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Formulation and Evaluation of an Antifungal Ointment Containing *Zingiber officinale* Roscoe (Zingiberaceae) Essential Oil as Active Principle

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Abstract

The increase incidence of fungal infections, the resistance of fungal strains to existing drugs and the toxicity assimilated to long term usage of antifungal drugs as prompted the search of new alternative from natural source. In this study we have evaluated the antifungal properties of the essential oil of the rhizome of *Z. officinale,* a plant known for its various biological properties and used in food industry as spice. An antifungal ointment with *Z. officinale* essential oil as active principle was also formulated and its antifungal properties evaluated. GC/MS analysis identified Zingiberene (22.36%), 1.8-cineole (15.54%), geranial (11.91%), camphene (11.09%) and geraniol (8.75%) as the main constituents of *Z. officinale* essential oil obtained by hydrodistillation. The oil showed a significant antifungal activity against clinical isolate of *Candida krusei* with MIC 800 ppm by microdilution method. The formulated ointment containing 10 % glycerol, 60 % vaseline, 8 % hydrosol, 20% lanolin and 2% *Zingiber officinale* essential oil (Formule E1) was found to follow pharmaceutical standards according to European Pharmacopeia and also showed good antifungal properties.

Keywords: Essential oil; Zingiber officinale; antifungal; ointments

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1 Introduction

Fungal infections constitute a real public health problem nowadays due to the increase in prevalence. Newborns, the elderly, pregnant women, immunocompromised people, people with diabetes and people with cancer are the more prone to infections [1]. In Cameroon, due to the precarious situation of a large part of the population, many neighborhoods are hotbeds to fungal infections. Despite the antifungal therapeutic arsenal available today, the treatment of mycoses is proving difficult due to the emergence of strains resistant to the usual antimycotics, their expensive cost, and their toxicity [2,3]. The high incidence of candidiasis combined with limited treatments and poor health coverage underscore the urgent need to search for alternative treatment pathways. According to the WHO, medicinal plants represent an alternative avenue for the search for new antifungal drugs. They have proven to be credible sources of bioactive compounds against many infectious diseases. Therefore, the exploration of plants used traditionally for the treatment of candidiasis could be an appropriate approach for the search for new treatments.

Zingiber officinale Roscoe, commonly called ginger, is a plant native to India widely used as spice and also as medicine for its various properties including anthelmintic, antifungal, anti-inflammatory and antioxidant [4]. It is a perennial herbaceous tropical plant which can grow up to 3 m in height with light green leaves measuring 20 cm in length and 2 cm wide. The plants most used part is the rhizome which is an underground stem in the form of an elongated cylindrical axis thickened and horizontal. It consists of branched globose tubers. It measures on average 10 cm in length, up to 2 cm in width and 1.5 cm in thickness [5]. *Z. officinale* is traditionally used for the treatment of digestive and gastric disorders, cough, migraine, nausea, vomiting, as a digestive stimulant and in enterocolic spasms [5]. Previous studies have demonstrated a good antibacterial and antifungal properties of *Z. officinale* against a range of bacteria and fungi strains [6]. *Z. officinale* rhizome is largely used in food industry as spice. No toxicity has been demonstrated in human for consumption of up to 2 g per day. However, some persons with very sensitive digestive tract may develop gastro-intestinal problems after consumption of ginger powder [7]. The present study deals with the extraction and evaluation of *Z. officinale* essential oils and formulation of an antifungal ointment following pharmaceutical standards. The harvest was done in the city of Loum, Cameroon, where the soil is very fertile, with a shallow water table and the climate is favorable for the cultivation of *Z.officinale* [5].

2 Materials and Methods

2.1 Plant material

The rhizomes of *Zingiber officinale* were harvested in Loum (Littoral, Cameroon) on January 2021 in strict compliance with the conservation of biodiversity. The plant was identified at the Cameroon National Herbarium where the Voucher specimen 43146/HNC was deposited.

2.2 Essential oils Extraction

Fresh rhizomes of *Z. officinale* (4.2 Kg) cut into small pieces were subjected to hydrodistillation for 4 h using a Clevenger-type apparatus. The obtained oils were dried using anhydrous sodium sulfate and weighed [8]. The obtained dried oil (7.88 g) was stored at 4 °C until further usage. The yield extraction was calculated as % (w/w) using formula in equation 1.

$$\text{Yield} = \frac{\text{Mass of essential oil (g)}}{\text{Mass of vegetable (g)}} \times 100 \quad \text{(Equation 1)}$$

2.3 Gas chromatography analysis

Quantitative and qualitative data of the essential oils were obtained by GC-FID and GC-MS. The GC-FID analysis was carried out with a Varian CP 3380 type chromatograph equipped

with a methylsilicone (DB5) column and FID. The oven temperature was set at 50°C with a gradient of 5°C/min up to 200°C, injector and detector temperature were set at 200°C. The injection volume was 0.5 µL of a pentane solution of essential oil (10%) and nitrogen (1 mL/min) was used as carrier gas. The relative concentration was calculated using the software STARWS by integrating measurements of peak areas and assimilating the obtained results to the percentages of the various constituents.

GC/MS analysis was carried out with Hewlett-Packard 5970 series apparatus equipped with a mass spectrometer and a molten silica DB1column (30 m × 0.3 mm i.d.). Helium was used as carrier gas with flow rate 0.6 mL/min. The mass spectrometer operating conditions were: ionization energy 70 eV, injection volume 0.1 µL of the essential oil prepared at 10 % in purified hexane, Temperature programming 70-200 ° C at 10 ° C / min, injection temperature 220 ° C,

carrier gas flow rate (helium): 0.6 mL/min. As the furnace temperature increased (10°C/min), the integrator-logger recorded the different peaks corresponding to each eluted compound. Each peak was characterized by its retention time and occupied area. The identification of the compounds was carried out by calculating linear retention indices and using the LIPIDS library with retention index. This database of lipid mass spectra contains 430 GC mass spectra recorded from a pure standard and classified into 11 lipid classes. The database provides significant support for maximum allocation in complex mixtures [4].

2.4 Antibacterial activity

The in vitro antibacterial power of the essential oil was tested against clinical isolates of *Candida albicans, Candida tropicalis, Candida glabrata, Candida krusei and Candida parapsilosis* obtained from the culture collection of the Laboratory for Phytobiochemistry and Medicinal Plants Studies of the University of Yaoundé 1, Cameroon. The liquid microdilution method was used for the determination of the Minimum Inhibitory Concentrations (MIC) and Bactericidal Concentrations (MBC) [9].

The tests were carried out in triplicate in sterile 96 well microplates. 150 µL of Sabouraud Dextrose Broth (SDB) was introduced into the wells of the first line and 100 µL into the rest of the wells. Then, 50 µL of essential oil concentrated at 100 mg/mL diluted in dimethylsulfoxide were introduced into the first line wells of the microplates followed by a series of 5 geometric dilutions of order 2. Then 100 µL of the fungal suspension was added into all wells except those of the sterility control. Fluconazole was used as a positive control. The negative control consisted of a fungal suspension and the culture medium. The sterility control consisted only of the culture medium. Concentrations of essential oil and fluconazole in wells ranged from 12.5 mg/mL to 0.390 mg/mL and from 6.1 µg/mL to 0.19 µg/mL, respectively. The plates were covered and incubated at 37 ± 0.1 °C for 48 hours. At the end of the incubation time, the smallest concentration that showed no visible growth was considered as the MIC of the essential oil. For MBC, 25 µL was taken from inhibitory wells and introduced into 175 µL of SDB contained in another microplate. After another 72 hours of incubation at 37 ± 0.1 °C, the highest concentration that showed no visible growth was considered as the MBC of the essential oil. The MBC/MIC ratio was calculated to determine the bactericidal power of the essential oil.

2.5 Preparation of the antifungal ointment

The antifungal ointment was prepared according to the standards of good manufacturing practice of phyto-medicines described by [10]. The formulation was made by mixing an aqueous phase (hydrosol containing sodium benzoate 0.3 %) with an oily phase (vaselin and lanolin) and then adding the active principle, *Z. officinale* essential oil, to the obtained mixture. Different compositions of the ingredients were utilized until obtaining the best formula. The different formulas (E1-E4) prepared including the proportion of each ingredient (in percentage) are showed in Table 1.

The ointment was prepared by incorporating in small fraction the aqueous phase into the oily phase followed by mechanical agitation until complete homogenization at room temperature. The essential oil was then introduced at room

temperature (25 ± 1 °C) followed by a continuous homogenization. The optimization of the formulation was done by varying the proportions of the components namely Lanolin, hydrosol and glycerol as showed in Table 1.

Table 1: Content in percentage of the different formulas prepared

Formula	Lanolin	Glycerol	Vaseline	Hydrosol	*Z. officinale* oil
E1	20	10	60	8	2
E2	10	15	60	3	2
E3	10	20	50	8	2
E4	15	20	50	13	2

2.6 Physicochemical analysis of the ointment

2.6.1 Homogeneity test

The homogeneity tests were carried out by evaluating both the macroscopic aspect (by a simple visual observation of the absence of aggregates and the correct distribution of powders) and microscopic aspect looking at the dispersion and particle size observed with optical microscope reference: B-510PH. The pH of the formula was measured using pH paper Wattman [11].

2.6.2 Thermal stability test

This test was done in order to determine the validity period expiration (2 years) and storage measures to be taken to conserve the ointment [12]. Samples of the formulated ointment were packaged in plastic jar inside the oven at two different temperatures, 45 ± 1 ºC and 50 ± 1 ºC. After 15 min, the samples were removed from the oven and centrifuged successively at 500 rpm, 1000 rpm, 2000 rpm, 3000 rpm and 4000 rpm. Small amount of the ointment were collected into test tube after each centrifugation period and observed at regular intervals to check any phase separations. The best formula was then chosen, packaged in pots of 20 g and stored at ambient temperature until further use.

2.6.3 Microbiological control

The test was carried out to check the presence or absence of bacteria such as *Escherichia coli, Pseudomonas aeruginosa, Salmonella Spp.* and *Staphylococcus aureus*. The deep seeding method was used. Different diluted solutions in peptone water at 1/10, 1/100, 1/1000 and 1/10000 (D1-D4). These solutions were prepared under laminar flow hood from solution A made of 10 g of ointment diluted in 90 mL of Trypticase soy broth. 1 mL of each dilution was taken and seeded aseptically in liquefied agar plate count (PC) or in Sabouraud Dextrose agar (SD) at 45 ± 1 °C for the detection of Colony-forming units germs and fungi, respectively. Then, petri dishes containing SD agar and those containing PC agar were incubated for 5 days at 25 ± 1 °C and 30 ± 1 °C, respectively. The test was carried out in triplicate. Only petri dishes with a load of less than 300 colonies were considered for the assay. The arithmetic means of the counts allowed the calculation of the number of colony-forming units per gram or per mL [13].

2.6.4 Search for pathogenic germs

The potential pathogenic germs targeted were *Escherichia coli, Pseudomonas aeruginosa, Salmonella Spp.* and *Staphyloccocus aureus*. A solution B was prepared from solution A at 0.1 mg/mL in the soy trypticase broth and both solutions were incubated at 30 ± 1 °C for 24 hours.

2.6.5 Case *Salmonella Spp*.

After 24 hours incubation, solution A (0.1 mL) were introduced into 10 mL of cystine selenite broth followed by another incubation of 24 hours at 30 ± 1 °C. After the incubation period, 0.1 mL of the obtained solution was seeded on Xylose Lysine Deoxycholate agar (XLD) and incubated under the same conditions as described above. The presence of red colonies or small pinkish transparent colonies marked the presence of *Salmonella Spp*.

2.6.6 Case of *Escherichia coli*

After 24 hours incubation, solution B (1mL) were introduced into 100 mL of MacConkey broth followed by another incubation at 42 ± 1 °C for 48 hours. After the incubation period, 0.01 mL of the obtained solution was seeded in MacConkey agar and incubated at 30 ± 1 °C for 48 hours. The presence of red colonies with areas of reddish

precipitation marked the presence of *Escherichia coli*.

2.6.7 Case of *Pseudomonas aeruginosa* and *Staphyloccocus aureus*

After 24 hours incubation, solution B was well homogenized and subcultures were made on cetrimide agar and mannitol salt Agar, respectively, for *Pseudomonas aeroginosa* and *Staphyloccocus aureus* strains, respectively. The cultures were then incubated at 30 ± 1 °C for 24 hours. The presence of colonies in cetrimide agar marked the presence of *Pseudomonas aeroginosa* strains in the ointment while the presence of golden yellow colonies in mannitol salt Agar showed the presence of *Staphylococcus aureus.*

2.7 Evaluation of the antifungal power of the ointment

The antifungal activity of the ointment was carried out according to the protocol described by Akakpo-akue et al. [14] with slight modifications. Strains used were *Candida albicans, Candida tropicalis, Candida glabrata, Candida kruse* and *Candida parapsilosis*. The ointment was prepared in distilled water (2% w/v) and a series of dilution of order 2 was made to obtain concentrations ranging from 10 mg/ mL to 0.156 mg/mL. Subsequently, 1 mL of inoculum prepared at $1.5x10^8$ cell/mL from the 5-day-old strains was introduced into each dilution. The control consisted of distilled water and ointment. The obtained preparation was incubated at 30 ± 1 °C for 5 days and at the end of the incubation period, 10 µL were taken and seeded in streak of 5 cm on Sabouraud agar supplemented with chloramphenicol. An ointment containing 2 % clotrimazole was used as positive control.

2.8 Statistical analysis

All experiments were carried out in triplicate. Data were expressed as means ± SEM (standard error of mean). Data were analyzed using Epidata Analysis software version 2.2.2.186 (Windows, Denmark) and Excel software (Microsoft, Wahsington-USA).

3 Results and Discussion

3.1 Essential oil composition and Yield

Hydrodistillation of the fresh rhizomes of *Z. officinale* afforded a yellow spicy liquid oil with a yield of 0.19 % having a density of 0.88. GC-FID (Figure 1) and GC/MS analysis of the oil allowed the determination of the oil chemical composition. The identification of the compounds was based on the comparison of their Kowat indices and mass spectra with literature and databases. This allowed the identification of 35 compounds (Table 2) with major compounds identified as zingiberene (1, 22.36 %), 1,8-cineol (2, 15.54 %), geranial (3, 11.91 %), camphene (4, 11.09 %) and geraniol (5, 8.75 %). Their structures are depicted in Figure 2.

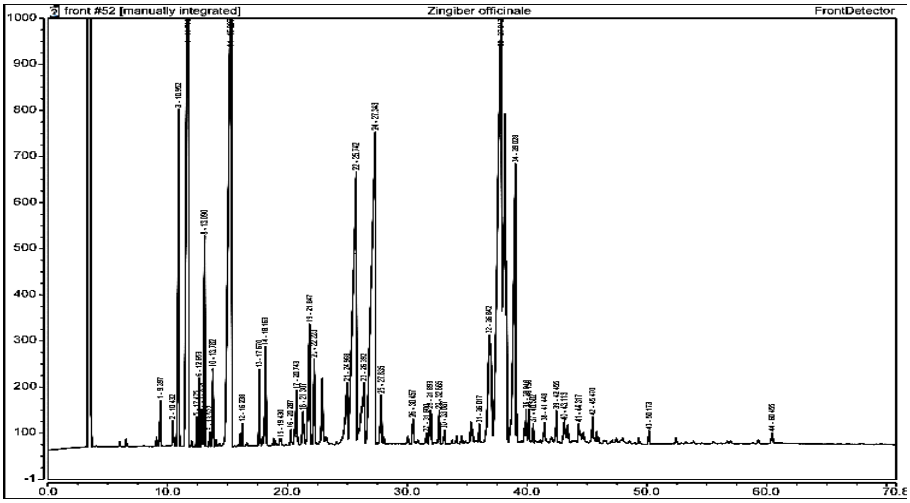

Figure 1. Chromatogram of essential oil of *Zingiber officinale*

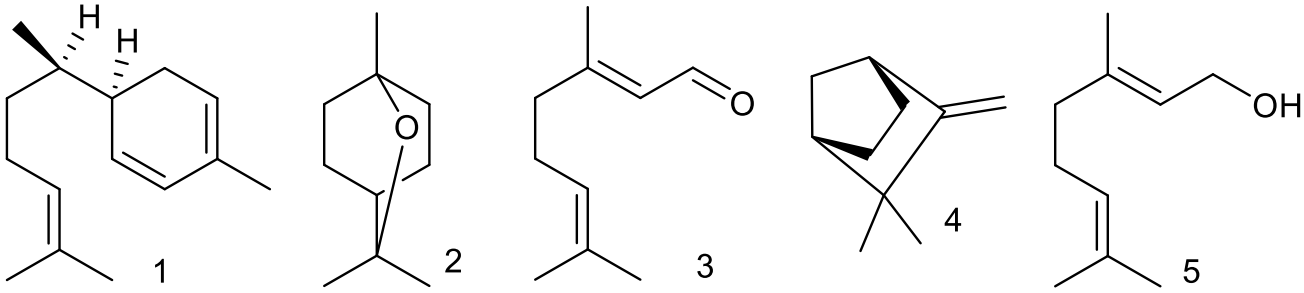

Figure 2 Chemical structure of some major compounds identified in the essential oil of *Z. officinale* rhyzomes (1) zingiberene, (2) 1,8-cineol, (3) geranial, (4) camphene and (5) geraniol

Table 2 Chemical composition of essential oil of *Zingiber officinale* rhizomes

N^0	Compound name and class	Kovat indice	Content (%)	TR (mn)
	Monoterpenes		59.99	
	Hydrocarbon monoterpenes		16.85	
1	*α*-thujene	921	0.25	6.76
2	*α*-pinene	934	3.81	6.89
3	Camphene	953	11.09	8.97
4	Sabinene	971	0.19	9.63
5	*β*-pinene	976	0.42	9.84
6	Myrcene	985	0.35	9.95
7	*α*-phellandrene	1004	0.61	10.32
8	*Y*-terpinene	1056	0.13	14.30
9	Isobornyl acetate	1262	/	20.34
	Oxygenated monoterpenes		43.14 %	
10	1,8-Cineol	1027	15.54	15.35
11	Linalool	1030	1.11	17.53
12	*Trans*-sabinene hydrate	1124	0.11	18.33
13	Camphor	1141	0.13	18.86
14	Camphene hydrate	1150	0.59	19.12
15	Isoborneol	1162	0.44	19.73
16	Borneol	1173	1.71	21.23
17	Terpineol-4-ol	1181	0.80	22.52
18	α-citronellol	1236	0.97	25.73
19	Geraniol	1252	8.75	28.35
20	Geranial	1284	11.91	28.89
21	Neryl acetate	1351	0.33	31.42
22	Geranyl butyrate	1530	0.44	40.92
23	Zingerone	1622	0.31	51.46
	Sesquiterpenes		40.73	
	Hydrocarbon sesquiterpenes		37.90	
24	*Z*-Caryophyllene	1401	0.29	32.53
25	Eudesma-3,7(11)-diene	1451	5.97	34.43
26	Ar-Curcumene	1484	0.12	34.85
27	*α*-muurolene	1491	3.19	36.9
28	Zingiberene	1498	22.36	38.75
29	*Trans,trans-α*-Farnesene	1514	5.97	40.12
	Oxygenated sesquiterpenes		2.83	
30	Cubenol	1562	0.40	40.8
31	Caryohyllene oxide	1577	0.90	43.54
32	*β*- Guaicol	1598	0.20	48.81
33	*Epi-α*-cadinol	1638	0.58	52.34
34	*β*-Eudesmol	1668	0.48	60.95
35	Z-Farnesol	1696	0.27	61.32
	Aliphatic and linear compounds		0.35	
36	6-Methyl-5-hepten-2-one	981	0.35	62.65

Table 3 Antifungal activity of the essential oil of *Z. officinale*

Fungi	*Z. officinale* essential oil			Amphotericin B[a)]	
	MIC[b)]	MFC	MFC/MIC	MIC	MFC
C. albicans	6400±0.11	6400±0.41	1	0,5±0,2	0,5±0,4
C. tropicalis	6400± 1.63	6400±0.81	1	1.00±0.00	1.00±0,05
C. glabrata	6400± 0.81	6400±0.63	1	0,25±0,21	0,25±0,14
C. krusei	800±0.33	800±0.42	1	0,125±0,33	0,125±0,3
C. parapsilosis	3200± 0.92	3200±1.67	1	0,5±0,23	0,5±0,12

[a)] amphotericin B was used as reference antibiotic. [b)] MIC (Minimum inhibitory concentration) and MFC (Minimum fungicidal concentration) are given in ppm.

3.2 Antifungal activity of Z. officinale essential oil

The results of the antifungal activity are shown in Table 3. It appeared that the MICs and MFCs of *Z. officinale* essential oil varied from 800 ppm to 6400 ppm. *Candida krusei* clinical isolates were the most susceptible (MIC = 800 ppm) and the most resistant were *C. albicans, C. tropicalis* and *C. glabrata* clinical isolates (MIC = 6400 ppm). The MFC/MIC ratios are identical for each strain and equal to 1 showing no significant difference with Amphotericin B (MFC/MIC = 1), suggesting the fungicidal nature of the essential oil.

3.3 Formulation and evaluation of the stability of the antifungal ointment

The ointment was prepared by incorporating the aqueous phase with the oily phase and then adding the active principle, *Z. officinale* essential oil. Four formulas were prepared with different contents of each ingredient as showed in Table 1 to afford ointments having a white coloration, with a pleasant smell inspired by the essential oil of *Z. officinale* and a creamy appearance without clumps. The pH of the obtained preparations varied from 5.6 to 6.

In the four different formulas prepared, the oily phase represents the largest proportion (65 to 80%) compared to the aqueous phase which represents the smallest proportion (20 to 35%). After thermal stability assay at 45 ± 1 ºC and 50 ± 1 ºC and centrifugation at different rate (500, 1000, 2000, 3000 and 4000 rpm), only formula E1 showed no visible separation of the two phases therefore confirming the good stability and homogenization of the ointment. Thus, Formula E1 was the best formula and was chosen for further analyses.

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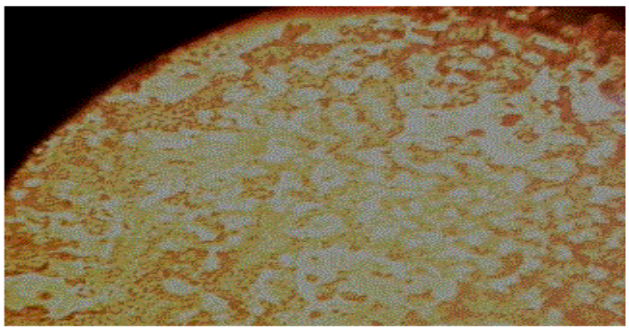

Figure 3 Microscopic structure of the formulated ointment, formula E1 observed with optical microscope reference: B-510PH

3.5 Microbiological quality of the ointment

Microbiological evaluation was carried out to search for potential pathogenic germs in the ointment. The Table 4 below showed results expressed in number of colonies found in the ointment solution after culture.

Table 4 Aerobic germ count on Plate Count Agar (PCA)

Dilutions	Number of colonies
D1	34.00±2,82
D2	1.00±0,00
D3	1.00±0,70
D4	0.00±0,00

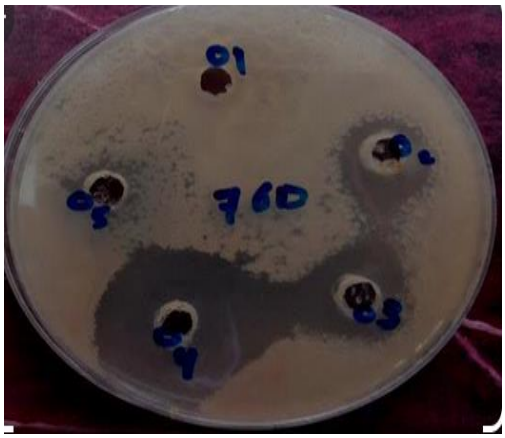

Figure 4 Illustration of inhibition of *Candida* species clinical isolates in presence of the ointment

It appears from Table 4 that in dilution D1 ($1/10^{th}$ of solution A), 34 potential germs were counted or 340 total aerobic germs in application of the factor dilution. In Sabouraud Dextrose Agar, a suitable environment for fungi growth, no colonies were observed. Since the total number of viable aerobic germs for herbal drugs is 10^7 bacteria per g [15], we can conclude that the ointment meets the standards. Moreover, no colonies were observed in cetrimide, mannitol and Mac conkey agars. This suggests the total absence of species of *Pseudomonas aeruginosa, Staphyloccoccus aureus* and *Escherichia coli* in the prepared ointment. Thus, the microbiological quality of *Z. officinale* essential oil ointment is acceptable according to the criteria of the European Pharmacopoeia [16].

3.6 Antifungal power of the ointment

The antifungal activity of the formulated ointment was evaluated on five clinical isolates of *Candida Spp. C. albicans*, *C. tropicalis*, *C. glabrata, C. krusei* and *C. parapsilosis*. It appears from this result that the fungal load decreases as the concentration of essential oil increases compared to the positive control which was amphotericin B and the negative control. Figure 4 showed the aspect of the petri dishes after the incubation of the *Candida* clinical isolates with the ointment.

The present work was carried out with the aim to develop a potential ointment formula with antifungal activity to fight against *Candida* infections using the essential oil of the fresh rhizomes of *Z. offcinale* as active principle. Hydrodistillation of the fresh rhizomes of *Z. officinale* afforded a yellow spicy oil with a yield of 0.19 %. The yield is close to that of Kamga *et al.* (2016) [17] after hydrosdistillation of fresh rhizomes cut into small pieces but very low compared to that of Andria *et al.* (2010) [18] who obtained a yield of 0.49 % after hydrodistillation of grated fresh rhizomes of *Z. officinale*. Therefore, it can be concluded that the extraction is best while the surface of penetration of plant by water is small allowing a total liberation of volatile constituents.

The identification of the oil composition by GC/MS allowed to characterized 35 compounds mainly terpenes represented by oxygenated monoterpenes (43.14 %) dominated by 1.8-Cineol (15.54 %) and hydrocarbon sesquiterpenes (37.90 %) dominated by zingiberene (22.36 %). A very small amount of aliphatic and linear compounds mainly 6-Methyl-5-hepten-2-one (0.35 %) was also identified. The oil obtained from rhizomes harvested in Cameroon showed an abundance of monoterpenes with camphene (14.5%), geranial (14.3%), and geranyl acetate (13.7%) as major constituents while from rhizomes harvested in Soa, Cameroon, zingiberene (23.9%), β -bisabolene (11.4%) and β-sesquiphellandrene (10.9%) were identified as the major compounds and a low content in 1,8-

cineole (8%) and zingiberene (7.5%) were observed from the rhizomes harvested in Spanish [19,20]. The observed differences in the chemical composition of the essential oils from the same species can be due to ecological conditions, and the variety of the plant in an intrinsic and extrinsic factor. These differences could also showed the existence of a significant number of chemotypes of this species. Indeed, the chemotypes of the same botanical species make it possible to obtain essential oils of different chemical compositions [21].

The antibacterial activity of *Z. officinale* essential oil was evaluated against five clinical isolates of *Candida* species, namely *Candida albicans, Candida tropicalis, Candida glabrata, Candida krusei* and *Candida parapsilosis*. A significant inhibition of bacteria growth was observed for *C. krusei* with MICs of 800 ppm, and a moderate inhibition for *C. parapsilosis* and for *C. albicans, C. tropicalis* and *C. glabrata* with MICs of 3200 ppm and 6400 ppm, respectively. The result was similar to those obtained by Pozatti *et al.* [19]. In addition, the observed activity could be due to the chemical composition of the essential oil mainly the presence of 1,8-cineole, geraniol and zingiberene. 1,8-cineole and geraniol are known known for their action on membrane integrity by depletion of intracellular K+ ions thus leading to cell death while zingiberene is one of the sesquiterpenoids present in essential oil of *Z. officinale* responsible for its antifungal activity mainly on *Candida* species [6, 22-23].

A yellow spicy and creamy ointments was formulated with *Z. officinale* essential oil as active principle. The pH of the ointments, varying from 5.6-6, was similar to that of the ointment formulated with black seed essential oil [24]. The pH values are in accordance with the recommendation of Good Manufacturing Practice according to European pharmacopeia which stipulates that the pH of a cream should be 5 to 6.8 to have no effect on skin [15]. Macroscopic observation of the ointment showed the absence of clumps and oil droplet confirming the homogeneous character of the ointment. While, microscopic observation (Figure 3) showed the presence of a two-phase system suggesting the presence of a lipophilic phase formed by petroleum jelly and lanolin, and a hydrophilic phase formed by *Z. officinale* hydrosol and glycerol. These results are in agreement with those of Akakpo-akue *et al.* [14] who obtained an ointment with similar microscopic and macroscopic homogeneity.

Microbiological control of the ointment showed the presence of 340 aerobic mesophilic germs on the PCA medium on Day 0 and 390 germs on Day 30. No germs were observed in the SDA, MacConkey, Mannitol and Cetrimide media suggesting the absence of *Pseudomonas aeruginosa, Staphyloccocus aureus, Escherichia coli* and *Salmonella Spp*. The physicochemical and microbiological parameters evaluation showed that the formulated ointment complies with the standards of the European Pharmacopoeia.

4 Conclusions

The emergence of fungal infections and the resistance of fungal strains to existing antifungal drugs has prompted the search of new alternative mainly from natural products such as from the plant *Z. officinale*. The present work has investigated the antifungal potential of Z. officnale essential oil and formulation of an antifungal ointment with Z. officnale essential oil as the active principle. GC/MS of *Z. officinale* essential oil has identified zingiberene, 1,8-cineole, geranial, campbene and geraniol as the major constituent. The essential oil demonstrated antifungal activity against *C. albicans, C. tropicalis, C. glabrata, C. krusei, C. parapsilosis clinical isolates*. The formulated antifungal ointment also showed antifungal activity against the clinical isolates previously cited and has physicochemical and microbiological characteristics acceptable to pharmaceutical standards

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6 Conflicts of Interest

The authors declare no conflict of interest.

7 Authors contribution

N.N and J.N. design, supervision, review and editing original draft. N.T.N. investigation, writing original draft, review and editing, data curation; S.T.G and S.L.N. data curation, writing original draft, review and editing.

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