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Research Article

Physicochemical characterization of medicinal essential oil made obtained from the rhizome of *Zingiber officinale* (ginger), grown in San Carlos, Costa Rica, in order to standardize future hydroponic

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Abstract

Introduction: Ginger is a medicinal plant native to India. Has been reported their potential use in cosmetics, medicines and natural products, however depending on crop conditions the medicinal components of the different parts of the plant not only changes in concentration but in its composition, this modifies its medicinal action. The aim of this study was to characterize by physicochemical methods 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. Materials and methods: 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).Results and Conclusions. The presence of flavonoids, alkaloids, saponins, tannins and triterpenes in the ethanolic extract was qualitatively determined. In characterizing the essential oil by GC-MS were identified as lead compounds the geranialdehyde (27.42%), nerol (20.11%), 1.8-cineole (13.35%), camphene (4.65%) and E-geraniol (3.92%). The composition obtained was compared with the composition reported in the literature, obtaining a clear difference with those reported in other studies, allowing predicting 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. It has been used since ancient times in culinary preparations and in traditional medicine (Park *et al.*, 2014) for the treatment of various diseases such as rheumatism, sore throat, cough, fever and gastrointestinal problems (Park *et al.*, 2014).

The smell of the rhizome *Zingiber officinale* (*ZO*) depends mainly on its essential oil. More than 50 components have been characterized among them i.e., β-phellandrene, 1,8-cineole, geraniol, citral, α-zingiberene, ar-curcumene, β-sesquifelandreno, amongst other (Ali *et al.*, 2008).The chemical composition of essential oil varies mainly by the growing conditions, environmental conditions and the extraction method (11).Besides volatile compounds, the *ZO* rhizomehas water-soluble substances (Nampoothiri *et al.*, 2012).

The objective of this research is to determine the chemical composition of the *ZO* essential oil from the area of San Carlos, Costa Rica. Rhizomes were used, *ZO* rhizomes from the San Carlos area.

MATERIALS AND METHODS

Vegetal material

Rhizomes from San Carlos, Costa Rica, were used.

Ethanolic extraction

1kg of *ZO* rhizome pieces was placed in a Soxhletextractor. In a 1 L balloon 500 mL of

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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 concentrated under reduced pressure using a rotary evaporator at 60°C.

Obtaining the essential oil

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.

Ferric chloride solution 4%: Add 4 g of ferric chloride in 100 mL of distilled water. Stir until completely dissolved ferric chloride and obtain an orange solution. Store in a jar with a lid to prevent oxidation of the solution, 5 mL of extract were added into a test tube Then 3 drops of ferric chloride (FeCl3) 4% were added. If the solution turns to a dark red color, the test is positive for the presence of tannins in the sample.

Saponins determination

4 mL of extract were added to a test tube with 8 mL of distilled water. Cover the test tube with your fingers and shake vigorously for 30 seconds. If foam is formed, the test is positive for the presence of saponins (Bhargava *et al.*, 2012).

Flavonoids determination

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. Allow to react for 5 minutes. If solution turns to a dark orange color, the test is positive for the presence of flavonoids (Bhargava *et al.*, 2012).

Alkaloids determination

Wagner reagent: 1.3 g of iodine and 2g of potassium iodide were added in 20mL of water in a 100 mL balloon. Then fill to volume with distilled water. Take 10 mL of extract and add 5 mL of 10% HCl. Boil for 5 minutes, for a positive test (Bhargava *et al.*, 2012), a whitish yellow precipitate is shown.

Steroids and terpenes determination

Liebermann-Buchard test: the reagent was prepared by adding a drop of sulfuric acid in a mixture of 1 mL of acetic anhydride with 1 mL of chloroform. The test is positive for steroids with the formation of a blue or green color; if positive for triterpenes, a red, violet or purple color (Bhargava *et al.*, 2012) is formed.

Characterization of the essential oil

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 detector (GC-MS).

RESULTS

Phytochemical screening

All tests for phytochemicals, performed to ethanolic extract, were positive (see table 1).

Characterization of the essential oil

A total of 39 major compounds were identified. 5 compounds generated a signal in the chromatogram was cannot be indentified and resulting essential oil component are shown in table 2 and figure 1.

Table 1: Phytochemicals screening of *Z. officinale* rhizome of the San Carlos area, Costa Rica.

Phytochemical group	Ethanolic extract
Flavonoids	+
Saponins	+
Tannins	+
Alkaloids	+
Steroids and / or triterpenes	+

* Interpretation of results: (+) positive test, (-) negative test.

Table 2: Chemical composition of essential oil of Zingiber officinale

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

The major compounds 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.

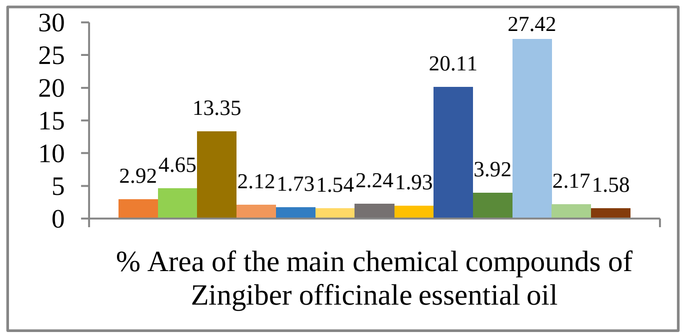

Figure 1: Main chemical percentage compounds *Zingiber officinale.*

Previously reported essential oil composition, comparing different sources are listed in table 3 and figure 2.

Table 3: 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

Compound	*Zingiber officinale* (Costa Rica) Area %	*Zingiber officinale* (Malaysia)[1] Area %	*Zingiber officinale* (Brazil)[2] Area %
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	-

Source Table 3

[1]Sivasothy, Y., Keng-Chong, W., Hamid, A., M.Eldeen, I., Sulaiman, S. F., &Awang, K. (2011). Essential oils of *Zingiberofficinale* var. rubrum Theilade and their antibacterial activities. *FoodChemistry. Elsevier*, 514-517.

[2]Yamamoto-Ribeiro et al (2013). Effect of *Zingiberofficinale* essential oil on Fusarium verticillioides and fumonisin production. *Food Chemistry. Elsevier* , 3147-3152.

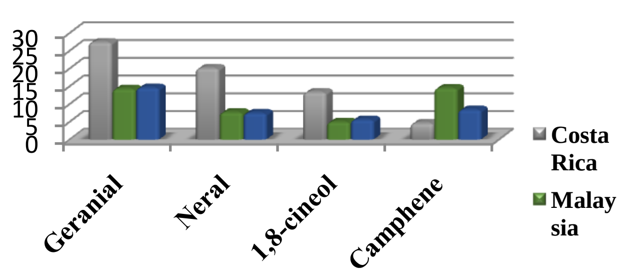

Figure 2: Comparison between the percentages of geranial, neral, 1, 8-cineol and camphene identified in the essential oil of Zingiber officinale grown in Costa Rica, Malaysia and Brazil.

DISCUSSION

Photochemical tests performed to ethanolic extract showed the presence of flavonoids, saponines, tannins, alkaloids and steroids and, or terpenes (Table 1). Other studies also found the presence of these phytochemical groups in the ethanolic extract. These water-soluble compounds from the ZO rhizome have anti-inflammatory activity, possibly by inhibiting cyclooxygenase (COX) (Ali *et al.*, 2008, Nampoothiri *et al.*, 2012, Bhargava *et al.*, 2012). 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 (Bhargava *et al.*, 2012). 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 (Singh *et al.*, 2008).

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, nerol, 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, these compounds are in a lower proportion than those reported in this analysis. Table 3 shows an interesting difference with previous works, on the chemical profile of *ZO* essential oil. Figure 3 shows the percentage comparison between geranial, nerol, 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. In literature reviews, we found that there are many variations in the reported chemical composition of *ZO* essential oil. Yamamoto-Ribeiro *et al.* (2013) reported that the main compound in *ZO* essential oil was the α-zingibereno, representing 23.85% of the essential oil. Papalia *et al.* (2011) also reported that the α-zingibereno was the main compound. Reported percentages differ with those obtained in this research, because the α-zingibereno represents only 1.58% of the characterized essential oil. However Singh *et al.* (2008) reported that the composition of the *ZO* essential oil was geranial (25.9%), α-zingibereno (9.5%), nerol (7.6%) and others (4)(Bhargava *et al.*, 2012). Majolo *et al.* (2014) reported a similar pattern in the chemical composition of the essential oil being geranial (23.9%), nerol (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 (Majolo *et al.*, 2014, Yamamoto-Ribeiro *et al.*, 2013, Padalia *et al.*, 2011, Bellik, 2014)

The importance of finding and characterizing OZ essential oil, lies in standardizing such composition with special features and use it in the preparation of pharmaceutical forms, then measure their biological effect. Essential oils have shown a number of applications in pharmacy and cosmetics, by a synergistic action those results from the activity combination of individual components, rather than the isolation of one component (Sivasothy *et al.*, 2011, Zarai *et al.*, 2011, Majolo *et al.*, 2014). 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 (Padalia *et al.*, 2011, Bellik, 2014, Bassolé and Juliani, 2012).

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 citralby its isomers, Nerol and Geranial, which represent most of their composition, have been associated with these effects (Bellik, 2014, Bassolé and Juliani, 2012, Marrufo *et al.*, 2013, Rocha *et al.*, 2012).

It would have to be performed a comparative study of 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.

CONCLUSIONS

The presence of flavonoids, alkaloids, saponins, tannins and triterpenes in the ethanolic extract was qualitatively determined. The ZO rhizome essential oil shows an interesting potential, as it presents a profile of photochemical composition different from those reported for the same plant, especially the high concentration of nerol, geranial and 1, 8-cineole and also the low concentration of α-zingibereno. 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.

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