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Chemical composition and antimicrobial activity of essential oils from leaves and rhizomes of *Curcuma zedoaria* obtained via supercritical fluid extraction

Composición química y actividad antimicrobiana de aceites esenciales de hojas y rizomas de *Curcuma zedoaria* obtenidos mediante extracción con fluido supercrítico

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ABSTRACT

Supercritical fluid extraction is a rapid, selective, and environmentally friendly method of particular interest in the extraction of essential oils from plants and herbs. *Curcuma zedoaria* is a member of the Zingiberaceae family widely cultivated in India, China, and Southeast Asia, including Vietnam. This study examined the chemical composition and antimicrobial activity of essential oils from the leaves and rhizomes of *C. zedoaria* from Vietnam obtained via supercritical fluid extraction. The main compounds of leaf essential oil were *iso*-borneol (31.2%), β-elemene (14.7%), β-caryophyllene (11.4%), 1,8-cineole (9.7%), and caryophyllene oxide (5.9%). In rhizome essential oil, 1,8-cineole (41.7%), β-elemene (13.6%), curzerenone (12.3%), camphor (10.2%), and germacrene B (5.2%) comprised the main compounds. The essential oils from *C. zedoaria* leaves and rhizomes displayed antimicrobial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus cereus*, and *Candida albicans*, with minimum inhibitory concentration values ranging from 100–400 µg/mL. Thus, our results support the need for further investigation of *C. zedoaria* essential oils as a potential source of antimicrobial agents.

Keywords: *Curcuma zedoaria*; Supercritical fluid extraction; Essential oil; Antimicrobial activity; Extraction method.

RESUMEN

La extracción con fluidos supercríticos es un método rápido, selectivo y respetuoso con el medio ambiente de particular interés en la extracción de aceites esenciales de plantas y hierbas. *Curcuma zedoaria* es un miembro de la familia Zingiberaceae ampliamente cultivada en India, China y el sudeste asiático, incluido Vietnam. Este estudio examinó la composición química y la actividad antimicrobiana de los aceites esenciales de las hojas y rizomas de *C. zedoaria* de Vietnam obtenidos mediante extracción con fluido

supercrítico. Los principales compuestos del aceite esencial de hoja fueron *iso*-borneol (31.2%), β-elemeno (14.7%), β-cariofileno (11.4%), 1,8-cineol (9.7%) y óxido de cariofileno (5.9%). En el aceite esencial de rizoma, 1,8-cineol (41.7%), β-elemeno (13.6%), curzerenona (12.3%), alcanfor (10.2%) y germacreno B (5.2%) comprendían los compuestos principales. Los aceites esenciales de las hojas y rizomas de *C. zedoaria* mostraron actividad antimicrobiana contra *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus cereus* y *Candida albicans*, con valores mínimos de concentración inhibitoria que oscilan entre 100 y 400 µg/mL. Por lo tanto, nuestros resultados respaldan la necesidad de una mayor investigación de los aceites esenciales de *C. zedoaria* como fuente potencial de agentes antimicrobianos.

Palabras claves: *Curcuma zedoaria*; Extracción de fluidos supercríticos; Aceite esencial; Actividad antimicrobiana; Método de extracción.

1. INTRODUCTION

The *Curcuma* L. is one of the largest genera in the family Zingiberaceae, containing about 100 species, and is native to tropical and subtropical regions (Ravindran *et al.*, 2007). This genus is known as an essential source of coloring and flavoring agents in Asian cuisines, traditional medicines, spices, dyes, perfumes, cosmetics, and ornamental plants (Dosoky and Setzer, 2018). Several *Curcuma* species are used medicinally for treating pneumonia, bronchial complaints, leucorrhea, diarrhea, dysentery, infectious wounds or abscesses, and insect bites (Dosoky and Setzer, 2018; Rahaman *et al.*, 2020). In particular, the essential oils obtained from *Curcuma* species are considered important sources of biologically active substances (Sun *et al.*, 2017).

Essential oils represent a small fraction of a plant's composition but confer the characteristic for which aromatic plants are used in the pharmaceutical, food, and fragrance industries (Pourmortazavi and Hajimirsadeghi, 2007). *Curcuma zedoaria* is a member of the genus *Curcuma* native to northeast India and Indonesia (Lim, 2016). Currently, this species is widely cultivated across subtropical regions, including India, China, and Southeast Asia. *C. zedoaria* is used both culinarily and medicinally because of its unique smell, as well as the presence of widely varying phytoconstituents (Lim, 2016). Chemical compositions of essential oils from different parts of *C. zedoaria* have been published (Mau *et al.*, 2003; Garg *et al.*, 2005; Purkayastha *et al.*, 2006; Rahman *et al.*, 2014). Extracts from *C. zedoaria* have been reported to exhibit anticancerous, anti-inflammatory, analgesic, antiallergic, antibacterial, and antifungal activities (Gharge *et al.*, 2021). Supercritical fluid extraction represents a selective, environmentally friendly technique for the extraction of essential oils from plants and herbs (Pourmortazavi and Hajimirsadeghi, 2007). This method has recently emerged as an efficient alternative to other methods for essential oil separation from plant materials. This study analyzed the chemical composition and antimicrobial activity of essential oils from *C. zedoaria* leaves and rhizomes obtained via supercritical fluid extraction.

2. MATERIALS AND METHODS

2.1. Plant material

Fresh leaves and rhizomes of *C. zedoaria* (2 kg of each) were collected from Lai Chau, Vietnam in November 2019. Prior to essential oil extraction, materials were air-dried under laboratory shade for two weeks to reduce moisture content. Additionally, sediments and other unwanted materials were separated from the samples. Afterward, dried materials were chopped and mechanically ground to a homogeneous powder using a laboratory mill.

2.2. Preparation of the essential oils

Essential oils from leaves and rhizomes of *C. zedoaria* were obtained by supercritical fluid extraction as described previously (Jing *et al.*, 2019). Experiments were conducted in a laboratory-scale SFE 1000 system (Waters, USA). All extractions were performed with supercritical CO_2 in dynamic mode. The volume of essential oils obtained were measured and filled up to 10 mL with hexane and stored in tightly closed dark vials at 4°C until use.

2.3. Analysis of the essential oils

Each of the essential oils was analyzed by gas chromatography (GC) and gas chromatography-mass spectrophotometry (GC-MS) with the conditions as previously reported (Thin *et al.*, 2021). The GC analysis was carried out on an Agilent Technologies 7890A GC attached to a flame ionization detector (FID), and fitted with an HP-5MS chromatographic column (i.d. 0.25 mm × 30 m, 0.25 μm film thickness). The GC/MS analysis was carried out on an Agilent GC 7890A chromatograph with the same column as in the GC analysis and coupled with a mass spectrometer (HP 5973 MSD). The identification of chemical compounds was made by comparison to their relative retention time and library data as described recently (Adams, 2007; Thin *et al.*, 2021; Thinh *et al.*, 2022). The identified constituents were arranged in order of retention time and quantity in percentage.

2.4. Antimicrobial screening

Five microorganisms were used to evaluate the antimicrobial activity of essential oils from leaves and rhizomes of *C. zedoaria* including *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 9027), *Staphylococcus aureus* (ATCC 6538), *Bacillus cereus* (ATCC 14579), and the yeast *Candida albicans* (ATCC 10231). The antimicrobial activity was determined using the microdilution broth susceptibility assay, as previously reported (Thin *et al.*, 2021; Thinh *et al.*, 2021). Dilution series were prepared in sterile distilled water in micro-test tubes from where they were transferred to the 96-well microtiter plates for the assays. The minimal inhibitory concentration (MIC) of the essential oil was determined as the lowest concentration that visibly inhibited the growth of the microorganisms.

2.5. Statistical analysis

All the analyses were carried out in triplicate. The results are presented as the mean value of the individual measurements with the corresponding standard deviation (SD).

3. RESULTS AND DISCUSSION

The supercritical fluid extraction experiment afforded light yellow essential oils in yields of 0.37 ± 0.01% (v/w) and 1.03 ± 0.02% for *C. zedoaria* leaves and rhizomes, respectively. These yields are consistent with data obtained from essential oils of *C. zedoaria* from other countries. For example, *C. zedoaria* rhizome essential oils from China and India were obtained in yields of 0.63% and 0.36%, respectively (Mau *et al.*, 2003; Purkayastha *et al.*, 2006), while *C. zedoaria* leaf essential oils from India and Bangladesh were obtained in yields of 0.2% and 0.8%, respectively (Garg *et al.*, 2005; Rahman *et al.*, 2014). These differing essential oil yields may be attributable to varying geographical origins, climatic conditions, environmental conditions, and extraction methods (Zantar *et al.*, 2015; Thinh *et al.*, 2022).

The chemical constituents of *C. zedoaria* essential oils obtained via supercritical fluid extraction are shown in Table 1. By using a combination of GC and GC/MS with HP-5MS column, 27 and 31 compounds representing 92.5% and 96.8% of the essential oil profile were identified in the *C. zedoaria* leaf and rhizome, respectively (Table 1). Oxygenated monoterpenes (49.5% and 56.6%), sesquiterpene

hydrocarbons (31.9% and 22.3%), oxygenated sesquiterpenes (9.5% and 16.5%), and monoterpene hydrocarbons (1.6% and 1.4%) were the main classes of compounds identified in *C. zedoaria* leaf and rhizome essential oils, respectively. The main constituents of *C. zedoaria* leaf essential oil were *iso*-borneol (31.2%), β-elemene (14.7%), β-caryophyllene (11.4%), 1,8-cineole (9.7%), and caryophyllene oxide (5.9%). The leaf oil contained sizeable amounts of borneol (3.6%), camphor (2.9%), α-cubebene (2.1%), α-copaene (1.7%), and spathulenol (1.7%). Significant compounds in the rhizome essential oil, however, were 1,8-cineole (41.7%), β-elemene (13.6%), curzerenone (12.3%), camphor (10.2%), and germacrene B (5.2%). The rhizome oil also demonstrated significant quantities of curzerene (3.2%), *iso*-borneol (2.1%), and germacrene D (1.6%). Chemical structures of the main compounds in the *C. zedoaria* leaf and rhizome essential oils are presented in Figure 1.

Table 1. Chemical compositions of essential oils from leaves and rhizomes of *Curcuma zedoaria*.

Compounds[a]	RI[b]	Leaves[c]	Rhizomes[c]
α-Pinene	939	0.1	0.3
Camphene	955	0.2	0.1
Sabinene	978	-	0.2
β-Pinene	984	0.2	0.3
Myrcene	992	-	0.1
α-Terpinene	1022	-	0.2
1,8-Cineole	1026	9.7	41.7
(*Z*)-β-Ocimene	1034	0.8	0.2
(*E*)-β-Ocimene	1046	0.3	-
Linalool	1105	1.1	0.5
Camphor	1156	2.9	10.2
iso-Borneol	1173	31.2	2.1
Borneol	1178	3.6	1.4
Terpinen-4-ol	1187	-	0.2
α-Terpineol	1200	0.8	0.3
Bornyl acetate	1294	0.2	-
δ-Elemene	1348	0.4	0.1
α-Cubebene	1360	2.1	-
Geranyl acetate	1377	-	0.2
α-Copaene	1389	1.7	-
β-Elemene	1403	14.7	13.6
β-Caryophyllene	1437	11.4	0.2
γ-Elemene	1445	0.4	-
(*E*)-β-Farnesene	1455	-	0.4
α-Humulene	1471	0.3	0.2
β-Selinene	1489	-	0.5
α-Selinene	1496	-	0.3
Germacrene D	1498	0.2	1.6
Curzerene	1502	-	3.2
β-Bisabolene	1517	-	0.2
Germacrene B	1577	0.7	5.2
Caryophyllene oxide	1580	5.9	0.2
Spathulenol	1598	1.7	-
Curzerenone	1615	-	12.3
Selin-11-en-4-one	1626	0.3	-
τ-Cadinol	1647	0.2	-
α-Cadinol	1652	0.6	-
neo-Intermedeol	1655	-	0.2
β-Eudesmol	1671	-	0.1
Germacrone	1709	0.8	0.5
Monoterpene hydrocarbons		1.6	1.4

Compounds[a]	RI[b]	Leaves[c]	Rhizomes[c]
Oxygenated monoterpenes		49.5	56.6
Sesquiterpene hydrocarbons		31.9	22.3
Oxygenated sesquiterpenes		9.5	16.5
Total		92.5	96.8

[a]Elution order on HP-5MS column; [b]Retention indices on HP-5MS column; [c]means of three replicate values, SD (±) omitted to avoid congestion; (-) not identified.

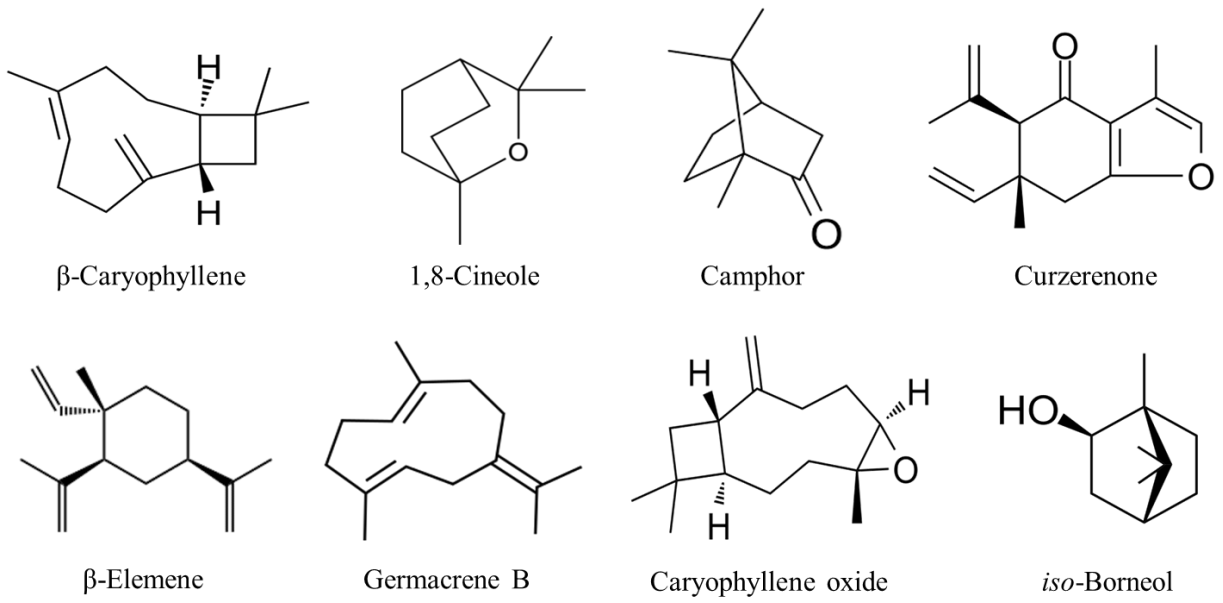

Figure 1. Chemical structures of main compounds in essential oils from leaves and rhizomes of *Curcuma zedoaria*.

The chemical compositions of *C. zedoaria* essential oils from Vietnam and other parts of the world have previously been characterized. Although the majority of these contain ubiquitous terpenes, the identities of these compounds differ from one another. For example, 1,8-cineole (43.49%), curzerenone (13.40%), and camphor (12.29%) were the main compounds of *C. zedoaria* rhizome essential oils from Brazil (dos Santos Lima Junior *et al.*, 2020). The main compounds identified in the essential oils of *C. zedoaria* rhizomes from India, however, were curzerene (22.3%), 1,8-cineole (15.9%), and germacrone (9.0%) (Purkayastha *et al.*, 2006). Lai *et al.* (2004) identified epicurzerene (46.6%), curdione (13.7%), and 5-isopropylidene-3,8-dimethyl-1(5H)-azulenone (9.2%) as the main compounds in *C. zedoaria* rhizome essential oils from China. Main compounds of *C. zedoaria* rhizome essential oils from Sri Lanka were debromofiliforminol (31.5%), camphor (11.8%), aromadendrene (11.8%), benzofuran (8.8%), and germacrone (5.2%) (Herath *et al.*, 2017). Regarding *C. zedoaria* leaf essential oils from India, main compounds included α-terpinyl acetate (8.4%), isoborneol (7.0%), dehydrocurdione (9.0%), and selina-4(15),7(11)-dien-8-one (9.4%) (Garg *et al.*, 2005), while eucalyptol (22.4%), α-caryophyllene (17.2%), 1-octen-3-ol (12.4%), β-elemene (9.6%), and caryophyllene oxide (8.3%) represented the main compounds of leaf oils from Bangladesh (Rahman *et al.*, 2014). Such variation can arise from several factors, such as fractionation, geographic origin of the plant, growing season, and the methods of harvesting, extraction, and distillation (Rao and Rout, 2003; Thinh *et al.*, 2022).

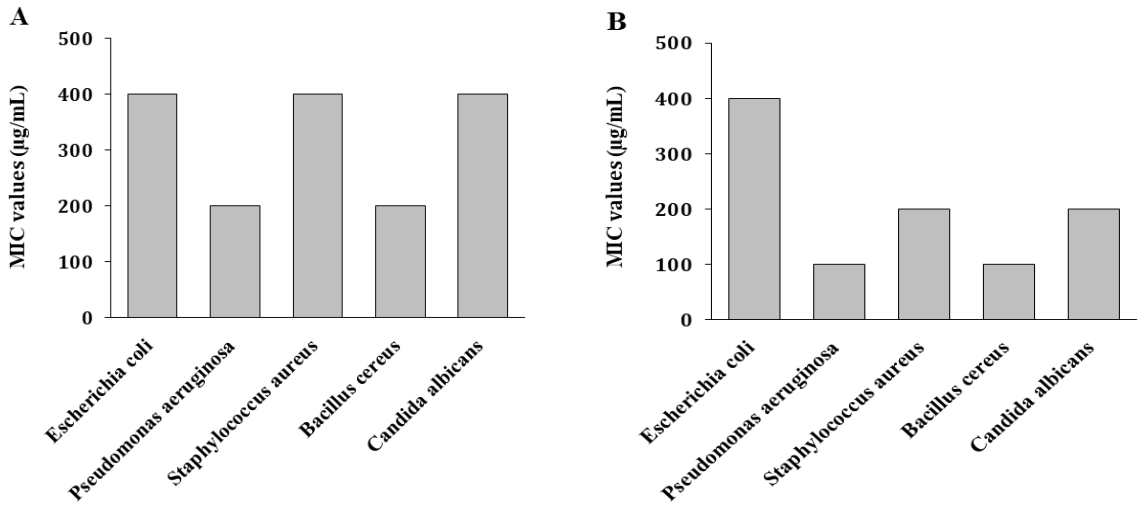

Figure 2. Minimum inhibitory concentration (MIC) values of essential oils from leaves (A) and rhizomes (B) of *Curcuma zedoaria* inhibiting the growth of microorganisms.

The essential oils of *C. zedoaria* obtained via supercritical fluid extraction were screened against a panel of microorganisms (Figure 2). MIC values were used to determine the minimum concentration that prevented the growth of test microbes. *C. zedoaria* rhizome essential oils showed the highest antibacterial activity against *P. aeruginosa* and *B. cereus*, with an MIC value of 100 µg/mL, while their MIC value against *S. aureus* and *C. albicans* was 200 µg/mL. Rhizome essential oil also demonstrated activity against *E. coli* at an MIC value of 400 µg/mL. In this study, leaf essential oil exhibited moderate antimicrobial action against the growth of *P. aeruginosa* and *B. cereus*, with an MIC value of 200 µg/mL, as well as displayed activity against *S. aureus*, *C. albicans*, and *E. coli*, with an MIC value of 400 µg/mL. The antimicrobial activities of *C. zedoaria* essential oils are likely related to the interaction between minor and major components and are conditioned by the activity of their components (Sharma *et al.*, 2020). The observed antimicrobial activity of *C. zedoaria* essential oils aligns with previous findings showing that essential oils of *Curcuma* species selectively inhibited the growth of different microorganisms (Wilson *et al.*, 2005; Dosoky and Setzer, 2018; Jena *et al.*, 2020).

4. CONCLUSIONS

In summary, this study sheds light on the chemical composition and antimicrobial activity of essential oils from the leaves and rhizomes of *C. zedoaria* obtained via supercritical fluid extraction. The major components of leaf essential oil were *iso*-borneol (31.2%), β-elemene (14.7%), β-caryophyllene (11.4%), 1,8-cineole (9.7%), and caryophyllene oxide (5.9%), while rhizome essential oil primarily comprised 1,8-cineole (41.7%), β-elemene (13.6%), curzerenone (12.3%), camphor (10.2%), and germacrene B (5.2%). Both essential oils exhibited antimicrobial activity, with MIC values ranging from 100–400 µg/mL. *C. zedoaria* essential oils obtained via supercritical fluid extraction may thus have potential applications as antimicrobial agents. However, further research concerning the effects of extraction methods on *C. zedoaria* essential oils is necessary to determine the optimal method for extracting essential oils from *C. zedoaria*.

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