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Study of the Antimicrobial Activity of Essential Oils from a Number of Medicinal Plants on Germs Involved in Skin Disorders

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A B S T R A C T

The development of skin infections and the phenomenon of antibiotic resistance among micro-organisms are now a particularly worrying public health problem. The use of medicinal plants is an interesting avenue to explore in order to discover and develop innovative new drugs to combat these microbial infections. It was against this backdrop that this study was carried out to investigate the antimicrobial power of essential oils of *Ocimum gratissimum L.*, *Ocimum americanum L.* and *Lippia multiflora Moldenke* on the in vitro growth of *Candida albicans, Trichophyton rubrum* and *Staphylococcus aureus,* the germs responsible for skin disorders. The essential oils were extracted by steam distillation using a Clevenger-type apparatus. The antimicrobial activity of these three essential oils was determined using the diffusion test and the solid double dilution method. The results obtained showed that *Lippia multiflora* essential oil was the most active on the three microorganisms tested with inhibition zone diameters of 34.33; 35 and 8.33 mm on *Staphylococcus aureus, Candida albicans,* and *Trichophyton rubrum* respectively. Antimicrobial activity resulted in MIC and MBC of 0.039 and >10 µL/mL respectively on *S. aureus.* The MIC and MFC values obtained for *C. albicans* and *T. rubrum* were identical (0.078 and 0.312 µL/mL respectively). With the exception of *Ocimum americanum*, all the essential oils showed good activity against the three strains studied.

Introduction

The skin is a complex organ and one of the most important in the body. It is a fully-fledged organ whose proper functioning is essential to the body. Its primary function is to ensure communication between the body and the surrounding environment and to protect the body against physical, chemical and biological aggression from the outside (Dréno, 2009). The skin is made up of a wide variety of micro-organisms known as the cutaneous flora, whose imbalance can lead to disease. Dermatoses are becoming increasingly common in Africa and are part

of routine consultations in general medicine, paediatrics and dermatology, and are mostly of bacterial, fungal, viral or parasitic origin (Kassi *et al.,* 2016). Infectious dermatoses account for 20% of cases, of which 9.52% are caused by fungi and 3.80% by bacteria (Kaboret *et al.,* 2019). Since the discovery of antibiotics at the beginning of the 20th century, their introduction and clinical use have considerably reduced the mortality of previously incurable diseases (Guinoiseau, 2010). Their effectiveness in controlling and limiting the distribution of pathogens has given rise to hopes of tackling all infectious diseases. Unfortunately, the phenomenon of antibiotic resistance in microorganisms has put an end to this wave of optimism (Guinoiseau, 2010). The acquisition of multiple resistances and the scarcity of new antibiotics can lead to a real risk of therapeutic impasse, prompting us to implement new therapeutic strategies based on the search for molecules with antimicrobial activity (Stéphane *et al.,* 2015). Among the avenues of research into new antimicrobials, the exploration of medicinal plants appears to be one of the most promising, since their biodiversity makes them the largest reserve of bioactive substances. The study of essential oils (EO) has aroused the interest not only of the general public but also of scientists because of their numerous antimicrobial properties (Stéphane *et al.,* 2015).

It is in this context that this study was carried out in order to study the antimicrobial power of essential oils on germs responsible for skin diseases and also to contribute to the valorisation of medicinal plants. Specifically, the aim was to :

-To evaluate the activity of *Ocimum gratissimum, Ocimum americanum* and *Lippia multiflora* essential oils on *Staphylococcus aureus* bacteria.
-To assess the activity of *Ocimum gratissimum, Ocimum americanum* and *Lippia multiflora* essential oils on the yeast *Candida albicans*
-To assess the activity *of Ocimum gratissimum, Ocimum americanum* and *Lippia multiflora* essential oils on the dermatophyte *Trichophyton rubrum.*

Materials and Methods

Plant material

The plant material used for this study consisted of fresh leaves of *Lippia multiflora, Ocimum gratissimum (n°UCJ008874)* and *Ocimum americanum (n°UCJ008877).* The *Ocimum gratissimum* leaves were harvested in the morning in Bingerville (Côte d'Ivoire). On the other hand, the essential oils of *Lippia multiflora* and *Ocimum americanum* were supplied by the Centre National de Floristique de Côte d'Ivoire.

Microbial strains tested

The microbiological material consisted of one bacterial strain: *Staphylococcus aureus* and two fungal strains: the yeast, *Candida albicans* and the dermatophyte, *Trichophyton rubrum,* supplied by the Institut pasteur de Côte d'Ivoire.

Chemical products

An organosulphur polar solvent, DMSO (Dimethysulphoxide), was used to make the EO soluble in the agar.

Extraction of essential oils

To extract the essential oil of *Ocimum gratissimum*, 1.3 kg of fresh leaves were used. The leaves were cut into small pieces. The essential oil was extracted by steam distillation using a clevenger distiller for 2 hours. The EO was separated from the water by decantation (Kassi *et al.,* 2020). The extraction yield was calculated according to the following formula :

$R=100 \times m / M$

R: extraction yield; m: mass of EO obtained; M: mass of leaf used.

Diffusion test on agar medium

Preparation of the discs

Ten microlitres (10 µL) of each pure HE was incorporated onto 6 mm diameter blotting paper discs (Bio-Rad°) and one disc was impregnated with sterile distilled water to act as a negative control (T-). After incorporation, all the discs were dried in a laminar flow hood for 20 minutes to avoid contamination.

Preparation of the bacterial inoculum

Two 18h-old colonies were picked using a platinum loop and bubbled through 10 mL of sterile distilled water. The turbidity of this suspension was adjusted to 0.5

Macfarland using an opacity control (Bio-Rad). This bacterial suspension, estimated at 108 cfu/mL, was diluted with 1/100 in EDS (0.1 mL suspension in 9.9 mL sterile distilled water) to give a bacterial inoculum of 106 cfu/mL (Bolou *et al.,* 2011).

Preparation of the fungal inoculum

The inocula were prepared from colonies 48 hours old for *C. albicans* and 5 days old for *T. rubrum*. A perfectly isolated colony was suspended in 10 mL of sterile distilled water to give an inoculum estimated at 106 cfu/mL. A 1:10 dilution of this suspension was performed by introducing 1 mL into 9 mL of sterile distilled water, to obtain a 10^5 cfu/mL suspension (Pkorou *et al.,* 2010; Kra, 2001).

Diffusion test

Inocula prepared previously in sterile distilled water were inoculated by flooding into petri dishes containing Mueller-Hinton and Sabouraud agars. The excess inoculum in the petri dishes was poured into a bleach tray, then the petri dishes were left ajar next to the flame for 3 to 5 minutes. The discs impregnated with the various essential oils were then placed on the agar, along with the negative control disc. Petri dishes were left for 2 hours at room temperature to pre-diffuse the EO, then incubated in an oven for 24 hours for *Staphylococcus aureus*, 48 hours for *Candida albicans* and 5 days for *Trichophyton rubrum* at 37°C. Antimicrobial activity was determined by measuring the diameter of the zone of inhibition around each disc (Adesokan *et al.,* 2007).

Determination of antimicrobial parameters (MIC, CMB or CMF)

Preparation of concentration ranges for essential oils

Concentration ranges were prepared in sterile test tubes using the double dilution method, in inclined tubes. Each test consists of 11 sterile test tubes numbered from 1 to 11. These tubes included 9 test tubes containing the essential oil and 2 control tubes without essential oils (one used as a control for germ growth and the other without germs used as a control for the sterility of the culture medium). The first T1 tube was concentrated to 10 µL/mL by adding 200 µL of HE, 200 µL of DMSO and 19.6 mL of sterile liquid agar (Mueller-Hinton or Sabouraud). The other 10 tubes contain only 10 mL of sterile agar. To carry out the double dilution, 10 mL were taken from the 1st tube (T1) and diluted in the 2nd tube (T2).

After shaking, 10 mL were also taken from the 2nd tube (T2) and diluted in the 3rd tube (T3). This operation was repeated successively up to tube 9 (T9), giving respective concentrations of 10; 5; 2.5; 1.25; 0.625; 0.312; 0.156; 0.078; 0.039 µL/mL. All the tubes, including the controls, were then tilted at room temperature to solidify.

Inoculation and incubation

After solidification of the agar and preparation of the inoculum, all tubes (T1 to T9 and the growth control) were inoculated by exhaustion with 10 µL of bacterial or fungal inoculum (106 cfu/mL or 10^5 cfu/mL). The inoculated tubes were incubated at 37°C for 24 hours for *Staphylococcus aureus*, 48 hours for *Candida albicans* and 5 days for *Trichophyton rubrum*.

Determination of the minimum inhibitory concentration (MIC)

At the end of the incubation time, microbial growth was observed in the tubes. The MIC (minimum concentration for which there is no growth visible to the naked eye) was determined.

Determination of MIC and FMC

After the MIC was read, the replicates of tubes with no visible growth were incubated for 24 h, 48 h and 5 days (depending on the germ) at 37°C. The BMC or FMC is the smallest concentration for which the subculture shows no growth.

Statistical analysis

The diameters of the inhibition zones of the essential oils were determined with three replicates (n=3) and the results were subjected to analysis of variance (ANOVA) using Turkey's multiple comparison test, with the threshold $p<0.05$ considered significant.

The software used to perform these analyses was STATISTICA 7.1. Each mean value is accompanied by the standard deviation (mean ± standard deviation). The software used to plot the graph is Graph Pad.

Results and Discussion

Essential oil extraction results

Extraction of *Ocimum gratissimum* EO gave a yield of 0.17%. Analysis of this result showed that the yield obtained is lower than those of Félicia *et al.,* (2018) (1.1%), and Bonou *et al.,* (2016) (0.92%). These observed differences in yield could be linked to the collection area, the nature of the soil, the harvesting period, the physical state of the plant material (dry or fresh) and the extraction method used (Soro *et al.,* 2015; Degnon *et al.,* 2016).

Results of antimicrobial activity

The in vitro antimicrobial activities of the EOs of *Lippia multiflora*, *Ocimum americanum* and *Ocimum gratissimum* against the three micro-organisms were defined by the presence or absence of zones of inhibition and the values of the antimicrobial parameters (MIC, BMC and FMC). The correlation between the two screening methods examined was generally as follows: larger zones of inhibition correspond to lower MICs (Boukhatem *et al.,* 2014).

Ocimum gratissimum essential oil

Inhibition tests carried out on solid media with *O. gratissimum* EO gave inhibition diameters of 22, 34.66 and 4.67 mm on *S. aureus, C. albicans* and *T. rubrum* respectively. The parameters (MIC and BMC or FMC) resulting from the antimicrobial activity were 0.156 and >10 µL/mL for *S. aureus*, 0.078 and 0.156 µL/mL for *C. albicans*. For *T. rubrum*, the MIC and MFC values were identical (5 µL/mL) (Table 1). Analysis of the results shows that *T. rubrum* is less sensitive to *O. gratissimum* EO than *C. albicans* and *S. aureus*. Of these two strains, the least sensitive was *S. aureus*. In the light of these results and by comparison with other studies, the EO tested is more active than theirs. Indeed, Koba *et al.,* (2009) obtained a MIC equal to 0.08 µL/mL on *T. rubrum* and 0.15 µL/mL on *C. albicans*. However, Bonou *et al.,* (2016) obtained an MIC of 0.078 mg/mL on *C. albicans*, which is identical to our own. Oussou *et al.,* (2004) revealed in their work that the antimicrobial activity of this essential oil is linked to its chemical composition (54.32% of compounds with antimicrobial activity) of which p-cymene (46.13%) and thymol (4.13%) were the main compounds. In the same vein, the work carried out by Koffi *et al.,* (2013) also showed that EO whose main compounds are thymol and p-cymene have a broad spectrum of action on *Candida albicans* and *Aspergillus fumigatus*.

Lippia multiflora essential oil

For *L. multiflora* EO, the inhibition diameters obtained during inhibition tests carried out on solid media were 34.33, 35 and 8.33 mm on *S. aureus, C. albicans* and *T. rubrum* respectively. Antimicrobial activity resulted in MIC and MBC values of 0.039 and >10 µL/mL respectively on *S. aureus*. The MIC and MBC values obtained on *C. albicans* and *T. rubrum* were identical (0.078 and 0.312 µL/mL respectively). Analysis of these results shows that *S. aureus* and *C. albicans* are sensitive to *Lippia multiflora* EO and confirms the traditional use of its leaves. According to Bassolé *et al.,* (2001), this EO is more active than the essential oil of the fruits of *Xylapia aethiopica* on *S. aureus*. Compared with the work of Baba-Moussa *et al.,* (2012) on the same species, our EO is more active than theirs. They obtained a MIC of 0.078 and 0.156 µL/mL on *S. aureus* and *C. albicans* respectively. Studies carried out on *L. multiflora* EO have revealed its chemical composition. Indeed, p-cymene (21.3%), thymol (14%), β-caryophyllene (12.9%), carvacrol (9.3%) and carvone (8.6%) were identified as the main constituents. The antimicrobial potential of this EO could be explained by the presence of thymol and carvacrol (Bassolé *et al.,* 2010).

Ocimum americanum essential oil

With O. americanum EO, respective diameters of 10.67 and 9 mm were observed on *S. aureus* and *C. albicans* during inhibition tests carried out on solid media. On the other hand, no inhibition diameter was observed on *T. rubrum*. The antimicrobial parameters (MIC and MBC) tested gave values equal to 2.5 and >10 µL/mL for *S. aureus*. For *C. albicans*, the MIC and MFC were identical (5 µL/mL). *T. rubrum* showed resistance at the maximum dose tested (10 µL/mL). Analysis of these results shows that *T. rubrum* is not sensitive to *O. americanum* EO, unlike *C. albicans* and *S. aureus*. This essential oil has very little antimicrobial activity compared to the other two EOs studied. Indeed, Jean-Pierre *et al.,* (2001) also showed that *O. americanum* EO was inactive on the growth of *T. rubrum* (>1000 µg/mL). However, this EO is widely used for its insecticidal properties.

Table.1 Antimicrobial parameters of Ocimum gratissimum EO

Strain	Diameters of inhibition zones (mm)	MIC (µL/mL)	MBC ou MFC (µL/mL)
Staphylococcus aureus	$22^{a} \pm 1,33$	0,156	> 10
Candida albicans	$34,67^{b} \pm 3,11$	0,078	0,156
Trichophyton rubrum	$4,67^{a} \pm 0,89$	5	5

Table.2 Antimicrobial parameters of *Lippia multiflora* EO

Strain	Diameters of inhibition zones (mm)	MIC (µL/mL)	MBC ou MFC (µL/mL)
Staphylococcus aureus	$34,33^{a} \pm 0,89$	0,039	> 10
Candida albicans	$35^{b} \pm 6$	0,078	0,078
Trichophyton rubrum	$8,33^{c} \pm 0,44$	0,312	0,312

Table.3 Antimicrobial parameters of *Ocimum americanum* EO

Strain	Diameters of inhibition zones (mm)	MIC (µL/mL)	MBC ou MFC (µL/mL)
Staphylococcus aureus	$10,67^{a} \pm 1,78$	2,5	> 10
Candida albicans	$9^{a} \pm 1,33$	5	5
Trichophyton rubrum	$0^{c} \pm 0$	> 10	> 10

Indeed, the insecticidal potential of *O. americanum* essential oil and its majority compound terpineol-4 was evaluated by Akantetou *et al.,* (2011) on adults of Aphis gossypii, a cotton aphid. The results of these tests revealed that the essential oil of *O. americanum* and terpineol-4 have remarkable aphicidal properties.

The values of the diameters of the zones of inhibition of the essential oil on the three strains are obtained statistically in the form of the mean standard deviation Diameters with the same letters are statistically identical.

This study enabled us to demonstrate the antimicrobial power of essential oils derived from a number of medicinal plants on germs responsible for skin disorders, namely *Staphylococcus aureus, Trichophyton rubrum* and *Candida albicans*. All the essential oils (*Lippia multiflora*, *Ocimum gratissimum*, and *Ocimum americanum*) tested inhibited the growth of the three strains with the exception of *Ocimum americanum* EO which was inactive on *T. rubrum*. *Lippia multiflora* EO stood out for its very strong activity on the three micro-organisms tested. *Candida albicans* and *S. aureus* were the most sensitive strains to the three essential oils. Ultimately, the search for natural substances with antimicrobial properties in the treatment of skin disorders led to the identification of *L. multiflora* EO as a potential source of active ingredients.

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Author Contribution

Agré Don Josette: Investigation, formal analysis, writing—original draft. Bolou Gbouhoury Eric-Kevin: Validation, methodology, writing—reviewing. Coulibaly Kiyinlma:—Formal analysis, writing—review and editing. Kouamé Adjoba Débora: Investigation, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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