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GC-MS ANALYSIS OF LEAVES AND RHIZOMES ESSENTIAL OILS OF *ZINGIBER PURPUREUM* ROXB. FROM BANGLADESH

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Abstract

Essential oils (EOs) are among the most important plant secondary metabolites. *Zingiber purpureum* Roxb. is a tropical ginger from Bangladesh, but its chemical constituents of leaves and rhizomes, both EOs, may have pharmacological effects. The aim of this study was carried out to analyze the qualitative composition of both Essential oils (EOs) of both leaf and rhizomes of *Z. purpureum*. The EOs from the leaves and rhizomes were extracted by hydrodistillation. Chemical constituents of the EOs were separated and identified by gas chromatography/mass spectrometry (GC/MS), and the relative percentage of each constituent was determined by area normalization. In total, 81 compounds were identified from leaf EOs, whereas 44 were from rhizome EOs. The common compounds identified from both rhizome and leaf EOs were triquinacene, 1,4, bis (methoxy), β-phellandrene and β-sesquiphellandrene, respectively. Besides, the major constituents of leaf EOs were sabinene (14.99%), β-pinene (14.32%), caryophyllene oxide (13.85%), and caryophyllene (9.47%), whereas the rhizome EOs confirmed (Z)-ocimene (21.97%), 4-terpineol (18.45%), γ-terpinene (3.86%) and cis-sabinene hydrate (3%). These EOs could be a promising source for the development of nutraceuticals, functional foods, and pharmaceutical products due to their potent activities and exploitation.

Introduction

Zingiber purpureum Roxb. is an herbaceous perennial plant belonging to the Zingiberaceae family widely distributed in Southeast Asia (Norikura *et al.* 2020). Plants originating from Indonesia and India can be grown in an area exposed to sunlight and adapted to an altitude of 1300 m above sea level (Taroeno *et al.* 1991). Generally, it is a tropical ginger used as food, spice, flavoring agent, and medicine in countries like Bangladesh, Thailand, Pakistan, and India (Devkota *et al.* 2021). EOs extracted from plants are complex mixtures of volatile terpenes and hydrocarbons (Spadaccino *et al.* 2021) and are defined as secondary metabolites (Baptista-Silva *et al*. 2020) from plants that produce fragrance with a broad scope of biomedical activities (Ban *et al.* 2020). The chemical compounds of extracted EOs from plants are influenced by geographical location, the techniques of cultivation, harvesting, extraction method, packaging, and storage conditions (Mahfud *et al.* 2017). Several researchers' studies showed a wide range of activities of EOs, such as natural food preservative, antibacterial, antioxidant, anticancer, anti-inflammatory, in the treatment of cardiovascular diseases, antiarthritic, antiatherosclerotic, antidepressant, diabetes, obesity, and in the prevention of neurodegenerative diseases (Arcusa *et al.* 2022, Ballester *et al.* 2023, Kamal *et al.* 2023, Mustafa and Chin, 2023, Tundis *et al.* 2023). However, these beneficial properties of *Z. purpureum* result from the presence of numerous bioactive compounds in its rhizome and leaf EOs. Among the major groups of chemical constituents, there are polyphenolic compounds (gingerols, paradols, shogaols, and their derivatives), terpene compounds divided into

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monoterpenes (camphor, phellandrene, geranial, neral, linalool), and sesquiterpenes (bisabolene, curcumene, zingiberene, farnesene, and sesquiphellandrene) (Mohammadi *et al*. 2021, Pagano *et al.* 2021) Thus, EOs can be used to formulate nutraceuticals, functional foods, and other medicinal purposes due to their availability, effectiveness, and low production cost (Zagorska *et al*. 2023). However, a literature survey showed that there is no report on the EO constituents of *Z. purpureum* of Bangladeshi origin; thus, we decided to investigate the chemical constituent analysis for the first time. Therefore, the aim of this study was to identify the different chemical constituents of EOs from *Z. purpureum* leaf and rhizome, as there are still many constituents whose potential is yet unknown.

Materials and Methods

Fresh leaf and rhizome of *Zingiber purpureum* Roxb. plant was collected from the experimental fields of the BCSIR Laboratories, Chattogram, during May 2023. One voucher specimen (J-134) was deposited in the herbarium of BCSIR Laboratory, Chattogram.

Fresh leaf and rhizome of *Z. purpureum* were collected from the BCSIR Laboratories' experimental field in Chattogram. The collected leaves and rhizomes were dried at room temperature. The dried leaf and rhizome samples were ground separately to a powder using a grinding machine (Retsch Mühle, Germany). Each 250 g portion of each powder was mixed in 750 ml of distilled water and soaked for 1 hour. This water mixture was placed in the Clevenger apparatus, followed by hydro-distillation for the extraction of EOs (Bhuiyan *et al*. 2011, 2023). The EO was subsequently extracted after the leaves and rhizomes were hydro-distilled for 4 hrs. The EO was then dried over anhydrous sodium sulfate to remove moisture. The two EOs (leaf and rhizome) were then sealed in separate vials and stored at 4°C under refrigeration prior to analysis.

The EOs from leaves and rhizomes were analyzed by gas chromatography-mass spectrometry (GC-MS) with the electron impact ionization (EI) method on a GC-17A gas chromatograph coupled to a GC-MS QP 5050A mass spectrometer (Shimadzu, Japan); a fused silica capillary column (30 m x 2.5 mm; 0.25 mm film thickness), coated with DB-1 (J&W); column temperature 100^{o}C (2 min) to 250^{o}C at the rate of 3^{o}C/min; carrier gas helium at flow rate of 1.0 ml/ min at constant pressure of 90 kPa. Acquisition parameters full scan; scan range 40-350 amu. Most constituents were identified by GC by comparison of their retention indices with those in the literature or with those of authentic compounds available in our laboratories. The retention indices were determined in relation to a homologous series of n-alkanes (C_8–C_{24}) under the same operating conditions. Further identification was made by comparison of their mass spectra with those stored in the NIST Library (NIST 147 and NIST 27) or with mass spectra from the literature (Adams 2007, Bhuiyan *et al*. 2008). Component relative percentages were calculated based on the normalization method without using correction factors.

Results and Discussion

The colourless EOs yield of *Z. purpureum* leaf was 0.20% (V/W) and the rhizome was 1.40% (V/W). A total of 81 constituents (Table 1) of the EO of leaf were identified, accounting for 98.67% of the total oil. The main constituents in the EO of the leaf were sabinene (14.99%), β-pinene (14.32%), caryophyllene oxide (13.85%), caryophyllene (9.47%), α-pinene (6.31%), methyl p-methoxycinnamate (5.02%), triquinacene, 1,4, bis (methoxy) (3.79%), camphene (3.56%), borneol 1(2.96 %), crypton (2.47%), β-mycerene (1.46%), apiol (1.40%), 1.6.10-dodecatriene, 7,11-dimethyl-3-methylene, (Z) (1.24%), (E)-ocimene (1.21%), β-sesquiphellandrene (1.21%) and β-phellandrene (1.04%). On the other hand, the major constituents in rhizome EOs were triquinacene, 1,4, bis (methoxy) (26.47), (Z)-ocimene (21.97%),

Table 1. GC-MS analysis of *Zingiber purpureum* leaf essential oil constituents.

S.N	Constituents	%	S.N	Constituents	%
1.	(-)-spathulenol	0.15	42.	Crypton	2.47
2.	(E)-ocimene	1.21	43.	Cubenol	0.13
3.	1(2H)-naphthalenone, octahydro-4-methoxy	0.08	44.	Cuminal	0.23
4.	1.6.10-dodecatriene, 7,11-dimethyl-3-methylene, (Z)	1.24	45.	Cuminol	0.21
5.	2-cyclohex-1-ol, 1-methyl-4-(1-methylethyl)	0.09	46.	Curcumene	0.16
6.	2-methyl-oct-2-enedial	0.05	47.	Cyclohexanone, 3-ethenyl	0.22
7.	2-pentenal, 2-ethyl	0.04	48.	Cyclohexene, 5-methyl-3-(1-methylethenyl)	0.10
8.	3,4,5-trimethoxybenzylchloride	0.11	49.	Damascone	0.32
9.	3-cyclobexen-1-one, 3(hydroxymethyl)-6-(1-methylethyl)	0.17	50.	Epi-13-manool	0.11
10.	3-cyclohexene-1-methanol	0.18	51.	Eremophilene	0.10
11.	4-terpineol	0.33	52.	Heptadecane	0.08
12.	5-caranol, trans	0.12	53.	Isogeraniol	0.24
13.	5-nonaol,-5-methyl	0.20	54.	Isolimonene	0.66
14.	7-hexadecenal	0.11	55.	Isothujol	0.07
15.	7-oxabicyclo (2.2.1) hept-5-en-2-one	0.10	56.	Juniper camphor	0.43
16.	Apiol	1.40	57.	α-caryophyllene	0.53
17.	Aromadendrene oxide	0.12	58.	α-methylfuran	0.10
18.	Asaraldehyde	0.63	59.	Longipinocarvone	0.18
19.	β-bisabolene	0.19	60.	α-pinene	6.31
20.	β-elemene	0.17	61.	α-selinene	0.03
21.	Benzen-1-methyl, 4-(1-methylethyl)	0.58	62.	α-thujene	0.04
22.	Bergamotol, z-α-trans	0.08	63.	Methyl p-methoxycinnamate	5.02
23.	β-linalool	0.39	64.	Methylvanillin	0.64
24.	β-myrcene	1.46	65.	Myrtanal	0.14
25.	Borneol	2.96	66.	Naphthalene	0.08
26.	Bornyl acetate	0.63	67.	Ocimene	0.83
27.	β-phellandrene	1.04	68.	Pentadecyne	0.53
28.	β-pinene	14.32	69.	Phellandral	0.42
29.	β-pinene oxide	0.04	70	Pinocarvone	0.11
30.	β-sesquiphellandrene	1.21	71.	Pseudo limonene	0.15
31.	Camphene	3.56	72.	Sabinene	14.99
32.	Camphor	0.07	73.	Squalene	0.07
33.	Carveol	0.04	74.	tau-muurolol	0.22
34.	Caryophyllene	9.47	75.	Tetracyclo [6.3.2.0(2.5),0(1,8)] tridecan-9-ol, 4,4-dimethyl	0.29
35.	Caryophyllene oxide	13.85	76.	trans-nerolidol	0.31
36.	Cedranoxide, 8,14	0.07	77.	trans-pinocarveol	0.25
37.	Cedrene	0.40	78.	Tricyclene	0.06
38.	Chamigrene	0.15	79.	Triquinacene, 1,4, bis(methoxy)	3.79
39.	Cholestan-3-ol, 2-methylene-(3B,5L)	0.88	80.	Triquinacene, 1,4,7-tris (methoxy)	0.79
40.	Cis-bicyclo (4.4.0) decan-1-ol-3-one	0.54	81.	Verbenone	0.07
41.	Cis-pulegone oxide	0.07			

4-terpineol (18.45%), γ-terpinene (3.86%), β-phellandrene (3.49%), cis-sabinene hydrate (3%), β-pinene (2.55%), β-sesquiphellandrene (2.45%), α-pinene (2.30%), 4-terpinyl acetate (2.10%), methyleugenol (2.07%) and 2-allyl-1,4-dimethoxy-3-methyl benzene (1.74%) (Table 2). The findings of this study are in accordance with the data reported earlier (Mahfud *et al*. 2017).

Table 2. GC-MS analysis of *Zingiber purpureum* rhizome essential oil constituents.

S.N	Constituents	%	S.N	Constituents	%
1.	Tricyclene	0.02	23.	(+)-sativen	0.02
2.	α-thujene	0.70	24.	1,6,10-dodecatrien,7,11-dimethyl-3-methylene (Z)	0.32
3.	α-pinene	2.30	25.	2,5-dibutylfuran	0.06
4.	Camphene	0.36	26.	2-propenoic acid, 3-phenyl-, ethyl ester	0.06
5.	(Z)-ocimene	21.97	27.	Germacrene D	0.15
6.	β-pinene	2.55	28.	γ-selinene	0.27
7.	β-myrcene	1.58	29.	α-selinene	0.12
8.	Bendolimonene	0.02	30.	α-bergamotene	0.41
9.	4-terpinyl acetate	2.10	31.	3,4-dimethoxyphenyl acetone	0.08
10.	m-cymene	0.46	32.	β-bisabolene	0.13
11.	β-phellandrene	3.49	33.	β-sesquiphllandrene	2.45
12.	(E)-ocimene	0.09	34.	Methyleugenol	2.07
13.	γ-terpinene	3.86	35.	Megastigmastriene	0.33
14.	Cis-sabinene hydrate	3.00	36.	Lachnophyllum ester	0.59
15.	2-carene	0.77	37.	2-allyl-1,4-dimethoxy-3-methyl benzene	1.74
16.	Linalool	0.04	38.	Caryophyllene	0.03
17.	Borneol	0.24	39.	Triquinacene,1,4-bis (methoxy)	26.47
18.	4-terpineol	18.45	40.	δ-cadinene	0.13
19.	Terpinyl acetate	1.10	41.	α-cadinol	0.03
20.	Trans-piperitol	0.34	42.	Juniper camphor	0.44
21.	Bornyl acetate	0.29	43	Cedrene	0.04
22.	Naphthalene,1,2-hydro-6-methoxy	0.02	44	2-propenoic acid, 3(4-methoxyphenyl), ethyl ester	0.26

The chemical constituents of EOs from the plant *Z. purpureum* are particularly interesting in current studies. These EOs are well known for their antifungal, insecticidal, and antibacterial activities, which enable them to perform satisfactorily in protecting stored goods (Begum *et al*. 2010, Rahman *et al*. 2012). EOs are typically less dense than water, liquid, volatile, and rarely contain colored lipid-soluble compounds (Bhuiyan and Nahid 2023). It is an important to note that Bangladeshi-origin EOs were analyzed by GC-MS. In total, 81 (Table 1) and 44 (Table 2) chemical constituents were identified and quantified in the leaf and rhizome EOs, respectively. In contrast, only 3 chemical constituents (triquinacene, 1,4, bis (methoxy) (26.47 and 3.79%), β-phellandrene (3.49 and 1.04%), and β-sesquiphellandrene (2.45 and 1.21%), respectively) are common in both rhizome and leaf EOs. Furthermore, the chemical constituents of both EOs were found to be responsible for their several activities, including natural food preservative, anticancer, antidiabetic, antimicrobial, antioxidant, and others (Guerrini *et al.* 2023, Tandirogang *et al.* 2022, Zhang *et al.* 2023). The study reveals that the chemical composition of both oils differs from the earlier reports and may therefore be treated as different chemotypes (Mahfud *et al.* 2017). Moreover, as *Z. purpureum* is growing widely in Bangladesh, it may be utilized as a natural source to contribute to pharmaceutical, nutraceutical, and functional food components because of the chemical compounds found in its leaf and rhizome EO.

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