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Comparison of the antifungal and antibacterial effect of the essential oil and ethanolic extract of the *Zingiber officinale* Rhizome (Ginger) cultivated in the San Carlos zone, Costa Rica in order to standardize a hydroponic medicinal cultivation of the same

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Abstract
Introduction: Ginger is a medicinal plant native to India. Its potential use in cosmetics, medicines and natural products has been reported, however depending on the conditions of cultivation the medicinal components of the different parts of the plant not only changes in its concentration, but in its composition, this modifies its action medicinal.
Objective: Characterize the chemical composition of the essential oil obtained from the rhizomes of *Zingiber officinale* cultivated in the San Carlos area, Costa Rica, and to compare its antifungal and antibacterial effect in order to standardize future crops of the plant. Hydroponic form and validate its pharmacological effect and, or Cosmetic a posteriori.
Materials and Methods: The rhizomes of the plant were used, the active ingredients were extracted by ethanolic extraction with Soxhlet and steam distillation, the analysis was performed by a qualitative phytochemical profile for the ethanolic extract, and the composition of the essential oil was studied by means of Gas Chromatography coupled to a mass detector (GC-MS). In addition, microbiological tests were performed by plate diffusion for ethanolic extracts and essential oil with strains of bacteria and fungi common in cutaneous pathologies. By means of a scanning electron microscope.
Results and Conclusions: The presence of flavonoids, alkaloids, saponins, tannins and triterpenes in the ethanolic extract was qualitatively determined. In the characterization of the essential oil by GC-MS, geranial (27.42%), neral (20.11%), 1,8-cineol (13.35%), camphene (4.65%) and E-geraniol%). The composition obtained with the composition reported in the literature was compared, obtaining a clear difference with that reported in other studies, which allows to predict an antimicrobial behavior different from most of the traditional essential oils of this rhizome. It was determined that the essential oil inhibited the growth of *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pyogenes*, *Stahylococcus epidermidis*, *Candida albicans*, *Enteroccocus faecalis*, but not against *Pseudomonas aureginosa*, the ethanolic extract showed no antifungal or antibacterial activity, in addition it was demonstrated that the oil Essential has a stronger anti-Candida Albicans effect than inhibition halo at the same concentration of essential oil and that its mechanism of action included destruction of the membrane by pore formation and an osmotic shock.

Keywords: *Zingiber officinale*, essential oil, gas chromatography, natural product, antimicrobial

Introduction
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 [1].
It is used in traditional medicine to treat different diseases such as rheumatism, odynophagia, 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 found, among which are: β-phelandrene, 1,8-cineole, geraniol, citral, α-Zingiberene, β-sesquifelandrene, ar-curcumene, among others [2]. The chemical composition of the essential oil mainly due to the cultivation conditions, the environmental conditions and the extraction method [1].
In addition to volatile compounds, the rhizome of ZO has water-soluble substances, which are responsible for the purgency of ZO [3]. The rhizome compounds of ZO have long been evaluated for their antibacterial properties, therefore these properties are widely known and

reported in many scientific articles [4], however this antimicrobial capacity varies greatly according to the composition of the essential oil.

There are multiple reports on the antimicrobial activity of the rhizome extracts of the ZO, but the species that were used to carry out these investigations are different from the species that is cultivated in Costa Rica. No reports were found on the chemical characterization of the rhizome of ZO grown in Costa Rica, therefore a comparison between the chemical profiles of the different species cannot be made to determine if there is any variance between them. However, it can be deduced that there is possibly a variation in the composition, both qualitative and quantitative, due to the aspects mentioned above. An important variation in the chemical composition can affect the antimicrobial activity, for this reason it is necessary to carry out tests with the rhizome extracts of ZO cultivated in Costa Rica to evaluate its antimicrobial action.

The objective of this research is to compare the antibacterial and antifungal activity of the essential oil of ZO from the area of San Carlos, Costa Rica in order to standardize a hydroponic medicinal crop of the plant.

Material and Methods

Vegetable matter

Rhizomes were used, ZO rhizomes are from the San Carlos area.

Biological matter

The tests for the evaluation of the antimicrobial activity of the essential oil were carried out in the nanotechnology laboratory. The antimicrobial activity against the following strains was evaluated: *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermitis* (ATCC 14990), *Streptococcus pyogenes* (ATCC 25923), *Pseudomona aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922ica) and *Enterococcus faecalis* (ATCC 29212). Culture media: Blood agar, Mannitol agar, Soy tripticase agar, Sabouraud agar, Nutritive agar and Muller Hinton agar.

Ethanolic extraction

1 Kg of ZO rhizome cut in pieces was placed in a Soxhlet extractor. In a 1 L balloon, 500 mL of ethanol was placed. The ethanol was heated to 70 ℃ by means of an electrical template. The extraction was carried out for 15 hours with continuous reflux of the solvent on the sample. The extract was concentrated under reduced pressure using a rotary evaporator at 60 ℃.

Essential oil obtention

5,000 g of ZO rhizome were placed in a 12 L balloon containing 5 L of water. It was considered steam distillation carried out for 12 hours. The essential oil was obtained by means of a trap for essential oils.

Phytochemical screening

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

Determination of Tannins

Ferric chloride solution 4%: Add 4 g of ferric chloride in 100 mL of distilled water. Stir until the ferric chloride is completely dissolved and obtain an orange solution. Store in a jar with a lid to prevent oxidation of the solution.

5 mL of extract was added to a test tube. Then add 3 drops of 4% ferric chloride (FeCl3). If the solution has a dark red color, the test is positive for the presence of tannins in the sample.

Determination of Saponins

4 mL of extract was 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 [5].

Determination of Flavonoids

5 mL of extract was added to a test tube. Then add a piece of magnesium and 3 drops of concentrated hydrochloric acid (HCl). Leave to react for 5 minutes. If solution takes a dark orange color, the test is positive for the presence of flavonoids [5].

Determination of Alkaloids

Wagner reagent: 1.3 g of iodine and 2 g of potassium iodide in 20 mL of water were added in a 100 mL balloon. Then fill with distilled water.

Take 10 mL of extract and add 5 mL of 10% HCl. Boiling for 5 minutes a whitish yellow precipitate is shown as a positive test [5].

Determination of Steroids and Terpenes

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 and if positive for triterpenes when a red, violet or purple color forms [5].

Essential oil characterization

The essential oil analysis was carried out at the Natural Products Research Center (CIPRONA) of the University of Costa Rica, through gas chromatography.

Evaluation of antimicrobial activity

Three different methods were used to evaluate the antimicrobial action of ethanol extract and essential oil. The first was done by directly applying essential oil and ethanolic extract on the surface of previously inoculated petri dishes. The second was agar diffusion described by Kirby Bauer and the third method was visual using the RPMI liquid medium.

Direct application of the extract and essential oil

500 µL of extract and essential oil was applied directly to the surface of solid blood agar media previously inoculated with *S. epidermidis* and *S. pyogenes*, Sabouraud dextrose agar with *C. albicans* and soy tripticase agar with *E. coli* and *P. aeruginosa*. The plates were incubated inverted at 37 ℃ for 48 hours.

Kirby Bauer's method

The pre-inoculars were grown in liquid medium of soy tripticase. For the agar diffusion method, the Mueller Hinton solid medium (MH) was used. The discs were made with Whatman number 1 filter paper. The diameter of the discs is 6 mm. The discs were impregnated with 40 µL of concentrated ethanol extract and 40 µL of ethanol extract and, or pure essential oil. The discs were placed on the surface of the agar with the help of a sterile clamp, gently pressing each disc to ensure complete contact with the agar. Plates were grown at 37 ℃ for 48 hours. After 48 hours of incubation, the inhibition halos were measured in millimeters (mm).

Microbiological test

The antimicrobial activity of the extracts and the essential oil is evaluated by the diffusion method in solid medium. Filter paper discs (6 mm in diameter) are impregnated with different concentrations of extracts and essential oil. The discs are placed on the surface of the culture medium. The impregnated discs are allowed to stand in the laminar flow chamber until the solvent evaporates. The plates were incubated at 37 °C for 24 hours.

Culture medium: Mueller Hinton agar / nutritive agar is used McFarland pattern preparation: to standardize the inoculum density the Mcfarland pattern is used. The McFarland standard is prepared by adding sulfuric acid to an aqueous solution of barium chloride. This reaction will produce a precipitation reaction of suspended barium sulfate, which results in turbidity. Verify the correct density of the standard using a spectrophotometer, whose absorbance at 625 nm is 0.08 to 0.10. Approximate formula for 100 mL of water: sulfuric acid (0.18 M) 99.5 mL, barium chloride (0.048 M) 0.5 mL. A MacFarland 0.5 corresponds to a development of 1.5X108 CFU/mL. Store in the dark.

Inoculum preparation: touch the surface of the container containing the strain with a handle, transfer one or two colonies to a test tube containing a sterile 0.9% saline solution (in the case of demanding bacteria such as Streptococcus spp transfer to a tube with Mueller Hinton broth), adjust the inoculum to a turbidity equivalent to 0.5 on the McFarland scale. Bacterial cultures are adjusted to approximately 105 CFU/mL. Within 15 minutes after the adjustment of the turbidity of the inoculum, a sterile swab is moistened with the microorganism suspension, the excess of the broth is removed by pressing and rotating the swab on the inner walls of the test tube. The swab is passed on the surface of the Mueller Hinton agar culture medium with the stria technique over the entire agar. To obtain a uniform inoculum, a last scan is made over the edge of the Petri dishes and the agar. The inoculum is allowed to dry at room temperature for 3-5 minutes.

Application of the disks: the diameter of the discs should 6 mm. Place the discs on the surface of the agar with the help of a sterile clamp, gently pressing on each disc to ensure complete contact with the surface of the agar. Do not remove any disc once it has contacted the surface of the agar.

Incubation: incubate the plates in an inverted position at 37 °C within 15 minutes after application of the discs.

Reading and interpretation of inhibition halos: measure the diameters of the entire inhibition zone (including the diameter of the disc), using a ruler or Vernier.

Sensitive

Intermediate

Resistant

Scanning micrographs

A colony of the microorganism to be studied was placed in the RPMI culture medium. It was incubated for 48 h at 37 °C. After 48 hours, it was centrifuged for 5 minutes at 3000 rpm, causing the bacteria to settle in the bottom. The supernatant (culture medium) is removed with a pipette and water is added. In an aluminum sample holder, a carbon tape is placed where a drop of sample is placed. Once the sample is placed, wait for the water to evaporate. Once the sample was ready, it was placed in the HITACHI® TM-3000 electronic scanning microscope.

Results

Phytochemical screening

All phytochemical tests performed on the ethanol extract were positive (see table 1).

Essential oil characterization

A total of 39 main compounds were identified. 5 compounds generated a signal on the chromatogram but were not identified. The chemical composition of the essential oil is shown in table 1.

The main compounds were geranial (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 the total of the compounds identified. The identified compounds can be classified as terpenes, ketones, alcohols, aldehydes and esters, with terpenes being the predominant compounds.

Table 1: Qualitive determination of the main phytochemical groups obtain by phytochemical screening to the extracts of the rhizome of *Zingiber officinale* of the area of San Carlos, Costa Rica.

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

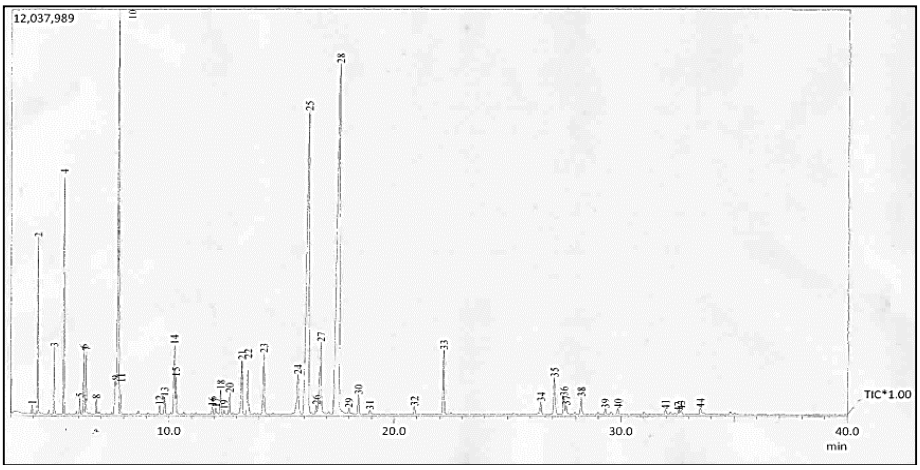
*Results interpretations: (+) positive test, (-) negative test.

Table 2: Chemical composition of *Zingiber officinale* essential oil cultivated in San Carlos zone, Costa Rica.

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-cineole (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

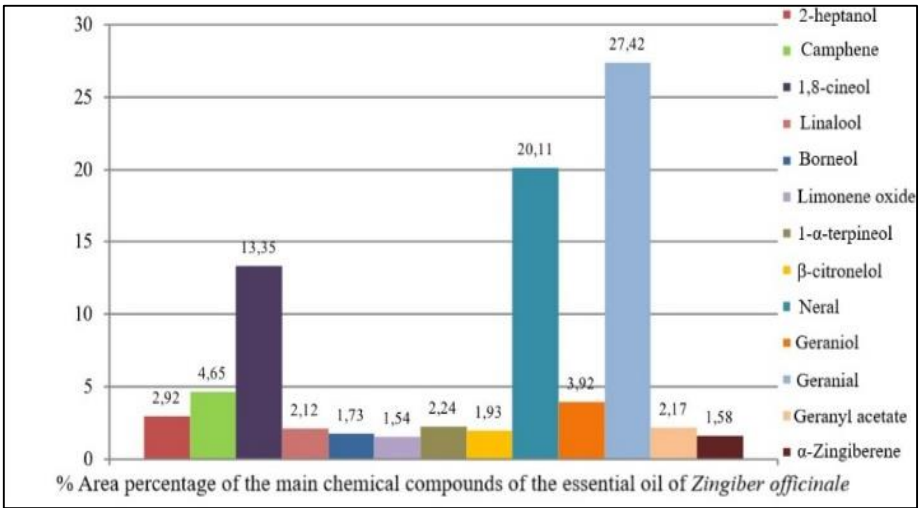
16	11.922	784295	0.24	-
17	12.077	641919	0.20	Camphor
18	12.289	2427627	0.75	Citronella
19	12.445	433679	0.13	camphene hydrate
20	12.684	2087606	0.64	-
21	13.223	5610468	1.73	Borneol
22	13.495	5014020	1.54	Limonene oxide
23	14.213	72888172	2.24	1-α-terpineol
24	15.715	6261331	1.93	β-citronellol
25	16.182	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-sesquifelandrene
39	29.313	656536	0.20	Elemol
40	29.871	666726	0.21	Nerolidol
41	31.972	566089	0.17	Sesquisabinen hydrate
42	32.525	440557	0.14	-
43	32.666	561066	0.17	-
44	33.516	902770	0.28	β-eudesmol

(-): undentified compounds



zone, Costa Rica.

Fig 2: Area percentage of the main chemical compounds of the medicinal essential oil of *Zingiber officinale* analyzed according to data in table 2.



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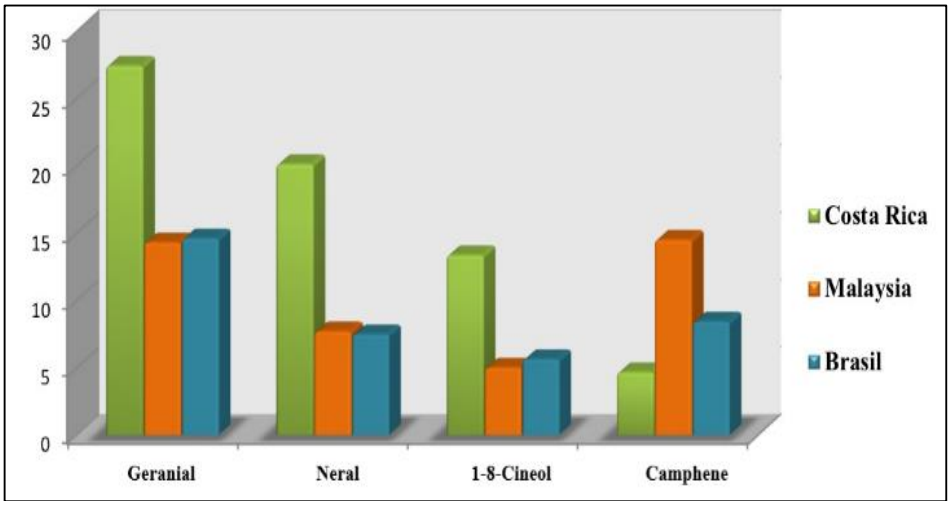

Fig 3: Comparison between the percentages of geranial, neral, 1,8-cineole and camphene identified in the essential oils of *Zingiber officinale* grown in Costa Rica, Malaysia and Brazil. Source: Table 3.

A comparative table of the essential oil composition obtained from other sources reported in the literature is described below.

Table 3: Comparison in the chemical composition of the essential oil of *Zingiber officinale* from different countries

Compound	*Zingiber officinale* (Costa Rica) % Area	*Zingiber officinale* (Malaysia) % Area	*Zingiber officinale* (Brasil) % Area
Camphene	4.65	14.5	8.43
β-Myrcene	1.30	2.0	0.54
α-felandrene	-	-	1.43
β-felandrene	-	-	7.73
1,8-cineole	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-sesquifelandrene	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	-
Citronelal	-	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	-	-
Linalil acetate	-	-	-
Bornyl acetate	0.17	1.4	-
2-heptanol	2.92	0.1	-
Sources: (6,7).			

Microbiological assays

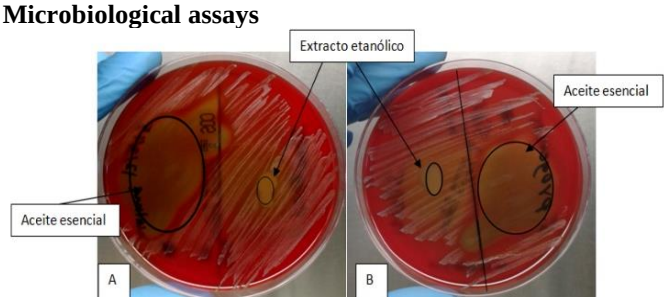

Fig 4: Preliminary evaluation of the antimicrobial activity of essential oil and ethanolic extract. 60 µL of essential oil and ethanolic extract was applied directly to the plate surface. (A) *S. epidermidis* (B) *S. pyogenes*. The ethanolic extract did not inhibit growth.

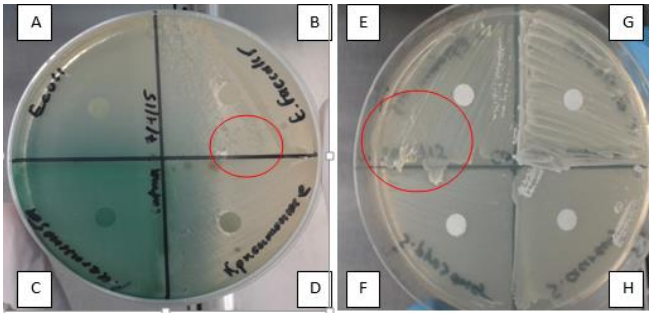

Fig 5: Evaluation of the antimicrobial activity of the ethanolic extract of the rhizome of *Z. officinale.* (A*) E. coli,* (B) *E. faecalis,* (C) *P. aeruginosa,* (D) *K. pnuemoniae,* (E) *S.epidermidis,* (F) *S. pyogenes,* (G) *C. albicans,* (H) *S. aureus.* The ethanolic extract showed no antimicrobial activity.

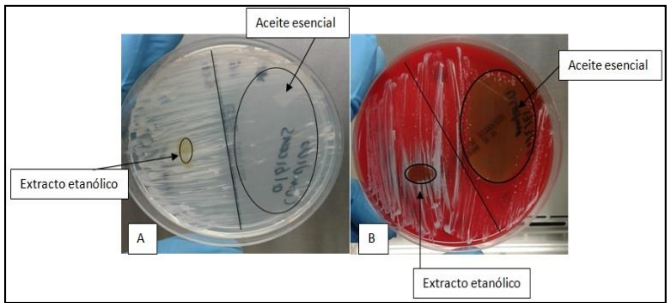

Fig 6: Preliminary evaluation of the antimicrobial activity of essential oil and ethanolic extract. (A) *C. albicans*. (B) *S. aureus*. The ethanolic extract showed no antimicrobial activity.

Table 4: Inhibition halos

Substance	Microorganism	Inhibition halo (mm)	Control
Ethanolic extract	*Staphylococcus aureus*	-	-
	Escherichia coli	-	-
	Streptococcus pyogenes	-	-
	Stahylococcus epidermidis	-	-
	Pseudomona aureginosa	-	-
	Candida albicans	-	-
Essential oil	*Staphylococcus aureus*	17	-
	Escherichia coli	14	-
	Streptococcus pyogenes	19	-
	Stahylococcus epidermidis	10	-
	Pseudomona aureginosa	-	-
	Candida albicans	30	-
	Enteroccocus faecalis	10	

Scanning micrographs

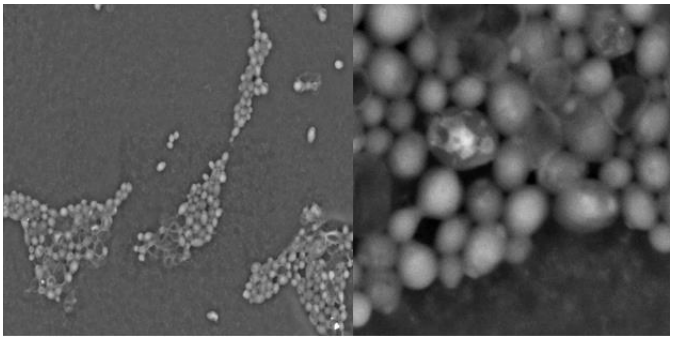

Fig 7: Scanning electron micrograph of C. albicans (control). The increase is (x4.0k) times that of the human eye.

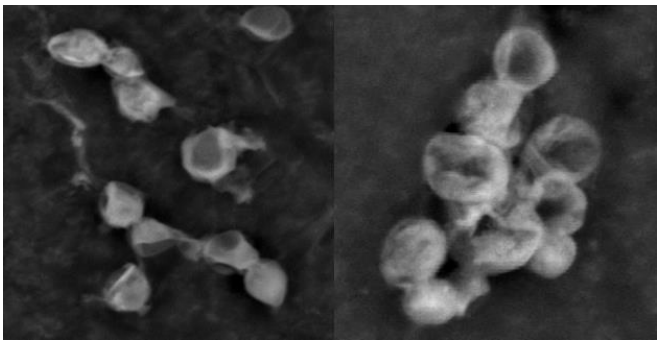

Fig 8: Scanning electron micrograph of *C. albicans* that was treated with 40 µL of essential oil. The increase of (x4.0k) times to that of the human eye. The structural damage caused by the essential oil of *Z. officinale* can be observed.

Discussion

The phytochemical tests performed on the ethanol extract show the presence of flavonoids, saponins, tannins, alkaloids and steroids and, or terpenes (table 1). Other studies have also found the presence of the same phytochemical groups in the ethanolic extract. These water-soluble compounds of the ZO rhizome have anti-inflammatory activity, possibly by inhibition of cyclooxygeneses (COX) [2, 3, 5].

It has also been shown that they are very effective in the treatment of chemotherapy-induced vomiting. However, in very few studies it is reported that ethanol extracts have antimicrobial activity [5].

The high percentages of neral and geranial possibly contribute to the aroma of this essential oil of ZO being similar to the aroma of the essential oil of Cympogon citratus (lemon grass), where neral and geranial are also the main compounds [8, 9].

The chemical composition of essential oils in general, varies according to the origin of the plant material used, the growing conditions, the environmental conditions and the method of obtaining, among other factors.

The main compounds of the essential oil of ZO cultivated in Costa Rica are geranial, neral, 1,8-cineole and camphene, representing more than 50% of the essential oil composition.

Although these compounds have also been reported in others, these compounds are found in a smaller proportion than reported in the present analysis. Table 3 shows an interesting difference with other studies in the literature on the chemical profile of the essential oil of ZO. Figure 3 shows the percentage comparison between geranial, neral, 1,8-cineole and camphene; of essential oils of ZO grown in Costa Rica, Malaysia and Brazil. In this comparison it can be verified that the chemical profile of the essential oil can vary according to the environmental conditions and the conditions of the crop, among others.

In the literature reviews it was found that there are many variations in the reported chemical composition of the essential oil of ZO. Yamamoto-Ribeiro *et al*. [7] reported that the main compound of the essential oil of ZO was α-Zingiberene, representing 23.85% of the essential oil. Padalia *et al*. [9] also reported that α-Zingiberene was the main compound. The percentages reported differ with those obtained in this investigation, because α-Zingiberene represents only 1.58% of the essential oil characterized. However, Singh *et al*. [8] reported that the composition of the essential oil of ZO was geranial (25.9%), α-Zingiberene (9.5%), neral (7.6%) among others [4]. Majolo *et al*. [10] reported a similar pattern in the chemical composition of the essential oil, with geranial (23.9%), neral (17.2%), 1.8-cineole (16.0%) and camphene (11.4%) being the majority compounds; having an order similar to the percentage ratio of the main compounds [7, 9-11].

The importance of finding and characterizing the essential oil of ZO lies in standardizing said composition with special characteristics and using it in the preparation of pharmaceutical forms, and then measuring its biological effect. Essential oils have shown a series of applications in Pharmacy, and Cosmetics, by means of a synergistic action that is to say combinations of individual components, and not so much with the isolation of a single component [6, 10, 12].

Among the main fields of interest for the application of essential oils are its preservative, antibacterial, antifungal, anti-inflammatory, expectorant, relaxing, analgesic and antioxidant effects [9, 11, 13, 14].

Due to the composition of the essence oil found in the ZO grown in San Carlos, it could be predicted that this essential oil has an important antimicrobial and relaxing action, since the citral in its Neral and Geranial isomers, which represent most of its composition, have been related to these effects [11, 13, 15, 16].

Comparative studies of the chemical profile of the essential oil of ZO grown in different regions of Costa Rica must be carried out to determine if there are variations in the composition of the essential oil within the same country.

The results of the tests are presented in table 4, where it is presented that pure essential oil inhibited all strains, except P. aeruginosa. The ethanolic extract in none of the tests performed showed antimicrobial action. There are many

reports about the in vitro antimicrobial activity of essential oil and ethanol extracts.

The reports on the antimicrobial activity of the ethanol extract are very controversial, since in some studies it does show moderate antimicrobial activity, while in others it is reported that it does not show activity, as is the case in this study. It has also been reported that methanol and acetonic extracts show greater antimicrobial activity than ethanol extracts. Research should be carried out using methanol and acetonic extracts of ZO grown in Costa Rica.

The antimicrobial activity reported in other studies cannot be compared with the results obtained in this research, because the chemical composition of the rhizome used in these studies is very different from the chemical composition of the ZO rhizome that is grown in Costa Rica, or At least that can be deduced by comparing the results of GC-MS that was performed in some studies when determining the antimicrobial activity of the essential oil (Table 3). If we rely on the results of the phytochemical screening performed on the ethanolic extract, no significant difference is found with the results of other sieves performed on the ethanol extracts in other studies, however this test is only qualitative. In addition, extraction methods and tests for the evaluation of antimicrobial activity are different.

The high content of citral and other oxygenated compounds causes the essential oil of ZO to possess a potent antimicrobial activity. The essential oil showed greater antimicrobial activity against C. albicans [17].

ZO essential oil has a broad spectrum for the required purposes, since it inhibited the main microorganisms that cause skin infections. Research should continue to evaluate the antifungal activity of the essential oil against the main dermatophyte fungi. The micrographs taken at C. albicans show the structural damage caused by the essential oil when treated with 40 µL, figure 8, in figure 7, the normal morphology of C. albicans is shown, which is spherical in shape. You can also see the moment when C. albicans is in binary fission. The mother C. albicans is dividing and transmitting all her genetic material to the daughter C. albicans. Figure 8 shows C. albicans when treated with 40 µL. It can be seen that the yeast totally loses its spherical shape and looks like "passes". It can also be seen that cell membrane disruption. Which indicates a bactericidal osmotic mechanism since it destroys the structure of the cell membrane making pores and therefore losing the cytoplasmic content.

In many studies the antimicrobial action of essential oils has been demonstrated, however, the mechanism of action is not yet fully understood. Generally, the antimicrobial activity is determined by the composition of the AE and by the concentration of each of the compounds. The antibacterial action of AE is due to many different mechanisms, but the mechanism that is most documented is the disruption of the cell membrane by lipophilic compounds present in EC. Terpenes and terpenoids are lipophilic compounds that cross the cell wall and the cytoplasmic membrane, causing a disruption in their structure, altering their permeability. Permeabilization usually generates a cascade of reactions, among which we can mention: the loss of ions, the reduction of membrane potential and the collapse of proton pumps [18].

In vitro tests it has been shown that terpenes have no antimicrobial activity when used in an isolated manner from each other [19].

Conclusions

The presence of flavonoids, alkaloids, saponins, tannins and triterpenes in the ethanolic extract was determined qualitatively.

The rhizome essential oil of ZO shows an interesting potential since it has a phytochemical composition profile, different from those reported for the same plant especially the high concentration of neral, geranial and 1,8-cineole, in addition to the low concentration of α -zingiberene, so it can enhance and standardize the culture conditions to produce a greater amount of essential oil with this composition, special characteristics to be used in phytopharmaceutical compositions, especially dermo-pharmaceuticals.

It was shown that the essential oil of the ZO rhizome shows antimicrobial activity against Staphylococcus aureus Escherichia coli, Streptococcus pyogenes, Staphylococcus epidermidis, Candida albicans, Enterococcus faecalis, but not against Pseudomona aeruginosa, the ethanolic extract showed neither antibacterial activity nor demonstrated antibacterial activity that the essential oil has a greater anti Candida Albicans effect greater inhibition halo at the same concentration of essential oil and that its mechanism of action included the destruction of the membrane through the formation of pores and an osmotic shock.

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