	1553	1549	2.38	0.18
49	24.911	β-Calacorene	1567	1563	0.09	-
50	25.335	Spathulenol	1581	1576	1.24	-
51	25.506	Caryophyllene oxide	1587	1581	0.55	1.28
52	26.261	Humulene epoxide II	1613	1606	-	0.64
53	26.868	γ-Eudesmole	1636	1631	0.36	-
54	27.005	Caryophylladienol II	1641	1636	-	0.07
55	27.148	τ-Cadinol	1646	1640	0.21	0.05
56	27.389	Eudesm-4(14)-en-11-ol	1654	1649	0.20	-
57	27.463	α-Eudesmol	1657	1653	1.23	-
		Total			**94.35**	**98.92**
		Monoterpene hydrocarbons			44.46	70.86
		Oxygenated monoterpenes			33.91	0.34
		Sesquiterpene hydrocarbons			9.58	25.32
		Oxygenated sesquiterpenes			6.17	2.22
		Others			0.23	0.18

RT: Retention time (min); [a] Elution order on HP-5MS Ultra Inert column; [b] Retention indices on HP-5MS Ultra Inert column; [c] Retention indices according to the NIST17 and Adams book; "-" Not identified

the reference compound, with MIC values ranging from 32-256 μg/mL. For rhizomes EO, MIC values were found lowest when testing with *Enterococcus faecalis* ATCC299212 (32 ± 0.05 μg/mL) and *Candida albican*s ATCC10231 (32 ± 0.06 μg/mL). The leaves EO was most active with *Enterococcus faecalis* ATCC299212 (8 ± 0.05 μg/mL) and least active with *Pseudomonas aeruginosa* ATCC27853 (128 ± 0.17 μg/mL).

The EOs from *H. yunnanense* also have higher antimicrobacterial activity compared with some other EOs from aroma plants. The EOs from *Thymus vulgaris* L. inhibited the growth of *Sarcina flava, Bacillus thuringiensis, Bacillus licheniformis,* and *Listeria innocua* for 20 h at a concentration of 400 ppm[32], while mint EOs showed a dose-dependent inhibition at 1000 ppm[33]. Morris *et al.* examined the antimicrobial activities of 521 fragrance materials and reported that only 23 materials were effective at 50 or 100 ppm, and approximately 4% had a MIC as low as 50 ppm[34].

Antimicrobial activities were different for Gram-positive and Gram-negative bacteria. Regarding the former, *Enterococcus faecalis* ATCC299212 was the most sensitive

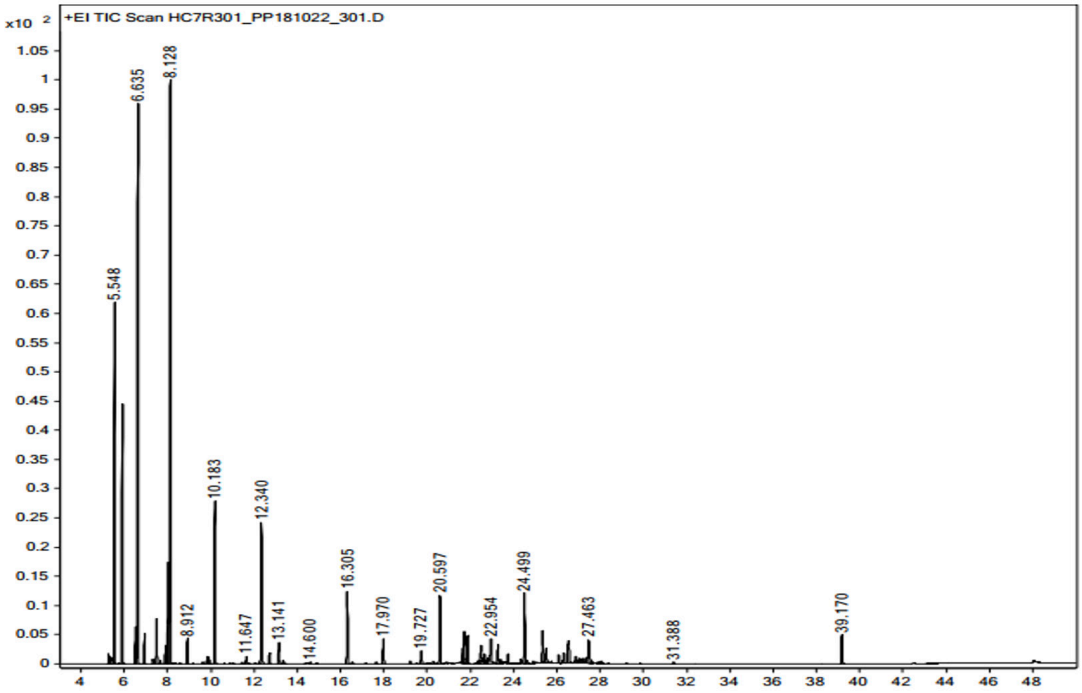

Figure 1. GC/MS chromatogram of the EO of *Hedychium yunnanense* rhizomes

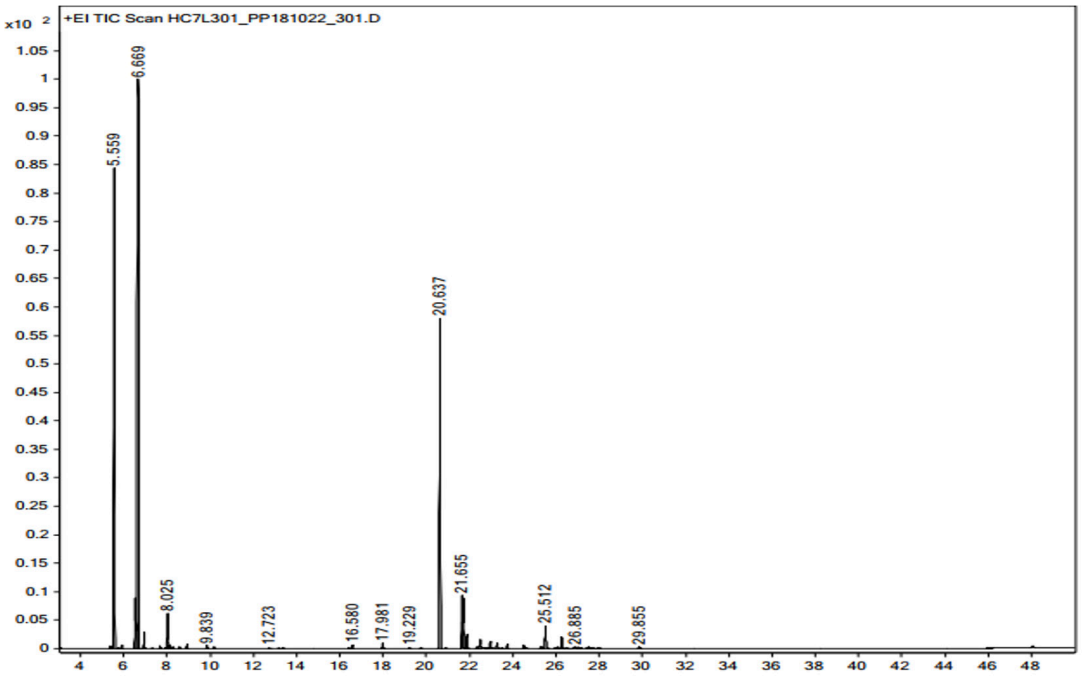

Figure 2. GC/MS chromatogram of the EO of *Hedychium yunnanense* leaves

species, with MIC values of 8 and 32 µg/mL for the leaves and rhizomes EOs, respectively. For *Staphylococcus aureus* ATCC25923 and *Bacillus cereus* ATCC14579, there were no significant differences in the antimicrobial activities of the EOs; the same MIC values of 32-64 µg/mL were recorded. As for the latter, *Escherichia coli* ATCC25922, *Salmonella enterica* ATCC13076 were determined to have the same sensitivity to the EOs (MIC values of 32-64 µg/mL), while the

Table 2. The antimicrobial activity of the EOs from rhizomes and leaves of *Hedychium yunnanense*

Microorganisms	Minimum inhibitory concentration (µg/mL)			
	Rhizomes EO	Leaves EO	ST	CY
Enterococcus faecalis ATCC299212	32 ± 0.05	8 ± 0.05	256	NT
Staphylococcus aureus ATCC25923	64 ± 0.06	32 ± 0.10	256	NT
Bacillus cereus ATCC14579	64 ± 0.17	32 ± 0.06	128	NT
Escherichia coli ATCC25922	64 ± 0.05	32 ± 0.05	32	NT
Pseudomonas aeruginosa ATCC27853	64 ± 0.24	128 ± 0.17	256	NT
Salmonella enterica ATCC13076	64 ± 0.21	32 ± 0.09	128	NT
*Candida albican*s ATCC10231	32 ± 0.06	32 ± 0.06	NT	32

ST: Streptomycin; CY: Cycloheximide; NT: Not tested; *: Mean ± SD, n = 3

MIC values against *Pseudomonas aeruginosa* ATCC27853 were 64-128 µg/mL. These results indicate that *Pseudomonas aeruginosa* ATCC27853 was the least sensitive species to EOs among test organisms and that Gram-positive strains seemed to be more susceptible to EOs than Gram-negative strains. These findings were consistent with previous studies. Sivropoulou *et al*. found that four EOs from *Mentha* species exhibited antimicrobial activity against all test microorganisms except *Pseudomonas aeruginosa* which appeared to be sensitive only to the pulegone-rich *Mentha pulegium* EOs[33]. Bosnić *et al.* showed that *Pseudomonas aeruginosa* was least susceptible to the EOs extracted from thyme, sage, rosemary, eucalyptus, melissa, and lavender. Those EOs showed MIC values ranging from 97-390 µg/mL against Gram-positive bacteria and 390-781 µg/mL against Gram-negative, which indicated their antimicrobial activities were more pronounced against Gram-positive bacteria than against Gram-negative bacteria[35]. These results could be explained by the fact that these EOs were easier to penetrate into cell walls of Gram-positive bacteria than those of Gram-negative bacteria. In fact, the mode of action of the EOs is generally considered to involve the disruption of the cytoplasmic membrane and its function groups[35,36].

In general, the EO from leaves showed higher antimicrobial activities (MIC from 8-32 µg/mL, except for *Pseudomonas aeruginosa* ATCC27853) than EO extracted from rhizomes (MIC 32-64 µg/mL). This could be explained based on the difference in their major compounds. The main components of EO extracted from *H. yunnanense* rhizomes were β-pinene (21.07%), 1,8-cineole (20.63%), α-pinene (8.80%) and camphene (6.35%), while major components of the EO from *H. yunnanense* leaves were β-pinene (45.92%), α-pinene (19.48%) and β-caryophyllene (18.26%). In previous studies, β-and α-pinene effectively inhibited various test bacteria, including *Pseudomonas putida, Staphylococcus epidermidis, Staphylococcus aureus, Escherichia coli, Micrococcus luteus, Klebsiella pneumoniae, Enterobacter aerogenes, Bacillus cereus, Streptococcus pneumoniae, Streptococcus pyogenes,* etc, fungal (e.g., *Candida albicans* or *Candida* spp.)[26,37]. In addition, when *α*-pinene was combined with antibiotics, it could be an effective modulator for antibiotic-resistant *Campylobacter jejuni* by significantly reducing the MIC values of antibiotics such as ciprofloxacin, erythromycin, and triclosan (512 times)[38]. Another well-studied active component was 1,8-cineole, which could show the inhibitory effects on Gram-positive and Gram-negative bacteria[39], and even antibiotic-resistant[40]. 1,8-cineole also showed synergistic antibacterial effects when combined with antibiotics, such as amoxicillin, clavulanic acid or gentamicin[41].

Conclusion

In conclusion, the present study reported the chemical compositions of rhizomes and leaves EOs of *H. yunnanense* and their antimicrobial

activities for the first time. The GC/MS analysis identified β-pinene (21.07%), 1,8-cineole (20.63%), α-pinene (8.8%) and camphene (6.35%) as the dominant compounds, present in the rhizomes EO, while the main components of the leaves EO were β-pinene (45.92%), α-pinene (19.48%), and β-caryophyllene (18.26%). These EOs exhibited antimicrobial activities against pathogenic bacteria and fungus, with MIC values of 8-128 μg/mL. This study has demonstrated potential applications against pathogenic bacteria for *H. yunnanense* EOs although further studies are needed.

Conflict of interest

The authors have declared no conflict of interest.

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