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The antimicrobial activity and chemical composition of *Elsholtzia blanda* (Benth.) Benth. essential oils in Lam Dong Province, Viet Nam

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ABSTRACT

This study aimed to assess the chemical composition of essential oils of Elsholtzia blanda (Benth.) Benth. in Lam Dong Province, Viet Nam, and evaluate their biological activities. Essential oils obtained by hydro-distillation of the aerial parts of E. blanda were analyzed by gas chromatography-mass spectrometry (GC-MS). Thirty-one constituents were identified in the oil, and the essential oil was predominantly monoterpenoid, with camphor (25.14%), camphene (22.64%), α-Pinene (11.53%), and cineole (9.89%) as the four most abundant constituents. The evaluation of antimicrobial activity using the agar wells diffusion method showed that the essential oil in all concentrations was active against the Gram-positive bacteria (Staphylococcus aureus), Gram-negative bacteria (Escherichia coli), and pathogenic yeast (Candida albicans), they are most sensitive and resistant to S. aureus strain.

1. INTRODUCTION

The genus *Elsholtzia* Willd. belongs to the family Lamiaceae (Bestmann et al., 1992; Raven & Hong, 1994; Guo et al., 2012). It is composed of about 40 species, distributed in East Asia, Africa, North America, and European countries, and is especially prevalent in China, Korea, Japan, and India (Haiyun et al., 2004; Guo et al., 2012). The plants in the genus are mostly aromatic plants and have been used in traditional medicine, food, spices, perfumeries, etc. (Guo et al., 2012). This genus has a diversified chemical profile regarding its secondary metabolites (flavonoid, phenylpropanoid, terpenoid, phytosterol, cyanogenic glycoside) and significant economic potential (Guo et al., 2012), making it a source of prospective studies on biologically active natural compounds.

In Viet Nam, the *Elsholtzia* genus is composed of about seven species including *Elsholtzia blanda* (Benth.) Benth., and *Elsholtzia ciliata* (Thunb.) Hyland, *Elsholtzia communis* (Coll. et Hemsl.) Diels, *Elsholtzia penduliflora* W.W.Sm.; *Elsholtzia pilosa* (Benth.) Benth.; *Elsholtzia rugulosa* Hemsl., *Elsholtzia winitiana* Craib., and most of them distribute along the altitude gradient from 700–2000 m (Chi, 2003; Ho, 2003). They are also used as domestic folk medicine, herbal tea, and food spices in Vietnamese life (Chi, 2003; Ho, 2003). Many *Elsholtzia* species have been examined for their oil constituents, but only a limited number have been thoroughly studied. The chemical composition of *Elsholtzia ciliata* (Thunb.) Hyland leaf essential oil growing in Vinh and Ho Chi Minh City is characterized by high amounts of geranial (19.5–26.5%), neral (15.2–20.5%), limonene (10.9–14.2%), and *(Z)*-p-farnesene (10.8–11.7%) (Dũng et al., 1996). Moreover, Lesueur et al. (2007) described the aerial parts oil compositions of three Vietnamese *Elsholtzia* species including *E. blanda*, *E. penduliflora,* and *E. winitiana* (Lesueur et al., 2007). In which, the essential oils of *E. blanda*, *E. winitiana,* and *E. penduliflora* growing in Son La and Lao Cai provinces were analyzed by GC, in combination with retention indices (RI), GC/MS, and ^{13}C NMR spectroscopy (Lesueur et al., 2007). The essential oils of *E. penduliflora* contained 1,8-cineole as the major component (71.7%) and the other important compounds were *β*-pinene (7.3%), *α*-pinene (3.9%), sabinene (2.8%) and limonene (2.4%), whereas essential oils of *E.blanda* contained Linalool as the main component (75.2–56.8%) and rosefuran was the major component (56.0%) of *E. winitiana* essential oil (Lesueur et al., 2007). Nhan and Huyen (2017) also reported chemical composition analyses of the essential oils of *E. ciliata* (Thunb.). Hyland growing in Thua Thien Hue Province consist of twenty-six compounds, in which the main constituents of the essential oil were found to be geranial (28.4%), *β-cis*-ocimene (23.0%), and neral (21.7%) (Nhan & Huyen, 2017).

Elsholtzia blanda (Benth.) Benth. is an aromatic perennial herb, widely distributed in Asia (Guo et al., 2012; Kotoky et al., 2017). In Viet Nam, *E. blanda* is found in many Provinces such as Lai Chau, Lao Cai, Son La, Ha Giang, Cao Bang, Lang Son, Bac Giang, Hoa Binh, Kon Tum, Lam Dong, and Khanh Hoa (Chi, 2003; Ho, 2003). This species has been used as traditional medicine by local people in their daily life. The chemical constituents of *E. blanda* and compositional variations in the essential oils of this species also have been reported in previous studies (Fang & Lin, 1990; Bestmann et al., 1992; Thappa et al., 1999; Ping et al, 2002; Lesueur et al., 2007; Rana et al., 2012; Kotoky et al., 2017), and indicating geranial acetate, 1,8-cineole, citral, and linalool are dominant components. However, significant variation in the essential oil content and its associated chemical components with time and geographical variation was also reported (Cheng et al, 1989; Lesueur et al., 2007; Saei-Dehkordi et al., 2010). Therefore, this study aims to chemically characterize and assess the antimicrobial activity against common bacterial pathogens of the essential oils of *E. blanda,* a plant species grown in the Lam Dong Province.

2. MATERIALS AND METHOD

2.1. Plant materials

The aerial parts of a wild population of *E. blanda* were collected from November to December 2019 in Bidoup-Nui Ba National Park, Lam Dong Province, Viet Nam.

The plant was identified by Dr. Hoang Thi Binh (Researcher at the Faculty of Biology, Dalat University, Viet Nam) and a voucher specimen of the collection (CT-26) was deposited in the Herbarium of the Dalat University (DLU).

2.2. Extraction and chemical composition of essential oils

Essential oils were extracted from 1.5 kg samples of fresh aerial parts of *E. blanda* by hydro-distillation for 120 min. Essential oils were properly collected, dried over anhydrous sodium sulfate, and kept in a freezer at 4ºC until the chemical composition was analyzed.

The gas chromatography-mass spectrometry (GC-MS) method was used to determine the chemical composition of the essential oils using an Agilent Technologies HP 6890N Plus Chromatograph connected to a mass spectrometer HP 5973 MSD. The GC-MS system was set up with the following parameters: Column: Agilent DB-5MS: Length: 30 m, Film: 0.25 µm, diam: 0.25 mm; MS transfer line temperature: 220ºC; Ion source temperature: 200ºC; Injector temperature: 220ºC; Temperature program: from 70ºC (15 min) up to 250ºC with increments of 10ºC/min: Flow: 1.2 ml/min; Mass range (m/z): 50−450. Compounds were identified based on the retention times (RT) and the mass spectra with those values found in the literature of Adams (2007) and by the computerized matching of the acquired mass spectra with those stored in the NIST08 and WILEY 275 mass spectral libraries of the GC-MS data system.

2.3. Antibacterial activity

The antimicrobial effects of the essential oil on *Staphylococcus aureus* ATCC 6538 (gram-positive bacteria), *Escherichia coli* ATCC 8739 (gram-negative bacteria), and *Candida albicans* (pathogenic yeast) were tested using the agar well diffusion method (Devillers et al., 1989; Valgas et al., 2007; Binh et al., 2020). Among those, two bacterial strains were supplied by the Institute of Drug Quality Control in Ho Chi Minh City, Viet Nam, and pathogenic yeast was provided by the General Hospital of Lam Dong Province, Viet Nam. Approximately 10^6–10^8 CFU/mL of the microorganisms were inoculated by the spread plate method in Nutrient agar (NA). Wells of 6 mm diameter were created in the center of the dish, and 40 µL of essential oil solution, dimethyl sulphoxide

(DMSO), and chloramphenicol were pipetted into the middle of each disk. In this study, the concentration of essential was diluted into five levels (12.5%, 25%, 50%, 75%, and 100%) and evaluated for antimicrobial activity. The essential oil of *E. blanda* was diluted by using sterile dimethyl sulphoxide (DMSO). DMSO and chloramphenicol 250 mg (Vidipha Central Pharmaceutical Joint Stock Company, Viet Nam) were used as negative control and positive control, respectively. The dishes were incubated at 4ºC for 12 hours for sample diffusion. They were then incubated at 30ºC for 24 hours to promote microorganism growth. After that, the inhibition zones (mm) were measured and analyzed. The experiments were repeated in triplicate.

2.4. Statistical analysis

Microsoft Excel for Mac 2021 (Microsoft Corporation) was used for the related statistical analyses. Mean values ± one standard deviation were calculated and presented from triplicate determinations. The mean difference was considered significant when $P < 0.05$ in the statistical analysis.

3. RESULTS AND DISCUSSION

In this study, the chemical compositions were examined along with the antimicrobial activities of essential oil obtained by hydro-distillation from aerial parts of *E. blanda*. The yield of light yellow-colored essential oil obtained from *E. blanda* was found to be 0.32%.

3.1. Chemical component analyses of the essential oil

The chemical constituents identified by GC-MS, retention times, and percentages are presented in Table 1 (and Supplement 1). The result shows that the essential oil of *Elsholtzia blanda* from Lam Dong including in a total of thirty-one compounds.

Among the compounds identified, camphor (25.14%) was the major compound. This is an oxygenated monoterpene, which has been used as medicine to treat inflammation-related diseases such as bronchitis, asthma, rheumatism, sprains, and muscular pain (Salman et al., 2012). Other components with significant concentrations were camphene (22.64%), α-pinene (11.53%), cineole (9.89%), and τ-terpinene (5.40%). The essential oil of this species is characterized by a predominance of monoterpenes and sesquiterpenes. The sesquiterpene composed 25.81%, while the monoterpene made up 74.19%, of which the monoterpene hydrocarbons had the most important contribution (56.52%).

Table 1. Chemical composition of essential oil of *Elsholtzia blanda* from Lam Dong, Viet Nam

No.	Component	RT	%
1	2-Thujene	4.81	2.40
2	α-Pinene	5.09	11.53
3	Camphene	5.63	22.64
4	Benzaldehyde	5.85	0.14
5	(+)-Sabinene	6.33	0.88
6	β-Pinene	6.55	2.57
7	β-Myrcene	6.95	0.74
8	α-Terpinene	8.29	0.62
9	*o*-Cymene	8.69	1.45
10	*D*-Limonene	8.99	3.75
11	Cineole	9.18	9.89
12	*Trans*-β-ocimene	9.38	1.15
13	*Cis*-ocimene	10.04	0.24
14	τ-Terpinene	10.87	5.40
15	1-Phenylethanone	11.11	0.31
16	Terpinolene	12.95	0.87
17	Linalool	14.41	0.32
18	Camphor	17.52	25.14
19	Endo-borneol	18.45	1.56
20	Terpinen-4-ol	18.77	0.79
21	α-Terpineol	19.25	0.66
22	β-Citral	20.45	0.13
23	α-Citral	21.14	0.20
24	Copaene	23.24	0.32
25	β-Bourbonene	23.38	0.28
26	β-Elemene	23.46	0.15
27	Caryophyllene	23.96	2.37
28	Humulene	24.50	0.37
29	Germacrene D	24.87	2.18
30	Lepidozene	25.06	0.50
31	Cadina-1(10),4diene	25.35	0.59

Note: RT (Retention times)

Although there are no previous studies on the chemical composition of *E. blanda* essential oil growing in Lam Dong Province, the chemical composition of essential oil of this species growing in Assam, Imphal, Meghalaya (India), Yunnan (China), Son La, Lao Cai (Viet Nam) has been previously reported. Several studies on the chemical composition of *E. blanda* have shown the predominance of monoterpenes and sesquiterpenes in the constitution of the essential oils of the sampled plants. However, a comparison of the chemical constituents between the essential oil of *E.*

blanda in Lam Dong Province and other areas in the world shows that those are differences, especially in the major compounds. Camphor (25.14%) and camphene (22.64%) are the dominant compounds in the essential oil of *E. blanda* in the present study, whereas 1,8-Cineole (27.58%), humulene (12.02%) and linalool (57.9%), geranial (43.5%) were found to be major components in the essential oil of this species distributed in China and India respectively (Fang et al., 1990; Bestmann et al., 1992; Ping et al., 2002; Rana et al., 2012; Kotoky et al., 2017). In addition, comparing the main compounds of essential oil of *E. blanda* growing in Son La, and Lao Cai Province of Viet Nam is 1,8-cineole (20.8%) and linalool (75.2%) (Lesueur et al., 2007). On the other hand, as can be seen from the previous study, camphor is minor or absent in the essential oil of *E. blanda* (Fang et al., 1990; Bestmann et al., 1992; Ping et al., 2002; Rana et al., 2012; Kotoky et al., 2017). Thus, it is also interesting to point out that important quantitative differences suggest that environmental factors strongly influence the chemical composition.

3.2. Antimicrobial activities of essential oil

So far, few studies have addressed the antimicrobial activity of *E. blanda* essential oils with microbial strains. In this investigation, we evaluated the *in vitro* antimicrobial activity of *E. blanda* essential oil against selected microorganisms, including *S. aureus* (gram-positive bacteria), *E. coli* (gram-negative bacteria), and *C. albicans* (pathogenic yeast) in Table 2.

Table 2. Antimicrobial activity of rosemary essential oil

Bacteria	**Inhibition zone diameters (mm)**						
	Chloramphenicol	**DMSO**	**Concentration (% of essential oil in DMSO)**				
			100%	**75%**	**50%**	**25%**	**12.5%**
E. coli	18.0 ± 0.26	-	12.6 ± 1.1	12.3 ± 0.5	10.6 ±0.5	8.6 ± 0.5	7.6 ± 0.5
S. aureus	21.0 ± 0.06	-	20.6 ± 1.0	15.6 ± 0.5	15.0 ± 2.0	13.6 ± 0.5	15.0 ± 1.0
C. albicans	17.0 ± 0.1	-	17.6 ± 0.5	17.0 ± 1.0	14.6 ± 1.1	14.3 ± 1.5	13.6 ± 0.5

Notes: "-": No antimicrobial activity

As can be seen from Table 2, each concentration of essential oils displayed antibacterial effects to different degrees against *S. aureus*, *E. coli*, *and C. albicans*. In which the pure essential oil (the oil at 100% concentration) had the best inhibition zones for all of the microorganisms tested, whereas the oil at 12.5% had the lowest. Besides, the antimicrobial activity decreased following the decrease of concentration of *E. blanda* essential oils. The results also illustrated that the antibacterial effects of the essential oil *E. blanda* against *S. aureus* are higher than for *E. coli* and *C. albicans* because the outer membrane of Gram-negative bacteria (*E. coli*) and pathogenic yeast (*C. albicans*) is composed of hydrophilic lipopolysaccharides and polysaccharides, fibrillar proteins (Lugtenberg, 1981; Chaffin, 2008). According to previous studies, these structures create a barrier toward macromolecules and hydrophobic compounds, providing *E. coli* and *C. albicans* with higher tolerance toward hydrophobic essential oil components (Nikaido et al., 1985; Trombetta et al., 2005). The results obtained in Table 1 showed that the essential oil samples were found to be rich in camphor (25.14%) and camphene (22.64%). Thus, the anti-microorganism activity of the essential oil of *Elsholtzia blanda* is related to its major components such as camphor and camphene. According to our literature survey, camphor and camphene have been reported to have significant antimicrobial activity (Pitarokili et al., 2003; Salamci et al., 2007). In addition, α-Pinene, β-Pinene, cineole, and germacrene D are well-known chemicals exhibiting strong antimicrobial activity (Sivropoulou et al., 1997; Magiatis et al., 1999; Filipowicz et al., 2003; Hichri et al., 2018) and these compounds also appeared with a relatively high ratio in the essential oil of *E. blanda*.

Staphylococcus aureus, *E. coli,* and *C. albicans* have long been recognized as one of important microorganisms that cause disease in humans, and in this study, they are sensitive organisms to *E. blanda* essential oil. Therefore, *E. blanda* oil may be promoted as an antibacterial and antifungal agent.

4. CONCLUSION

In conclusion, this study revealed that there are 31 compounds in the essential oil of *E. blanda* growing in Lam Dong Province, Viet Nam. The major components are camphor (25.14%) and camphene (22.64%). The chemical composition of this essential oil was different from previously published works from different regions of Viet Nam and other countries. Antimicrobial assays of this essential oil demonstrated that gram-positive bacteria (*S. aureus*) and pathogenic yeast (*C. albicans* were more sensitive to this essential oil compared to gram-negative bacteria (*E. coli*). Therefore, this is an interesting finding in view of their eventual application as natural antimicrobial compounds to replace the use of traditional antibiotics.

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