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CHEMICAL COMPOSITION OF ESSENTIAL OILS OF *EUGENIA CARYOPHYLLA* AND *MENTHA SP CF PIPERITA* AND THEIR *IN VITRO* ANTIFUNGAL ACTIVITIES ON SIX HUMAN PATHOGENIC FUNGI

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Abstract

Background: Many fungal infections are responsible for human skin damages, to control their negative action, some aromatic and medicinal plants are traditionally used by local population in Cameroon. The present study was carried out to determine the chemical composition of essential oils of *Eugenia caryophylla* and *Mentha sp cf piperita* and their antifungal activity on some human pathogenic fungi.
Materials and methods: Essential oils from *Eugenia caryophylla* and *Mentha sp cf piperita* were extracted by steam distillation using Clevenger apparatus and the antifungal activity was evaluated on six human pathogenic fungal strains; two yeasts (*Candida albicans* 1 and *Candida albicans* 2) and four dermatophytes (*Tricophyton rubrum* 1, *T. rubrum* 2, *T. violaceum*, and *T. soudanensis*) using modified broth microdilution method M27-A3 and M38-A respectively.
Results: The essential oils obtained yielded of 5.9 for *Eugenia caryophylla* and 0.2% *Mentha sp cf piperita* respectively. The chemical composition was assigned by GC and GC/SM and showed that *E. caryophylla* was mainly composed of eugenol (80.0 %), □-caryophyllene (8.3%), and eugenol acetate (6.7%) while *Mentha sp cf piperita* was characterized by piperitone (67.5 %), menthol (10.0 %) and ß-phellandrene (5.8%). The result showed that essential oil of *E. caryophylla* exhibit the highest antifungal activity with MICs and MFC of 0.25µL/mL and 0.125µL/mL for filamentous fungi and MIC of 0.5 µL/mL for both yeast strains while MFC value was 1 µL/mL for one yeast strain and not determined for the second. MFCs *Mentha sp cf piperita* essential oil showed a weak activity with a MIC of 2.5 µL/mL on *Tricophyton* strains while no activity was exhibited on *Candida albicans* strains.
Conclusion: The results of this work can be used to confirm their traditional uses and can also be proposed as natural ingredients to some industries to treat superficial infections.

Keys words: Essential oil, *Eugenia caryophylla, Mentha sp cf piperita,* antifungal activity, Human pathogenic strains, fungistatic and fungicide.

Introduction

The skin, first protective barrier against biotic and non biotic attacks is usually affected by environmental changes which usually cause damage on beauty and health (Gupta et al., 2007; Shweta and Swarnlata, 2011). Superficial fungal infections represent almost 70 % of fungal infections among human beings every year. The incidence of fungal infection is very high in tropical areas where the climate is warm and humid, favourable for the development of dermatitis (Rodrigues et al., 2009). In order to overcome these problems, industries and researchers in alternative and complementary medicine have come out with many products. Most of them are synthetic molecules (Tala and Feda, 2003), which are not very appreciated by consumers and do not respect ethics and the environment. Considering people's needs and scientific progress, industries and researchers are looking forward for new substances which are less toxic, safer and respectful to human beings and their environment like plant natural substances. Many of these molecules are synthesized and stored by plants as essential oil and are recognized to be safe. Essential oils are volatile and odorant liquids with many constituents responsible for their smell and make them important ingredients in many preparations used in aromatherapy, cosmetics and perfumes (Talal and Feda, 2003). In addition to the functions described above, essential oils are known to be responsible for many biological activities (Willem, 2002; Nyegue, 2006). The aim of this work was to contribute to the valorisation of natural resources**,** by investigating the chemical composition and *in vitro* bioactivity of essential oils of *E. caryophylla* and *Mentha sp cf piperita;* two aromatics plants growing in Cameroon on six human pathogenic fungi.

E. caryophylla (Myrtaceae) (clove) is a tree that originates from Molu archipelago, precisely in the region of Bandah ache, Indonesia. However, it is also harvested in African countries like Cameroon, Madagascar and Tanzania. Its height is between 10 and 12 metres but can sometimes reach 20 metres; its leaves are oval, persistent and tough with flowers having four white-pink petals characterized by their persistent sepals. Before their floral buds reach 1.5 to 2 centimetres, they are harvested and dried for one month to have the final spices. Traditionally, buds are used as culinary spices, to cure toothache and some infections (Nurdjannah and Bermawie, 2000).

Mentha sp cf piperita is an herbal plant belonging to the Lamiaceae family, native from temperate regions with a fresh sweet smell. Nowadays it is widely found all over the world. This is as a result of a triple hybridization hence is sterile and that is why it multiplies by budding. Its height is between 30 to 90 cm and is characterised by a menthol flavour, dark-green cog and opposite leaves of 2.5 to 5 cm. traditionally, it is used to cure gastric disorder, toothache and muscular pains (Saha and D'Mello, 2004). Before everything, it is known for its pretty and fresh menthol taste used in the kitchen and in cosmetic industries.
Many studies have been carried out on the selected plants of this study, but to the best of our Knowledge no investigations have been done on those from Cameroon and their activity on human pathogenic fungi.

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Materials and Methods

Plant Material

Dried buds of *E. caryophylla* were bought at Mfoundi market (Yaoundé), while the fresh leaves of *Mentha sp cf piperita* were harvested in the Nkoldom locality (Yaoundé) in October 2010. Their identifications were carried out on in the Botanic Laboratory of Faculty of Science of the University of Yaoundé I and later confirmed at the Cameroon National Herbarium (Yaoundé), where voucher specimens were deposited as *E. caryophylla* (Myrtaceae) **Ref: TSN: 506167** and *Mentha sp cf piperita* (Lamiacae) **Ref: 25t45/SRF-Cam.**

Fungal strains

Fungi strains were gratefully given by Centre Pasteur du Cameroon (Yaoundé) and were made up of four filamentous fungi (dermatophytes): two *Trychophyton rubrum* (1 and 2), *T. violaceum,* and *T. soudanense)* and two pathogenic yeasts (*Candida albicans* 1 and 2).

Essential oil extraction (Lamaty et al., 1987)

The plant material (200 g) was subjected to steam distillation using a Clevenger apparatus for 3 hr (three replicates). The essential oils were collected by decantation and finally dried over anhydrous sodium sulfate and keep at 4 °C in opaque bottles.

Chemical analysis (Afnor, 1985).

All samples were analyzed by gas chromatography (GC) and gas chromatography coupled with mass spectrometry (GC/MS).

Gas chromatography

The essential oil was analyzed using a CP-3380 gas chromatography (GC) equipped with a FID detector and two silica capillary columns: HP5 J&W Agilent (5%-Phenyl-methylpolysiloxane (30 m x 0.25 lm, film thickness 0.25 lm) and Supelcowax 10 (polyethylene glycol) fused (30 m x 0.25 lm, film thickness 0.25 lm). Injector and detector temperatures were set at 220 and 250°C respectively. Column temperature was gradually increased from 50 to 200°C with a rate of 5°C/min. Nitrogen was the carrier gas at a flow rate of 0.8 ml/min. Diluted samples of oils (1/100 in acetone, v/v) of 1.0 l were injected manually in the split-less mode. The linear retention indices of the components were determined relative to the retention times of a series of *n*-alkanes.

Gas chromatography/mass spectrometry

GC-MS analyses were performed using a Hewlett–Packard GC5890 series II equipped with a HP5 (5%-Phenylmethylpolysiloxane) fused silica column (30 m x 0.25 mm; film thickness 0.25 µm) and a DB-Wax fused silica column (30 m x 0.25 mm; film thickness 0.25 µm) interfaced with a quadrupole detector (Model 5972); temperature program (50-200°C at 5°C/min); injector temperature, 220°C; MS transfer line temperature, 180°C; carrier gas, helium at a flow rate of 0.6 mL/min; injection type, split, 1:10 (1 µL 10:100 CH_2Cl_2 solution); ionization voltage, 70 eV; electron-multiplier 1460 eV; scan range 35-300 amu; scan rate, 2.96 scan/s.
Qualitative analysis: The identification of the constituents was based on comparison of their relative retention times with either those of authentic samples or with published data in the literature (Adams, 2012) and by matching their mass spectra with those obtained from authentic samples and/or the NBS75K and Wiley 7th NIST 98 EPA/NIH libraries spectra and literature data (Adams 2012).
Quantitative analysis: The percentage composition of the essential oils was computed by the normalization method from the GC-FID peak areas, assuming an identical mass response factors for all compounds. All determinations were performed in duplicate and averaged.

Anti-fungal assays

The preliminary screening

The preliminary screening for antifungal activity was performed using agar incorporation method (dilution on a solid medium) described by Sing *et al.* (1993), Lahlou (2004) and Nyegue (2006). The strains were cultivated on SDA medium for 7 days for filamentous and 48 hr for yeasts before the screening tests. Five concentrations from 1 to 0,0625 µL/mL of *E. caryophylla* oil were tested while for *Mentha sp cf piperita* ten different concentrations were used respectively from 2 to 0,125 µL/mL for filamentous fungi strains and from 3 to 0,935 µL/mL for yeast. The content of each tube was poured after homogenization in Petri dishes of 55mm and dried at 35°C for 30 min. For inoculation, mycelium disks of 7 mm coming from subcultures (7 days old, 25°C) were laid in each Petri dish for each filamentous fungi strains, while for yeast 1 mL of inoculums size of $N=4.10^5$ conidia/mL (2 days old at 37°C) was used for flood. The inoculated dishes were incubated at 25°C for 7 days for filamentous strains and 48 h at 37°C for yeast. Three replicates for each concentration and microorganism were carried out and the data were analysed by the STATGRAPHIC 5.0 software**.** The antifungal activity of the essential oil was evaluated according to the method of Singh *et al.*(1993) and Nyegue (2006) by calculating the percentage of inhibition (I%) from the diameter values of the colony in the control plate (dC) and from that obtained in the plates added with the essayed essential oil (dEO): I%= 100(dC-dEO)/dC. The efficiency of the essential oil was evaluated according to the strongest activity they exhibit: the essential oil has strong activity when > 50%, low activity when <50% or are not active when no growth was detected. The evaluation was applied for filamentous fungi while in the case of yeast strains; the activity was noticed by the presence of confluent tablecloth (Nyegue, 2006).

Determination of the Minimal inhibitory concentrations (MICs) and Minimal fungicidal concentrations (MFCs)

The broth micro-dilution method M27-A2 and M38-A (**NCCLS, 2002a and b**) was used to determine the minimal inhibitory concentrations (MICs) and minimal fungicidal concentrations (MFCs) **(Torres-Rodriguez and Carrillo-Munoz, 1995; Rodrigues *et al.*, 2009)**. The EO was diluted

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to the highest concentration in Sabouraud both at the first well of 96-well micro-titer plate. A serial doubling dilution was prepared over the range of 1 to 0, 0625 µL/mL for *Eugenia caryophylla* essential oil for all tested strains while *Mentha sp cf piperita* essential ranged from 2 to 0,125 µL/mL for filamentous strains and from 3 to 0,935 µL/mL for yeasts. A dilution of 7 days culture of 5.10^4 cells/mL of filamentous strains and 48 h culture dilution of 4.10^5 cells/mL of yeast were then added to every well. In the same micro-liter plate two controls were made of with a negative control containing the Sabouraud broth only and the positive control containing the sabouraud broth and microorganism. The plates were then incubated for 7 days at 25°C for filamentous strains and for 48 h at 37°C for yeast strains and maintained over shaking conditions and the growth was observed from the second to the seventh day for filamentous fungi and on the second day for yeast. Each assay was carried out in triplicate. Ketokonazole was used as the reference and the solution was prepared with 200 mg dilution in 10 mL of sterile distilled water. A stock solution of 20 mg/mL was then two fold serial diluted in Sabouraud broth to obtain final concentrations of 0,128 to 0.004 mg/mL. The MICs were determined as the lowest concentration of oil inhibiting the visible growth of each organism on the agar plate. The presence of one or two colonies was disregarded.

To determine the minimal fungicidal concentrations (MFC), a volume of 50 µL was taken in the micro well where no growth was observed and inoculated in Petri dishes poured with SDA. Then, the dishes were incubated at 25°C for 3 days for filamentous and 37°C for a day for yeasts. When no growth was observed (less than 0.1% of the initial inoculums survived), the activity was fungicidal and in the contrary, the activity was fungistatic (Carta and Arras, 1987; Thompson, 1989 and Lahlou, 2004).

The inhibition parameters was based on the Avril and Fauchere (2002) method adapted to fungals by Nyegue, (2006) where the MFC/MIC ratio was calculated and according to the values obtained in inhibition tests, the essential oil can be fungicidal, fungistatic or tolerant to the tested strains. Nyegue, (2006) proposed the following classification based on MFC/MIC values: when the MBC of the tested sample is comparable to the MIC (MFC/MIC < 4), the sample may be classified as "fungicidal", when the values are in the range 4< (MFC/MIC) <16, the sample is considered as "fungistatic", and finally when MFC/MIC > 32, we talk about "tolerance".

Results

Extraction yields and chemical composition

A pale yellowish oil with a sweet and fresh odour was extracted from fresh leaves of *Mentha sp cf piperita* with a yield of 0.2% (w/w) and a density of 0.9 (g/L) while a deep yellowish oil with sweet warm sent with 5.9% (w/w) and a density of 1 (g/l) was obtained from dried buds of *E. caryophylla*. The analysis of the chemical composition of essential oils extracted from dried buds of *Eugenia caryophylla* and fresh leaves of *Mentha sp cf piperita* using GC and GC/SM method are found in table 1 where components are listed according to their index on HP-5 and CBW columns.

Table I: Chemical composition of essential oils of *Eugenia caryophylla (E. c)* and *Mentha sp cf piperita (M. p)*

Components	**LRI**		**Percentages (%)**		**Method of identification**
	A	**B**	***M. p***	***E. c***	
Monoterpenes					
α-Pinene	934	1004	0.3	tr	GC, MS, LRI
Sabinene	974	1108	0.4		GC, MS, LRI
ß-Pinene	978	1093	0.7		GC, MS, LRI
p-Cymene	1018	1272	tr		GC, MS, LRI
ß-Phellandrene	1029	1209	5.8		GC, MS, LRI
Limonene	1032	1218	0.7		GC, MS, LRI
Oxygenated monoterpenes					
Menthol <neo>	1161	1233	0.3		GC, MS, LRI
Menthol	1168	1245	10.0		GC, MS, LRI
Pulegone	1233	1360	0.6		GC, MS, LRI
Carvacrol methyl ether	1241	1377	2,3		GC, MS, LRI
Piperitone	1249	1381	67.5		GC, MS, LRI
α-Terpineol acetate	1345	1402	0.2		GC, MS, LRI
Eugenol	1388	2186		80.0	GC, MS, LRI
Hydrocarbonated sesquiterpenes					
ß-Cubebene	1390	1454	0.5		GC, MS, LRI
α -Bourbonene	1399	1476	0,6		GC, MS, LRI
α Ylangene	1410	1491		0.1	GC, MS, LRI
β- caryophyllene	1414	1571	1.9	8.3	GC, MS, LRI
β-copaene	1431	1626	0.35		GC, MS, LRI
α-Guaiene	1510	1687	0.2		GC, MS, LRI
Germacrene - D	1514	1712		4.0	GC, MS, LRI
γ-Cadinene	1517	1748	0.2		GC, MS, LRI
δ-Cadinene	1526	1738	0.1		GC, MS, LRI

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α-Copaene	1531		0.35		GC, MS, LRI
Oxygeneted sesquiterpenes:					
Eugenol acetate	1543			6.7	GC, MS, LRI
Caryophyllene oxyde	1576	2000	1.7	0.1	GC, MS, LRI
TOTAL			**94,7**	**99,2**	

The components and the percentages are listed in order of their elution on the apolar column (HP5)
A = Linear retention indices on HP5 column (a 5%-Phenyl-methylpolysiloxane phase); temperature program 50-200°C at 5°C/mn, 200°C for 10 min.
B = Linear retention indices on Supelcowax 10 (polyethylene glycol phase)
M.p: *Mentha sp cf piperita*
E.c : *Eugenia caryophylla*
Methods of identification: GC, identification based on co-injection with authentic sample, MS, identification based on comparison of mass spectrum with literature data, LRI, identification based on comparison of retention index with those of published data.

Antifungal assays

The antifungal screening tests of essential oils samples using agar dilution method are represented in figures 1 and 2.

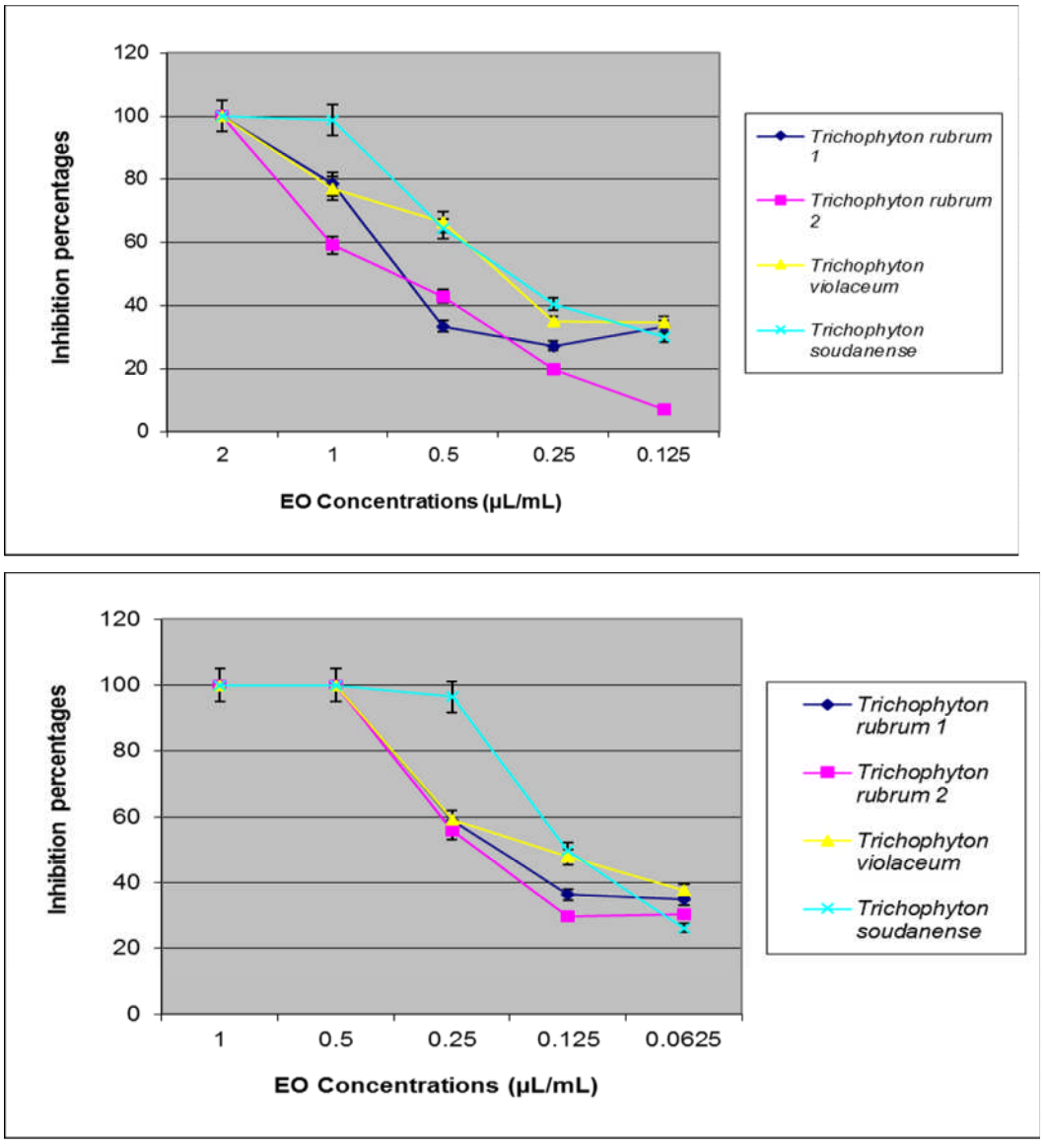

Figure 2: Inhibition Activity of *Eugenia caryophylla* on filamentous fungal strains

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The results of the screening test of *Mentha sp cf piperita* essential oil was active on all tested strains with a percentage inhibition of 100% at 2 µL/mL and 59% at 1 µL/mL showing that, this essential oil is active on all filamentous fungal strains. Statistical analysis with ANOVA in statgraphic 5.0 showed that at 1 µL/mL the difference between filamentous fungal strains was not significant ($p<0.05$). The antifungal screening on *Candida albicans* strains showed total inhibition at concentrations of 3 µL/mL, 1.5 µL/mL and 0.75 µL/mL.

The MICs were evaluated for all strains because of the high activity (>50%) exhibited in a preliminary screening tests. The results showed that MICs of *Mentha sp cf piperita* essential oil on all filamentous strains are the same (MIC=2 µL/mL) while no inhibition was detected on *C. albicans* strains. Concerning MFC, no values were determined because no growth was observed for all tested strains, so MFC/MIC was not determined meaning that this essential oil inhibited the action but did not kill the fungal strains.

Regarding the results of *Eugenia caryophylla* essential oil, MICs varied from 0.125 to 0.5 µL/mL for all strains. *T soudanense* have the lowest MIC (MIC=0.125 µL/mL) confirming the result of the preliminary screening tests. MFCs values were the same for all filamentous strains while for yeast, MFC was 2 µL/mL for one strain and was not determined for the last one. Acording to the classification of Nyegue (2006), *E. caryophylla* EO showed the best effect; hence it exhibits a fungicidal activity against all the filamentous fungi and one strain of *Candida albicans* with a ratio of MFC/MI**C**=1 and 2 respectively (Table 2). On their side, the nature of the activity of *Mentha sp cf piperita* EO and Ketokenazole® have not been determined (table 2) meaning that strains were inhibited rather not killed and making the activity to be described as fungistatic. So comparing the activity of tested essential oil with ketokenazole®, we can say that *Eugenia caryophylla* is more active than the reference.

Table 2: Expression of inhibition parameter of EO on all the strains

EO/ Antifungal	**Inhibition Parameters**	**Tested fungal strains**					
		TR 1	*T R 2*	*T V*	*T S*	*CA1*	*CA2*
Menth.sp cf piperita	MI**C** (µL/mL)	2	2	2	2	Nd	nd
	MFC (µL/mL)	nd	Nd	Nd	nd	Nd	nd
	MFC/MIC	nd	Nd	.nd	nd	Nd	nd
Eugenia caryophylla	MIC (µL/mL)	0.25	0.25	0.25	0.125	0.5	0.5
	MFC (µL/mL)	0.25	0.25	0.25	0.125	1	nd
	MFC/MIC	1	1	1	1	2	nd
Ketoconazole ®	MIC (µL/mL)	0.128	0.128	0.128	0.128	Nd	nd
	MFC (µL/mL)	nd	Nd	nd	nd	nd	nd
	MFC/MI**C**	nd	Nd	nd	nd	nd	nd

nd: not determined; **EO** CMI and CMF in µL/mL; **Ketoconazole** CMI and CMF in mg/mL

Discussion

The results of extraction were very different from those reported by Crouzet and Muchamad, (1982); Nurdjannah and Bermawie, (2000); Alma et al., (2007), where the yields varied from 14-20% and can be due to the conservation time of clove buds. Nurdjannah and Bermawie, (2000); Alma et al., (2007) showed in their investigations that the more the buds are conserved, the weaker will be the yield. Our sample was purchased in a local market, so, no information was available about the date of harvesting and the treatment applied on the sample. On the other hand, some treatments before drying buds highly reduce the yield of essential oil (Robert et al., 1996).

The yield of *Mentha sp cf piperita* essential oil was lower than those reported by Benayad (2008); Derwich *et al*. (2010); Mohammedi (2010) whose values were respectively 1.7%, 1.02% and 1.01%. This difference can be explained by the period and the area of harvesting (Brunetton, 1993) and the treatment of samples before the extraction of essential oil (Pitarevic et al., 1985) moreover due to plant parts used (Arslan et al., 2004). In fact, in our investigation only fresh leaves have been used, while dried aerial parts were used in reported study.

Eight components were identified in *Eugenia caryophylla* essential oil representing 99.2% and mainly made up of eugenol (80.0%), β-caryophyllene (8,3%), and eugenol acetate (6.7%). These results are in accordance with those reported by Crouzet and Muchamat (1982); Nurdjannah and Bermawie (2000), Alma et al., (2007); Eugenia et al., (2009) who showed in their investigations that, despite the different region and date of harvesting, the chemical composition remain the same in term of main components. The values vary between 70 and 95 % for eugenol, 17% for eugenol acetate and 12-15% for β- caryophyllene, but in our study the last two components are less than those in reported study. Nurdjannah and Bermawie (2000) demonstrated that, these values can change according to the time of treatment and conservation of buds, so eugenol increases when eugenol acetate decreases.

Considering the chemical composition of *Mentha sp cf piperita*, 15 components representing 94,7% were identified. The main constituents were piperitone (67.5%), Menthol (10.0%) and β- phellandrene (5.8%) which are different from those published by Behnam *et al*., (2006), Benayad (2008), Socovic *et al*., (2009); Derwick et al., (2010) where the main constituents were respectively menthone, menthol, linalool, and methanol. Menthol appears in both samples as main constituents but in our study (10.0%), it is four times less than those from the literature. The variability

observed can be attributed to the geographical origin of the plant material and the species. The presence of piperitone as major component should be taken into account for confirming the identification of this species as *Mentha sp cf piperita*.

The results of the screening test of *Mentha sp cf piperita* essential oil was active on all tested strains with a percentage inhibition of 100% at 2 µL/mL and 59% at 1 µL/mL showing that, this essential oil is active on all filamentous fungal strains. Statistical analysis with ANOVA in statgraphic 5.0 showed that at 1 µL/mL the difference between filamentous fungal strains was not significant ($p<0.05$).

The efficiency of the essential oil of *Mentha sp cf piperita* can be attributed to the synergetic action of monoterpenes hydrocarbons such as pinene-type which are well-known chemicals having antimicrobial potentials (Dorman and Deans, 2000) and phenol components (menthol, menthol <neo>, carvacrol methyl ether) which may interfere with cell wall enzymes like chitin synthase/chitinase as well as with the α- and β-glucanases of the fungus (Adams et al., 1996; Hammer *et al*., 2003). Many authors among them Koba et al. (2004) have demonstrated that piperitone alone had no significant effects on fungi in general. The activity observed in our study can also be explained by the synergetic action between ketone, some pinene-type and phenol-type which destroy host cells by eliminating the filamentous mycelium that produce fungi with differing specificity and levels of activity, which is in connection with the functional groups present and also associated with hydrogen-binding parameters.

This result of the screening tests of essential oil from *E. caryophylla* is in accordance with that obtained by Eugénia et *al*. (2009), who tested the activity of the essential oil of *E. caryophylla* on some dermatophytes strains. At 0.25 µL/mL the degree of inhibition was the same and significant ($p<0.05$) for all filamentous fungal strains but *T. soudanense* appeared to be more active than the other strains. The screening tests on *Candida albicans* revealed that the EO inhibited the strains at 1 µL/mL and 0.5 µL/mL confirming the results of (Fouazi (2005) and Eugénia et *al.*, (2009). This activity can be due to the presence of eugenol (80.0 %) which is a phenolic compounds known for their high antimicrobial power in general and antifungal in particular (Dorman and Deans, 2000 ; Aligiannis *et al.*, 2001; Franchomme et al., 2001; Nostro *et al.*, 2004). The higher the percentage of phenols, the higher the antimicrobial power of EO (Cosentino et *al.*, 1999). The mechanism of toxicity of phenols against fungi is based on the inhibition of fungal enzymes containing the -SH group in their active site or based on their non-specific interactions with proteins (Cowan, 1999; Mohammedi *et al*., 2010). All the EOs has shown an anti-fungal activity against all the strains tested.

According to the results of *Eugenia caryophylla* essential oil, MICs varied from 0.125 to 0.5 µL/mL for all strains. *T soudanense* have the lowest MIC (MIC=0.125 µL/mL) confirming the result of the preliminary screening tests. MFCs values were the same for all filamentous strains while for yeast, MFC was 2 µL/mL for one strain and was not determined for the last one. According to the classification of Nyegue (2006), *E. caryophylla* EO showed the best effect; hence it exhibits a fungicidal activity against all the filamentous fungi and one strain of *Candida albicans* with a ratio of MFC/MI**C**=1 and 2 respectively (Table 2). On their side, the nature of the activity of *Mentha sp cf piperita* EO and Ketokenazole® have not been determined (table 2) meaning that strains were inhibited rather not killed and making the activity to be described as fungistatic.

The traditional use of these plants as medicine provides the basis for indicating where and how the essential oils may be useful for specific conditions or target. It is however known since decades that, many essential oils have been used as topical antiseptics or have been reported to have antimicrobial properties (Nyegue, 2006; Hockmeni, 2011), so it is now interesting to use them in Africa especially in Cameroon as potential sources of actives ingredients in some cosmetics and pharmaceutical industries for dermatophytosis which are known to be serious skin disorders. In the case of *Mentha sp cf piperita*, this is the first result obtained about the species we use in our study and it was interesting to finally confirm the identification species. According to the activity exhibited by *E. caryophylla* and with respect to the potential toxicity of eugenol acetate (6.7%), the use of this essential oil on human skin can restricted for adults and animals products.

Conclusion

At the end of this study we can conclude that essential oils from these plants have antifungal activity with the oil from *E. caryophylla* having a higher activity. Therefore, the result of our study can be exploited in complementary and alternative medicine in cosmetics purposes to treat some dermathophytosis such as ringworm and onyxis.

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References

1. Adams, R.P. (2012). Identification of essential oil components by Gas Chromatography/ Mass Spectrometry, 4th Ed. Allured Business Media: Carol Stream, IL 60188 USA, 804 p.
2. Adams, S., Kunz, B. and Weidenbörner, M. (1996). Mycelial deformations of *Cladosporium herbarum* due to the application of Eugenol and Carvacrol. J. Essent. Oil Res. **8**: 535-540.
3. Aligiannis, N., Kalpoutzakis, E., Mitaku, S. and Chinou, I. B. (2001). Composition and antimicrobial activity of the essential oils of two Origanum species. Journal of Agricutural Food Chemistry, **49**: 4168–4170.
4. Alma Hakki, M., Ertas M., Nitz, S. and Kollmannsberg, H. (2007). Chemical composition and content of essential oil from the buds of cultivated Turkish clove (*Syzygium aromaticum L*. Bio Ressources, **2**(2): 265-269.
5. Arslan, N., Gurbuz B. et Sarihan, E. O. (2004). "Variation in essential oil content and composition in Turkish anise (*Pimpinella anisum L*.) Populations," Turkish Journal of Agriculture and Foestry, **28**: 173-177.
6. Avril, J.L and Fauchère, J. L. (2002). Bactériologie générale et médicale. Ed. Ellipses. 365 P.
7. Benayad N. (2008). Les huiles essentielles extraites des plantes médicinales marocaines : moyen efficace de lutte contre les ravageurs des denrées alimentaires stockées. Projet de thèse de Doctorat de l'Université Mohammed V – Agdal. 61 P.
8. Brunetton, J. (1993). Pharmacognosie, phytochimie, plantes médicinales. 2eme édition technique et Documentation, Lavoisier. Paris. 915 P

9. Carta, C. and Arras, G. (1987). Azione inhibitrice *in vitro* di olii essenzialinei confronti di alcani di pathogeni di plante ornementali. La difensa Delle Plante, **10**: 195-202.
10. Consentino, S., tuberose, C.I.G., Pisano, B., Santa, M., Mascia, V., Arzedi, E. and Palmas, E. (1999). *In-vitro* antimicrobial activity and chemical composition of *Sardinian thymus* essential oil. Letter Applied Microbiology, **29**(2):130-135.
11. Cowan, M. M. (1999). Plants products as Antimicrobial Agents. Clinical Microbiology Reviews, **12**(4): 564-582.
12. Crouzet, J. and Muchamad, M. (1982). Composition de l'huile essentielle de clous de girofle de différentes provenances. *VIIIth* International Congress on essential oils. Fedarom, Cannes-Grasse, 255-258.
13. Derwich, E., Benziane, Z., Taouil, R., Senhaji, O. and Touzani, M. (2010). Aromatic Plants of Morocco: GC/MS. Analysis of the Essential Oils of Leaves of *Mentha piperita*. Advanced Environnemental Biology, **4**(1): 80-85.
14. Dorman, H. J. D. and Deans, S. G. (2000). Antimicrobial agents from plants: antibacterial activity of plant volatile oils. Journal of Applied Microbiology, **88**: 308–31.
15. Eugénia, P., Vale-silva, L., Cavaleiro, C. and Salgueiro, L. (2009). Antifungal activity of the clove essential oil from *Syzygium aromaticum* on *Candida*, *Aspergillus* and dermatophyte species. Journal of Medical Microbiology, **58**: 1454–1462.
16. Franchomme, P., Jollois R., and Pénoel D. (2001). L'aromathérapie exactement. Encyclopédie de l'utilisation thérapeutique des extraits aromatiques. Eds. Roger Jollois, 490p.
17. Gupta, A., Ashawat, M.S., Shailandra, S. and Swarlanta, S. (2007). Phytosome: A Novel Approch towards Functional Cosmetics. *Journal of Plants Sciences*, **2**(6): 644-649.
18. Koba, K., Sanda, K., Raymond, C., Nenonen, Y.A., Millet, J. and Chaumont, J.P. (2004). Activités antimicrobiennes d'huiles essentielles de trios *Cymbopogum sp*. africains vis à vis de germs pathogens aux animaux de compagnie. Annal of Emergency Medecine veterinary **148** :202-206.
19. Hammer, K.A., Carson, C.F. and Riley, T.V. (2003) antifungal activity of compounds of malaleuca alternifolia (tea tree) oil. Journal of Applied Microbiology, **95**:853-860.
20. Hockmeni, T. C. (2011). Composition chimique et évaluation *in vitro* de l'activité antifongique des huiles essentielles de : *Syzygium aromaticum* et de *Mentha sp cf pipérita* sur quelques germes pathogènes à l'Homme. Mémoire de Master, 49p.
21. Hoffman, D. L. (1987). The Herb User's Guide. Wellingborough, UK Thorons publishing group.
22. Lahlou, M. **(**2004). Methods to study the photochemistry and bioactivity of the essential oils. Phytotherapy Research, **18**: 435-448.
23. Lawless, J. (1995). The illustrated Encycopedia of Essential oils. Shaftesbury, UK Element Book. Ldt
24. Mohammedi, Z. (2006). Etude du pouvoir antimicrobien et antioxydants des huiles essentielles des et flavonoïdes de quelques plantes de la région de Tlemcen. Thèse de Doctorat de l'Université Abou Bakr Belkaid Tlemcen.140 P.
25. Mohammedi, Z., Bachik, S. and Belkaroube, N. (2010). Potentiel antifongique et antiaflatoxinogène des huiles essentielles d'une plante endémique *Thymus fontanesii* Boiss. et Reut. Les technologies de laboratoire, **5**(19) : 10-15.
26. NCCLS (2002a): National Comity for Clinical Laboratory Standards. Reference methods for broth dilution antigungal susceptibility testing of filamentous fungi. Approved standard M38-A, Wayne.
27. NCCLS (2002b): National Comity for Clinical Laboratory Standards. Reference methods for broth dilution antigungal susceptibility testing of yeasts. 2nd Eds. Approved standard M27-A2, Wayne
28. Nostro, A., Blanco, A. R., Cannatelli, M. A., Enea, V., Flamini, G., Morelli, I., Roccaro, A. S. and Alonzo, V. (2004). Susceptibility of antifungal activity of clove essential oil methicillin-resistant *staphylococci* to oregano essential oil, carvacrol and thymol. *Fédération* Eeuropéenne des Médecins Salariés Microbiology Letter, **230**:191–195.
29. Nurdjannah, N. and Bermawie, N. (2000). Clove, Research Institute for Spice and Medicinal Crops Indonesia, **12**: 154-163.
30. Nyegue, M.A. (2006). Propriétés chimiques et biologiques des huiles essentielles de quelques plantes aromatiques et/ou médicinales du Cameroun : Evaluation de leurs activités antiradicalaires, anti-inflammatoire et antimicrobienne. Thèse de Doctorat de l'Université de Montpellier II.194P.
31. Pitarevic, I., Kustrak, D., Kuftinec, J., and Blazevic, N. (1985). "Influence of ecological factors on the content and composition of the essential in *salvia officinalis*" In: *Proceeding of the 15th International symposium on essential oils, Svendsen, A.B andeScheffer, J.J.C. (eds.)* Martinus Nijhoff/Dr W Junk Publishers,Boston, 199-202.
32. Robert, P.A., Barrero, A.F. and Lara, A. **(**1996). Comparisons of the Leaf essential oils of *Juniperus phoenicea.* Journal of Essential Oil Resource, **8**: 367-371.
33. Rodrigues, C. A., Miranda, K. C., Fernandes, O. F. L., Soares, A. J. and Silva, M. R. R. (2009). *In vitro* susceptibility testing of dermatophytes isolated in goiania, brazil, against five antifungal agents by broth micro dilution method. Review of the Sao Paulo Institute of Tropical Medicne, **51**(1):9-12.
34. Saha, P. and D'Mello, P.M. (2004). A review of medecinal uses and pharmacological effect of *Mentha piperita*. Natural product Radiance, **3**(4): 214-221
35. Shweta, K. and Swarnlata, S. (2011). Topical Alternative and Complementary choice to Combat Acne. *Research* Journal of Medecinal Plant, ISSN 1819-3455, 20 P.
36. Singh, G., Upodhyay, R. K., Narayanan, C. S., Padm kumara, K. P. and Rao, G. P. **(**1993). Chemical and fungitoxic investigation on the essential oil of *Citrus sinensis, pers. z. p. flanzenkr.* Pflanzenschuts, **100**: 69-74.
37. Talal, A. and Feda, M. N. (2003). Plants Used in cosmetics**.** Phytotherapy Researchs, **17**: 987-1000.
38. Thompson, D.P. (1989). Fungitoxic activity of essential oil components on food storage fungi. *Mycologia*, **81**:151-153.
39. Torres-Rodriguez, J. M. and Carrillo-Munoz, A. J. (1995). *In-vitro* antifungal activity of sertaconazole, econazole and bifonazole against Candida spp. Journal of Antimicrobial Chemotherapy, **36**: 713-716.
40. Willem, J-P. (2002). Les huiles essentielles médecine d'avenir. Dauphin, Paris. 318 P.