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Original Article

Physicochemical characterization of medicinal essential oil from the rhizome of *Zingiber officinale* (ginger), grown in San Carlos, Costa Rica

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Abstract: Ginger is a medicinal plant native to India. Their potential use in cosmetics, medicines and natural products has been reported, however depending on crop conditions, the medicinal components of the different parts of the plant may not only go through changes in concentration but in composition, what modifies its medicinal potential. The aim of this study was to characterize by the chemical composition of essential oil obtained from rhizomes of *Zingiber officinale* grown in the area of San Carlos, Costa Rica, in order to standardize future hydroponic cultivations of the plant and validate their subsequent pharmacological or cosmetic effects. The rhizomes of the plant were used, the active principles were extracted by ethanolic extraction with Soxleth and distillation by entrainment with vapor, analysis was performed by using a qualitative phytochemical profile for the ethanolic extract, and the composition of the essential oil was studied by gas chromatography coupled to mass spectrometry detector (GC-MS). The presence of flavonoids, alkaloids, saponins, tannins and triterpenes in the ethanolic extract was qualitatively determined. In characterizing the essential oil by GC-MS we identified as lead compounds geranialdehyde (27.42%), neral (20.11%), 1.8-cineole (13.35%), camphene (4.65%) and E-geraniol (3.92%). The composition we obtained presented a clear difference with those reported in other studies, allowing the prediction of an antimicrobial behavior unlike most traditional essential oils of the rhizomes.
Keywords: *Zingiber officinale*, essential oil, gas chromatography, natural product, antimicrobial.

Introduction

The *Zingiber officinale* (ginger) is a plant that belongs to the family of Zingiberaceae. It is native to Asia and has been used since ancient times in culinary preparations and in traditional medicine[1] for the treatment of various diseases such as rheumatism, sore throat, cough, fever and gastrointestinal problems[1]. The smell of the rhizome of *Z. officinale* (*ZO*) depends mainly on its essential oil. More than 50 components have been characterized among them: β-phellandrene, 1,8-cineole, geraniol, citral, α-zingiberene, β-sesquifelandrene, ar-curcumene, amongst other[2]. The chemical composition of essential oil varies mainly by the growing conditions, environmental conditions and the extraction method[11]. Besides volatile

compounds, the *ZO* rhizome has water-soluble substances, such as tannins and flavonoids[3].

The aim of this research was to determine the chemical composition of the *ZO* essential oil from the area of San Carlos, Costa Rica. This essential oil, obtained specifically from the crops at this area, was not chemically characterized so far. By doing this research, we obtained parameters in order to standardize the future production of the rhizome, possibly by hydroponic cultivation.

Materials and Methods

Vegetal material

Rhizomes from San Carlos, Costa Rica, were used. The plant was collected in the environment, by Jean Guevara, October 2014, latitude: 10.4709; longitude: -84.6453. It was deposited in the Laboratory of Pharmacognosy, Universidad Latina of Costa Rica, with the number ULCR101.

Ethanolic extraction

A total of 1Kg of *ZO* rhizome pieces was placed in a Soxhlet extractor. In a 1 L balloon, 500 mL of ethanol were placed. The ethanol was heated at 70°C with an electric template. The extraction was performed for 15 hours with continuous reflux of the solvent on the sample. The extract was concentrated under reduced pressure using a rotary evaporator at 40°C.

Essential oil

A total of 5000 g of *ZO* rhizome were placed in a 12 L ball containing 5 L of water. Steam distillation was used, and held for 12 hours. The essential oil was obtained through a trap for essential oils.

Phytochemical screening

Tests for qualitative determination of the main phytochemicals groups present in the ethanolic extract were performed.

Tannins determination.

A total of 5 mL of extract were added into a test tube Then 3 drops of ferric chloride ($FeCl_3$) 4% were added. If the solution turns to a dark red color, the test was considered positive for the presence of tannins in the sample.

Saponins determination

A total of 4 mL of extract were added to a test tube with 8 mL of distilled water and shaked vigorously for 30 seconds. If foam was formed, the test is considered positive for the presence of saponins[4].

Flavonoids determination

A total of 5 mL of extract were added into a test tube. A piece of magnesium and 3 drops of hydrochloric acid (HCl) concentrated were added afterwards and allowed to react for 5 minutes. If solution turned to a dark orange color, the test was considered positive for the presence of flavonoids[4].

Alkaloids determination

Wagner reagent was used in this test: 1.3 g of iodine and 2g of potassium iodide were added in 20 mL of water in a 100 mL balloon, then dissolved with distilled water. 10 mL of extract were taken and 5 mL of 10% HCl were added. The mixture was boiled for 5 minutes; for a positive test, a whitish yellow precipitate is shown[4].

Steroids and terpenes determination

The test was considered positive for steroids with the formation of a blue or green color by the reaction of the Liebermann-Buchard reagent and the extract, and was considered positive for triterpenes with the formation of a red, violet or purple color[4].

Characterization of the essential oil by Gas Chromatography coupled to Mass Spectrometry

The analysis of the essential oil was performed at the Research Center for Natural Products (CIPRONA) of the University of Costa Rica, through gas chromatography coupled to a mass spectrum detector (GC-MS). The analyses were performed using a Shimadzu GC-17A gas chromatograph coupled with a GCMS-QP5000 apparatus and CLASS 5000 software with Wiley 139 and NIST databases. The data were obtained on a 5% phenyl-/95% dimethylpolysiloxane fused silica capillary column (30 m x 0.25 mm; film thickness 0.25 μm), (MDN-5S). Operating conditions were: carrier gas He, flow 1.0 mL/min; oven temperature program: 60-280°C at 3 °C/min; sample injection port temperature waa set at 250°C; detector temperature waa set at 260°C; ionization voltage: 70 eV; ionization current 60 μA; scanning speed 0.5 s over 38-400 amu range; split 1:70.

Results

Phytochemical screening and Characterization of the essential oil

All qualitative tests for phytochemicals were positive for our sample. A total of 39 major compounds were identified by GC-MS, and 5 compounds generated a signal in the chromatogram, but were not identified.

The chemical composition of essential oil is shown in Table 1. The major compounds we found were geraniol (27.42%), neral (20.11%), 1,8-cineole (13.35%), camphene (4.61%) and E-geraniol (3.92%). These 5 main compounds represent 69.41% of all the compounds identified. The identified compounds can be classified as terpenes, ketones, alcohols, esters and aldehydes; terpenes being the predominant compounds.

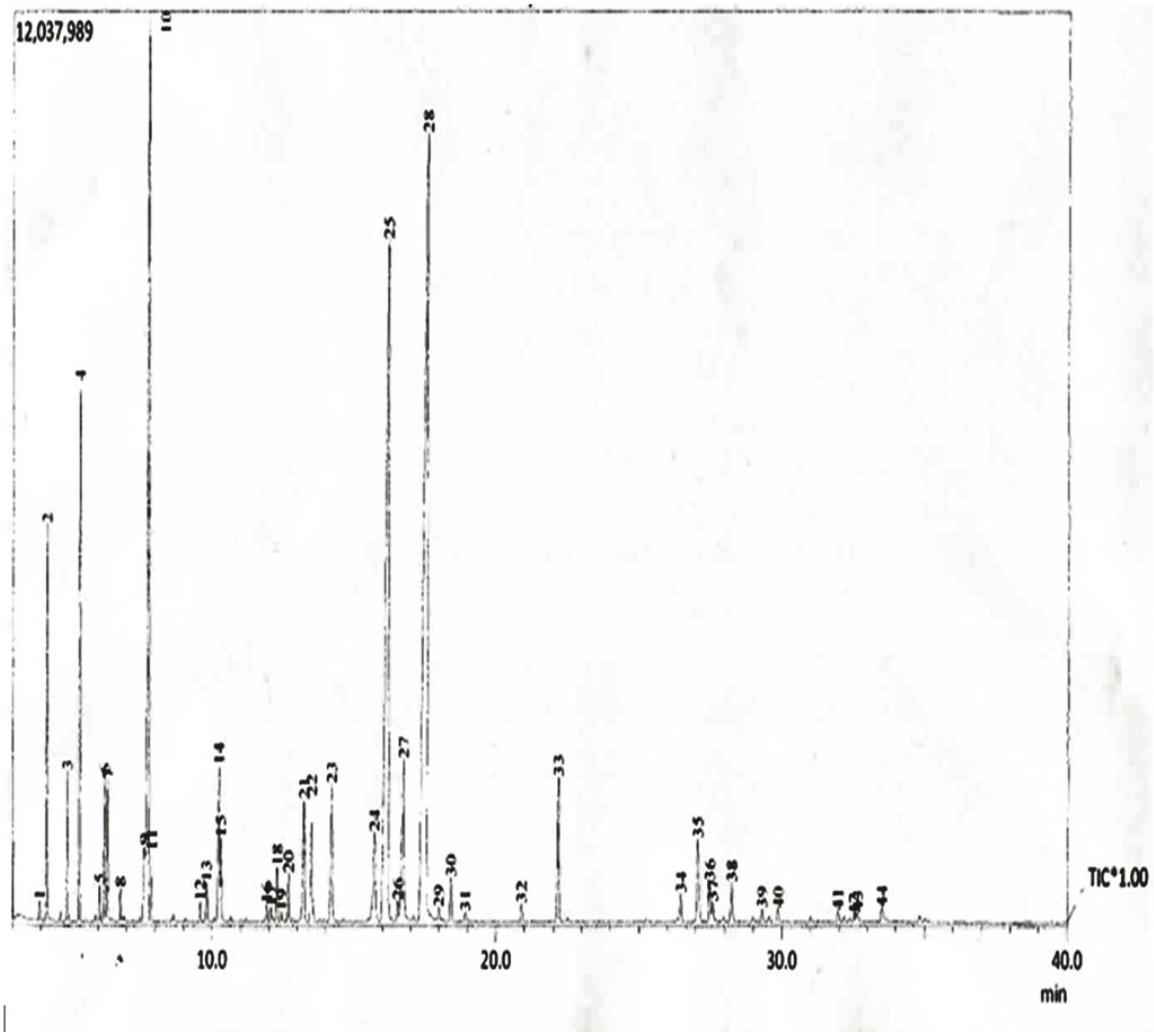

Fig.1: Mass spectrum of the *Z. officinale* essential oil cultivated in the San Carlos area, Costa Rica.

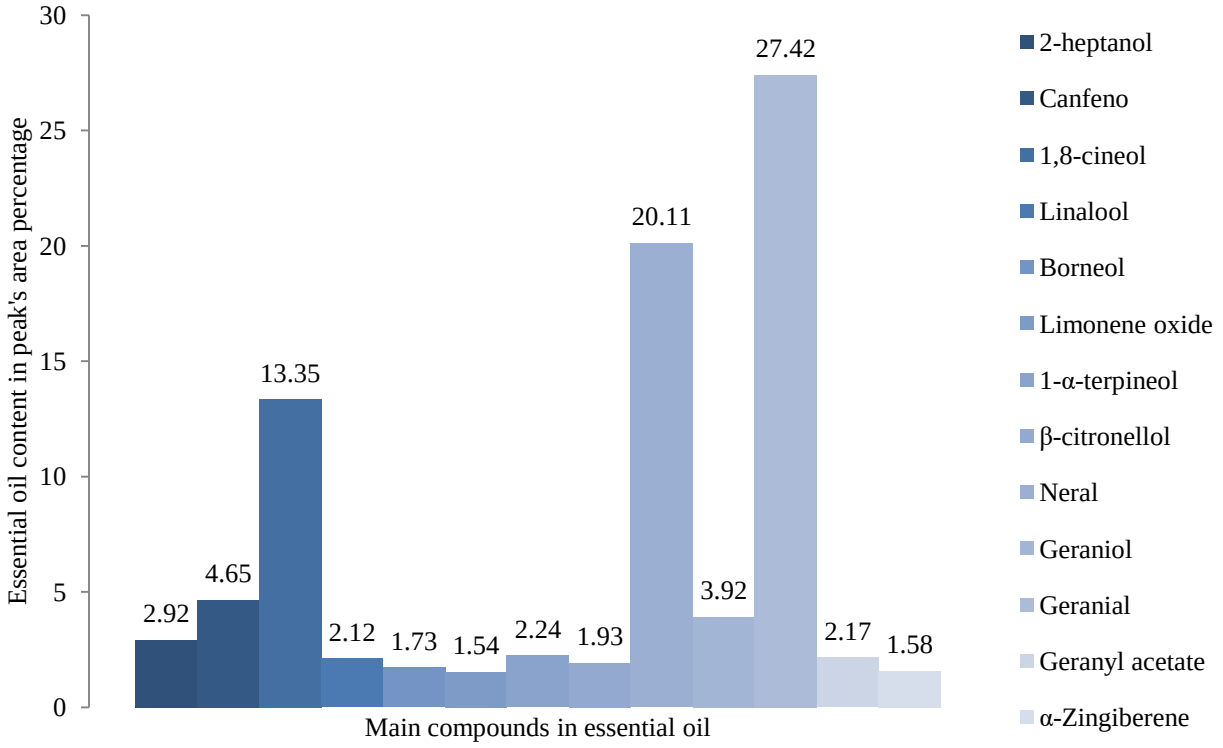

Figure 2. Composition of *Zingiber officinale* essential oil, according to data in Table 2.

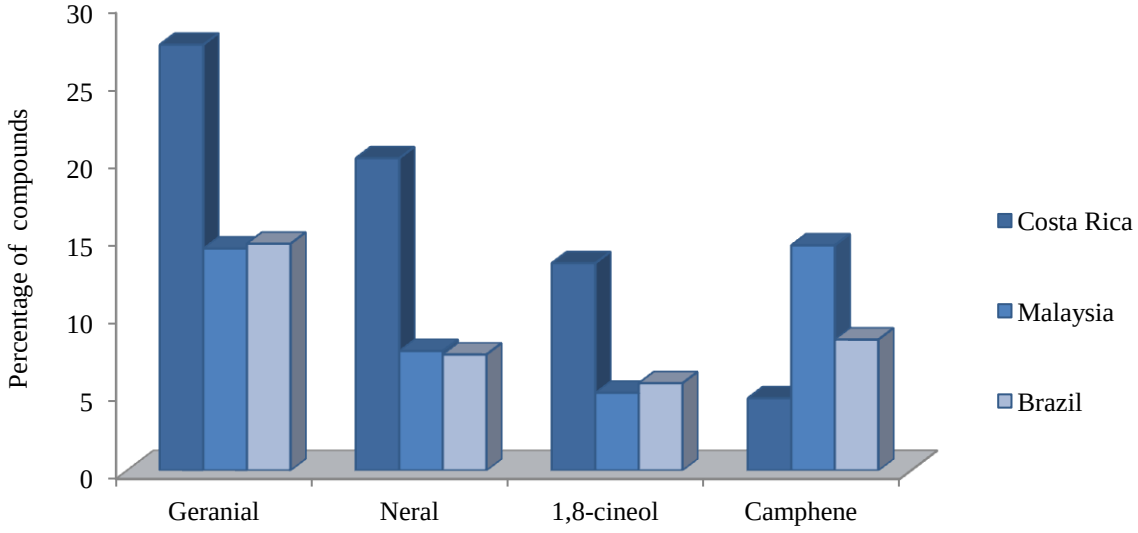

Figure 3. Comparison between the percentages of geranial, neral, 1,8-cineol and camphene identified in the essential oil of Zingiber officinale grown in Costa Rica, Malaysia and Brazil. Source: Table 3.

Table 1. Chemical composition of the essential oil of *Z. officinale* grown in the San Carlos area, Costa Rica, obtained by GC-MS.

Peak	Retention time	Area	% Area	Name
1	3.946	503352	0.15	2-heptanone
2	4.193	9480181	2.92	2-heptanol
3	4.905	3811043	1.17	α-pinene
4	5.334	15117380	4.65	Camphene
5	6.047	1024998	0.32	β-pinene
6	6.215	4387279	1.35	6-methyl-5-hepten-2-one
7	6.321	4226797	1.30	β- myrcene
8	6.773	1055130	0.32	Octanal
9	7.617	3911066	1.20	Limonene
10	7.756	43378642	13.35	1,8-cineol (eucalyptol)
11	7.870	1475827	0.45	acetic acid, sec butyl ester
12	9.580	705928	0.22	α-terpinolene
13	9.808	1677909	0.52	2-nonanone
14	10.235	6881837	2.12	Linalool
15	10.300	3004078	0.92	2-nonanol
16	11.922	784295	0.24	-
17	12.077	641919	0.20	Camphor
18	12.289	2427627	0.75	Citronella
19	12.445	433679	0.13	Camphene hydrate
20	12.684	2087606	0.64	-
21	13.223	5610468	1.73	Borneol
22	13.495	5014020	1.54	Limonene oxide
23	14.213	72888172	2.24	1-α-terpineol
24	15.715	6261331	1.93	β-citronellol
25	16.182	65356738	20.11	Neral (Z-citral)
26	16.539	862009	0.27	-
27	16.733	12726771	3.92	Geraniol (E-geraniol)
28	17.554	89140466	27.42	Geranial (E-citral)
29	17.995	538720	0.17	Bornyl acetate
30	18.416	1946501	0.60	2-undecanone
31	18.932	474167	0.15	2-undecanol
32	20.888	846209	0.26	Citronellyl acetate
33	22.172	7037756	2.17	Geranyl acetate
34	26.480	1264525	0.39	Curcumene
35	27.063	5126157	1.58	α-zingiberene
36	27.488	1988128	0.61	α-farnesene
37	27.583	836176	0.26	β-bisabolene
38	28.243	1916310	0.59	β-sesquifelandreno
39	29.313	656536	0.20	Elemol
40	29.871	666726	0.21	Nerolidol
41	31.972	566089	0.17	sesquisabinene hydrate
42	32.525	440557	0.14	-
43	32.666	561066	0.17	-
44	33.516	902770	0.28	β-eudesmol

Table 2 shows the essential oil composition, comparing different sources reported in the literature.

Table 2. Comparison in chemical composition of Z. officinale essential oil from different countries.

Compound	***Zingiber officinale*** **(Costa Rica)** **Area %**	***Zingiber officinale*** **(Malaysia)** (1) **Area %**	***Zingiber officinale*** **(Brazil)** (2) **Area %**
Camphene	4.65	14.5	8.43
β- myrcene	1.30	2.0	0.54
α-phellandrene	-	-	1.43
β- phellandrene	-	-	7.73
1,8-cineol	13.35	5.0	5.62
γ-terpinene	-	0.1	0.58
Linalool	2.12	2.3	0.79
Borneol	1.73	2.9	0.50
Citronellol	1.93	0.4	0.92
Geraniol	3.92	7.3	0.80
ar-curcumene	0.39	1.0	6.09
α-zingiberene	1.58	3.2	23.85
β-sesquiphellandrene	0.59	1.6	7.04
(E,E)-α-farnesene	0.61	1.8	9.98
α-pinene	1.17	3.6	-
β-pinene	0.32	3.6	0.03
Limonene	1.20	2.5	-
Octanal	0.32	-	-
α-terpinolene	0.22	0.4	-
2-nonanone	0.52	0.2	-
Borneol	1.73	2.9	-
Citronellol	1.93	0.4	-
Citronellal	-	0.1	-
Neral	20.11	7.7	7.47
Geranial	27.42	14.3	14.16
β-bisabolene	0.26	-	-
Elemol	0.20	0.6	-
Nerolidol	0.21	0.1	0.50
β-eudesmol	0.28	0.1	-
2-heptanone	0.15	-	-
Linalyl acetate	-	-	-
Bornyl acetate	0.17	1.4	-
2-heptanol	2.92	0.1	-

Numbers (1) and (2) are related to the references used for this comparison

Discussion

The importance of the findings on characterizing OZ essential oil lies in standardizing such composition with special features and its use it in the preparation of pharmaceutical forms, then assess their biological effect. Essential oils have shown a number of applications in pharmacy and cosmetics, by a synergistic action that is a result from the combination of individual components, rather than the isolation of one component[5-7]. Among the main areas of interest for the application of essential oils are: their preservative, antibacterial, antifungal, anti-inflammatory, expectorant, relaxing, analgesics and antioxidant activity[8-10].

Phytochemicals tests performed to ethanolic extract showed the presence of flavonoids, saponines, tannins, alkaloids and steroids and, or terpenes. Other studies also found the presence of these phytochemicals groups in the ethanolic extract. These water-soluble compounds from the ZO rhizome have anti-inflammatory activity, possibly by inhibiting cyclooxygenases (COX)[2-4,11,12]. In addition, it has been shown to be very effective in the treatment of chemotherapy-induced vomiting. However, very few studies reported that ethanol extracts possess antimicrobial activity[4].

The high percentages of nerol and geraniol possibly contribute to the similarities between the scent of the ZO essential oil and the *Cympogon citratus* (lemongrass) essential oil, where the nerol and geraniol are also the main compounds[13]. The chemical composition of essential oils in general, varies with the origin of the vegetal material used, cultivation conditions, environmental conditions and the obtaining method, among other factors. The major compounds of the ZO essential oil grown in Costa Rica are the geranial, neral, 1,8-cineol and camphene, representing more than 50% of the composition of essential oil. Although these compounds have also been reported in other studies, they are in a lower proportion than those reported in this analysis. Table 2 shows an interesting difference with previous works, on the chemical profile of OZ essential oil. Figure 3 shows the percentage comparison between geranial, neral, 1,8-cineol and camphene of ZO essential oils produced in Costa Rica, Malaysia and Brazil. In this comparison, it can be seen that chemical profile of the essential oil can vary depending on environmental and crop conditions, among others.

Yamamoto-Ribeiro et al.[14] reported that the main compound in ZO essential oil was the α-zingiberene, representing 23.85% of the essential oil. Padalia et al.[8] also reported that the α-zingiberene was the main compound. Reported percentages differ with those obtained in this research, because the α-zingiberene represents only 1.58% of the characterized essential oil. However, Singh et al.[13] reported that the composition of the ZO essential oil was geranial (25.9%), α-zingiberene (9.5%), neral (7.6%) and others[4]. Majolo et al.[7] reported a similar pattern in the chemical composition of the essential oil being geranial (23.9%), neral (17.2%), 1.8-cineole (16.0%) and camphene (11.4%) the major compounds; having a similar order in the percentage ratio of the main compounds[7,9].

Due to the composition of the essential oil found in the ZO grown in San Carlos, it could be predicted that this essential oil has significant antimicrobial and relaxing action, as citral by its isomers, Neral and Geranial, which represent most of their composition, have been associated with these effects[9, 15- 18].

Conclusion

The presence of flavonoids, alkaloids, saponins, tannins and triterpenes in the ethanolic extract was confirmed. The ZO rhizome essential oil shows an interesting antibacterial, antifungal and anti-inflammatory potential, as it presents a different profile of phytochemical composition from those reported for the same plant, especially the high concentration of neral, geranial and 1,8-cineole and also the low concentration of α-zingiberene. So, cultivation conditions can be enhanced and standardized to produce a greater amount of essential oil with this composition and special features for use in phytopharmaceutical compositions. Future studies should be conducted for comparing the chemical profile of ZO essential oil grown in different regions of Costa Rica, to determine whether there are variations in the composition of the essential oil within the same country.

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