Vol. 13(9), pp. 173-187, 13 May, 2019
DOI: 10.5897/JMPR2019.6768
Article Number: 2F8D44C61007
ISSN 1996-0875

http://www.academicjournals.org/JMPR

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Journal of Medicinal Plants Research

Full Length Research Paper

Chemical composition and antimicrobial activity of essential oils from *Aframomum citratum*, *Aframomum daniellii*, *Piper capense* and *Monodora myristica*

Sylvie Carolle Meffo Dongmo[1], Jean-De-Dieu Tamokou[1#], Guy Sedar Singor Njateng[1], Pierre Tane[2] and Jules-Roger Kuiate[1*]

[1]Research Unit of Microbiology and Antimicrobial Substances, Faculty of Science, University of Dschang, P. O. Box 67 Dschang, Cameroon.
[2]Research Unit of Natural Products Chemistry, Faculty of Science, University of Dschang, P. O. Box 67 Dschang, Cameroon.

Received 4 April, 2019; Accepted 6 May, 2019

This study was initiated to evaluate the chemical composition and *in vitro* antimicrobial activity of essential oils from four Cameroonian spices and to determine the therapeutic effect of a cream based on essential oil from *Aframomum citratum*. Essential oils were extracted from seeds by hydrodistillation and analyzed by gas chromatography coupled with mass spectrometry (GC/MS). The broth microdilution method was used for the minimum inhibitory concentration (MIC) and minimum microbicidal concentration (MMC) determinations. The therapeutic effect of a cosmetic cream based on essential oil from *A. citratum* seed (1.25, 2.5 and 5% w/w) was evaluated against dermatosis induced with a Methicillin-Resistant *Staphylococcus aureus* (MRSA) in rats. The main identified compounds in the essential oils are geraniol for *A. citratum*; eucalyptol, α-terpineol and geraniol for *Aframomum daniellii*; β-pinene, germacrene D, trans-β-caryophyllene, α-pinene, naphthalene and sabinene for *Piper capense*; α-phellandrene, germacradienol and δ-cadinene for *Monodora myristica.* Essential oil of *A. citratum* (MIC = 8-4096 µg/ml) was the most active against bacteria and fungi, following in decreasing order by those of *A. daniellii, P. capense* and *M. myristica.* The antibacterial activity of the essential oil of *A. citratum* against MRSA and *Escherichia coli* S2(1) (MIC = 8 µg/ml) was higher than that of amoxicillin used as reference drug (MIC = 128- 256 µg/ml). The combination of essential oils of *A. citratum* and *A. daniellii* (1:1) displayed a synergistic effect. The cream based on essential oil of *A. citratum* (5%) and Baneocin (reference drug) eradicated the dermatosis induced with MRSA in rat after two weeks of treatment. These results indicate that the tested essential oils possess antimicrobial activities which could be a function of either the individual or the additive effects of the identified phytoconstituents.

Key words: Spices, hydrodistillation, essential oils, gas chromatography/mass spectrometry (GC/MS), antibacterial, antifungal, synergistic effect.

INTRODUCTION

The skin can be infected by different types of microorganisms, most often by Gram-positive bacteria such as *Staphylococcus* species. Bacterial skin infections are widespread all over the world and many are caused by *Staphylococcus aureus*. The treatment of *S. aureus* infection, particularly Methicillin-Resistant *S. aureus*

(MRSA), is a challenge in clinical practice (Malachowa et al., 2013; Song et al., 2017).

In developing countries, the main difficulties that accompany their treatments with conventional medicines are the high cost and toxic effects of the common antibiotics, as well as the development of multi-resistant pathogenic microorganisms to the treatments (Yang et al., 2016). Complementary and alternative medicines (CAMs) are used by 60-80% of developing countries and are also the most widespread sources of medicines in the world (Lee et al., 2012). Indeed, among all the CAMs, essential oils represent the most popular choices for the treatment of fungal skin infections (Millikan, 2002) and one of their main applications is their use in dermatology (Reichling et al., 2009).

Essential oils are primarily used as natural preservatives, flavorings and fragrances in cosmetic products (Fernandes et al., 2013). They are potential sources of antioxidants and natural antimicrobials, in addition to their multiple properties such as antiparasitic, analgesic and cytotoxic properties (Mith et al., 2014).

Among the potential sources of essential oils, spices have long been investigated because they contain volatile bioactive compounds that can be of interest in therapy and nutrition. Indeed, essential oils from medicinal spices and vegetables are important sources of antimicrobial agents in addition to their ability to stimulate the digestive system (Rahman et al., 2011). Medicinal spices and vegetables have traditionally been used as food additives, coloring, flavoring and preservative agents as well as antiparasitic, antihelmintic, analgesic, expectorant, sedative, antiseptic and antidiabetic substances in many parts of the world (Rahman et al., 2011; Dzoyem et al., 2014).

Aframomum daniellii (Hook.f.) K. Schum belonging to Zingiberaceae family is a large, robust, perennial plant that is about 3-4 m tall and is usually found under shades in plantations near riverine areas. Its seeds are used for flavouring traditional dishes as well as food additives, laxative, anti-helmintic, antibacterial and antifungal agents. The rhizome juice of this plant is effective in the treatment of body odor and toothache (Pamela et al., 2016). The leaves and seeds are used in the treatment of internal and external piles (Focho et al., 2009). This plant is also used in traditional food preparations for its flavoring, coloring and preservative properties (Tajkarimi et al., 2010), as well as to cure malaria, dysentery, dysmenorrhea, infertility, rubella, leprosy and cancers (Titanji et al., 2008).

Aframomum citratum (Zingiberaceae) K. Schum is a perennial herbaceous producing leafy stems up to 3 meters tall from a rhizomatous rootstock (Burkil, 2000). Young shoots are eaten as a vegetable while the seeds are eaten as a spice in Cameroon. The plant is traditionally used as an aphrodisiac and also to treat bacterial infections, malaria and cancers (Titanji et al., 2008; Kuete et al., 2011).

Piper capense Lin. f (Piperaceae), known as long black pepper, is an endemic plant of East Africa found in wet highlands where it is produced traditionally for human consumption and medical uses (Kokwaro, 1976; Van Wyk and Gericke, 2000).

M. myristica (Gaertn) Dunal is a perennial edible plant of the Annonaceae family. It is mostly found in the humid tropical forests of West and Central Africa and commonly known as African nutmeg and calabash nutmeg. The seeds of this plant are used to cure constipation, uterine hemorrhage, diuretic and fever (Dzoyem et al., 2014). Some biological activities such as cytotoxic, antiprotozoal, antibacterial, anti-inflammatory and antioxidant activities have been documented for *A. citratum, A. daniellii, P. capense* and *M. myristica* (Kuete et al., 2011; Dzoyem et al., 2014).

However, from literature search, no scientific investigations have been conducted till date to verify the *in vivo* antimicrobial activities of the above plant species and there is a paucity of data on their essential oil composition. This work was therefore carried out to evaluate the chemical composition and *in vitro* antimicrobial activity of essential oils from the seeds of *A. citratum*, *A. daniellii*, *P. capense* and *M. myristica* as well as to determine the therapeutic effect of a cream based on essential oil from *A. citratum*.

MATERIALS AND METHODS

Plant materials

Dried seeds of *A. citratum, A. daniellii, P. capense* and *M. myristica* were purchased in February 2016 at market “B” in Bafoussam, situated in the Western region of Cameroon. The plant species were identified using their seeds by Mr. Fulbert TADJOUTEU, a Botanist of the National Herbarium of Cameroon by comparison with specimens whose voucher numbers were 37736/HNC, 43130/HNC, 6018/SRF/NHC and 2949/SRF/NHC for *A. citratum, A. daniellii, P. capense* and *M. myristica*, respectively.

Extraction of essential oils

The essential oils were obtained by hydrodistillation using a Clevenger-type apparatus. 500 ml of distilled water was separately added to seeds of *A. citratum* (100 g), *A. daniellii* (100 g), *P. capense* (200 g) and *M. myristica* (200 g). A magnetic stirrer was introduced in the apparatus and the mixture was heated on a hot plate. Hydrodistillation was carried out for 6 h with *A. citratum* and *A. daniellii* and for 1 h with *P. capense* and *M. myristica*. The essential oils were dried over a column of anhydrous sodium sulphate (Sigma-Aldrich, St. Louis, MO, USA) and then stored in

*Corresponding authors. E-mail: jrkuiate@yahoo.com; jtamokou@yahoo.fr

amber tubes at 4°C until analyses.

Determination of the chemical composition of essential oils

The essential oils were analyzed by gas chromatography coupled with mass spectrometry (GC/MS), using an Agilent apparatus (6890 N series), fitted with a HP-5MS fused silica capillary column (30 m × 0.25 mm, film thickness 0.25 µm) and coated with 5% phenyl 95% dimethylpolysilosane. The initial temperature was set at 50°C and the oven was heated up to 110°C at a rate of 3°C/min, then from 110 to 300°C at a rate of 10°C/min. The carrier gas was pure helium at a flow rate of 1.2 ml/min. The injector temperature was 250°C, applying the split ratio of 1:5. Mass spectra were obtained using electron ionization source at 70 eV. Ion source temperature was maintained at 230°C and the mass range was m/z 40-400 u. A scan interval of 0.5 s and fragments from 40 to 550 Da were maintained. The essential oil was solubilized in pentane at concentration of 2 mg/ml and 1 µl was injected on the chromatographic system. The relative quantity of the compounds present in the essential oils was expressed as a percentage based on the peak area produced in the chromatogram. From the obtained chromatograms, retention indices (RI) of components were determined relatively to the retention times of a series of *n*-alkanes (C_8-C_{40}) with linear interpolation. Compounds were identified by comparing their retention indices and their mass spectra with those of Wiley Library data 2009.

Microorganisms

The used microorganisms in this study included fungal and bacterial species involved in skin infections and wound contaminations. These microorganisms included reference strains from American Type Culture Collection and clinical isolates. Fungal strains were made of two dermatophytes: *Microsporum gypseum* E1420 and *Trichophyton violaceum* obtained from *Ecole Nationale Vétérinaire d'Alfort* in France; seven yeast strains: *Candida albicans* ATCC 1663, *C. albicans* ATCC 9002, *C. albicans* IS1*, Candida parapsilosis* ATCC 22019*, Candida tropicalis* ATCC 750*, Candida krusei* ATCC6258, *Cryptococcus neoformans* IP90526; four amphotericine B and nystatin sensitive *Candida spp* isolates: *Candida krusei, Candida parapsilosis*, *Candida lipolithica* and *Candida haemophilus*; twenty amphotericine B and nystatin resistant isolates of *C. albicans*: *Ca* Da11, *Ca* E01, *Ca* F021, *Ca* F045, *Ca* K42, *Ca* D10, *Ca* F066, *Ca* F005, *Ca* F017, *Ca* F057, *Ca* K14, *Ca* F015, *Ca* F023, *Ca* F040, *Ca* F002, *Ca* F041, *Ca* K072, *Ca* F049, *Ca* F026, *Ca* K22 and eight nystatin resistant isolates of *C. neoformans*: CN, CN169, CN173, CN047, CN091, CN046, CN 096, CN158, obtained from Pasteur Institute (IP, Paris-France). Bacteria were constituted of eight Gram-positive species: *S. aureus* ATCC 25923, methicillin resistant *S. aureus* MRSA03, methicillin resistant *S. aureus* MRSA04, methicillin sensitive *S. aureus* MSSA01, *Staphylococcus aureus* ST120, *Enterococcus aerogenes* ATCC 13048, *Enterococcus aerogenes* and *Enterococcus adecarboxylate* and eight Gram-negative bacteria: *Escherichia coli* ATCC 10536, *Escherichia* coli, entero-aggregative *Escherichia coli*, *Pseudomonas aeruginosa* ATCC 27853, *Pseudomonas aeruginosa* PA01, *Bacillus subtilis, Klebsiella pneumoniae* ATCC2513883, *Shigella flexneri* obtained from our laboratory collection. The bacteria and yeasts were maintained at +4°C on agar slants.

Experimental animals

In this study, 42 *Wistar albino* rats (21 males and 21 females; 10-12 weeks old; 150-200 g) were used. They were bred in the animal house of the Department of Biochemistry, University of Dschang, Cameroon. The animals were fed with a standard diet. Food and water were given *ad libitum* to all animals used for the experiments. Animals were maintained at room temperature (22 ± 2°C). The study was conducted according to the ethical guidelines of Committee for Control and Supervision of Experiments on Animals (Registration no. 173/CPCSEA, dated 28 January, 2000), Government of India, on the use of animals for scientific research.

Determination of the minimum inhibitory concentration (MIC) and minimum microbicidal concentration (MMC)

MIC was determined by broth micro dilution method as previously described (Tamokou et al., 2009; Fogue et al., 2012) with slight modifications. The microbial inocula were prepared from 24 h, 48 h and 7 days old broth cultures from bacteria, yeasts and dermatophytes, respectively. The absorbance was read at 600 nm (for bacteria), 530 nm (for yeasts) and 450 nm (for dermatophytes) using a spectrophotometer (Jenway™ 6305 UV/Visible Spectrophotometer, Fisher scientific, UK).

From the prepared microbial solutions, other dilutions with sterile physiological solution were prepared to give a final concentration of 2 $\times 10^5$ colony-forming units (CFU)/ml for bacteria and 2×10^5 spores/ml for yeasts and dermatophytes. Stock solutions of essential oils were prepared in 5% tween 80 at concentrations of 16.38 (for essential oils) and 2.04 mg/ml (for pure reference drugs). The antimicrobial susceptibility test was performed in a 96-well microplate. The twofold serial dilutions of test samples were made in Mueller Hinton Broth (MHB) (Conda, Madrid, Spain) for bacteria and Sabouraud Dextrose Broth (SDB) (Conda, Madrid, Spain) for yeasts and dermatophytes.

The final concentrations ranged from 4.096 to 0.032 mg/ml for the essential oil and from 128 to 0.50 µg/ml for the reference drugs. For every experiment, a sterility check (5% tween 80 and medium), negative control (5% tween 80, medium and inoculum) and positive control (5% tween 80, medium, inoculum and reference drug) were included. The plates were covered with the sterile sealer and incubated at 35°C for 24 h (for bacteria) and 48 h (for yeasts). Dermatophytes were incubated at 27°C for 5 days. Bacterial growth was monitored colorimetrically using iodotetrazolium chloride (INT). Viable bacteria change the yellow dye of p-iodonitrotetrazolium violet to a pink color. Yeasts and dermatophytes growth in each well was determined by observing and comparing the test wells with the positive and negative controls. The absence of microbial growth was interpreted as the antibacterial or antifungal activities. The MIC was the lowest concentration of the essential oil that prevented change in color or visible growth of micro-organisms.

Minimum Bactericidal Concentrations (MBCs) and Minimum Fungicidal Concentrations (MFCs) were determined by adding 50 µl aliquots of the well (without INT), which did not show any microbial growth after incubation during MIC assays, into 150 µl of essential oil-free Mueller Hinton Agar (for bacteria) and Sabouraud Dextrose Agar (for yeasts and dermatophytes). MBCs or MFCs were defined as the lowest concentration yielding negative growth. All the experiments were performed in triplicate. Amoxicillin, nystatin and griseofulvin were used as positive controls for bacteria, yeasts and dermatophytes, respectively.

***In vivo* antibacterial assay**

The *in vivo* antibacterial activity was determined with the essential oil of *A. citratum* seed which displayed the most *in vitro* antimicrobial activity.

Formulation of cosmetic cream

Cosmetic cream was made using the modified formula of Banker

and Rhodes (1995). Two mixtures were prepared separately: mixture A made up of water (97.4%), glycerol (1.7%) and hydroxyl-propyl-methyl-cellulose (HPMC) (0.9%) and mixture B made up of wax (15.1%), kernel oil (82.9%) and shea butter (2.0%). The two mixtures were mixed at 70°C in a water bath for 4 min and then cooled on ice bath for 2 min, followed by addition of sodium benzoate (1%) as cream preservative. Finally, 1.25, 2.5 and 5 g of essential oil of *A. citratum* were added to 100 g of cream to yield the final concentrations of oil in the cream of 1.25, 2.5 and 5% respectively. Control cream was made up of cream without any antibacterial and without essential oil.

Bacterial infection induced with *S. aureus* MRSA03

Prior to infection, rats were starved for 12 h and anesthetized using ketamine (100 mg kg^{-1} body weight) under sterile conditions. The dorsal fur of the animals was shaved with an electric clipper and the site of the infection (3 cm diameter) was outlined on the back of the animals using a marker pen, then disinfected with ethanol 95° and abraded with sandpaper (N°120) 1 min before inoculation. Then, rats were inoculated at the site of infection with 10^8 CFU/ml of Methicillin-Resistant *S. aureus* (MRSA) suspension prepared from an overnight culture (Kugelberg et al., 2005). One group was not infected and not treated (uninfected group). Infected rats were divided into six groups of three animals each (three control groups and three test groups). The first control group was not treated (untreated group), the second and third control groups received cream without essential oil (blank group) and Baneocin® (2%) 250 UI/5000 UI (Baneocin group), respectively. The three other groups were treated with cream-based essential oil from seed of *A. citratum* at 5, 2.5 and 1.25% (w/w), respectively. Treatment started 24 h after the establishment of the infection by dermal application of 0.1 g of cream-based on essential oil and Baneocin® once per day for 14 consecutive days.

Evaluation of *in vivo* antibacterial activity

The efficacy of the treatment was evaluated on a clinical and mycological basis. Clinical efficacy was based on changes observed at the site of infection during the test. These observations were based on measurements taken at the site of infection from two perpendicular lines drawn on the site and measuring on the one hand, the evolution of the inflammation (inflammation percentage) and on the other hand, the evolution of epithelialization (epithelialization percentage). These parameters were noted every three days.

$$\text{Inflammation } \% = \frac{\text{Horizontal diameter} + \text{Vertical diameter}}{2} \quad (1)$$

$$Epithelialization\ \% = \frac{\text{Horizontal diameter} + \text{Vertical diameter}}{2} \quad (2)$$

The epithelialization time, that is, the number of days required for the scar to fall off without residual gross injury, was determined as the epithelialization period (Ameri et al., 2013). For the mycological efficacy, animals were anesthetized with chloroform vapors at the end of the treatment and a skin sample (4 g) was taken, ground in a porcelain mortar in the presence of 4 ml of physiological saline (NaCl) 0.09%) and the ground product obtained was centrifuged at 3000 rpm for 15 min. The supernatant obtained after centrifugation was decanted and used for culture on Mannitol Salt Agar medium in order to count the number of Colony Forming Units (CFU) of bacteria per gram of skin. Body weights of animals were measured before sacrifice. The organs (liver, kidneys, lungs, heart and spleen) were carefully dissected out, blotted, observed macroscopically and weighed immediately using a Sartorius electronic balance. The relative organ weight (ROW) of each animal was then calculated as follows:

$$\text{ROW} = \frac{\text{Absolute organ weight (g)x } 100}{\text{Body weight of rat on day of sacrifice (g)}} \quad (3)$$

Statistical analysis

Data were subjected to the one-way analysis of variance (ANOVA) and recorded as mean ± standard deviation (SD) and where differences exist, means were compared using Waller Duncan test at 0.05 significant levels. All analyses were performed using the Statistical Package for Social Sciences (SPSS, version 12.0) software.

Ethics

The experiments were carried out observing the welfare of animals as recommended by World Health Organization (WHO). Moreover, all procedures involving animals were carried out in strict compliance with the rules and regulations of local Ethics Committee.

RESULTS

Yield of extraction and chemical composition of essential oils

The essential oils of *A. citratum*, *A. daniellii* and *M. myristica* were translucent with extraction yields of 1.0 ± 0.1%, 1.1 ± 0.1% and 2.4 ± 0.5%, respectively whereas that of *P. capense* seed was green with the extraction yield of 1.6 ± 0.3%. A total of 6 compounds were identified in the essential oil of *A. citratum* seeds (Table 1). These compounds were mainly composed of monoterpenes with a marked predominance of oxygenated monoterpenes (geraniol, 96.80%) and the absence of sesquiterpenes. Eighteen compounds were identified in the essential oils of *A. daniellii* belonging mainly to oxygenated monoterpenes (Table 1). The essential oil of *A. daniellii* seed was predominantly constituted of eucalyptol (48.8 ± 9.9%), α-terpineol (21.7 ± 2.6%) and geraniol (10.5 ± 1.2%). A total of 25 compounds were identified in the essential oil of *P. capense*. The essential oil of this plant species was slightly more monoterpenic (56.2 ± 4.4%) than sesquiterpenic (41.4 ± 4.8%) and was mostly composed of hydrocarbonated compounds (90.7 ± 5.9%) than oxygenated compounds (6.8 ± 5.8%). The major compounds found in the essential oils of *P. capense* are β-pinene (37.3 ± 1.7%), germacrene D (9.8 ± 4.1%), trans-β-caryophyllene (8.8 ± 3.8%), α-pinene (8.6 ±

Table 1. Qualitative and quantitative compositions of essential oils from the studied spices.

Compound	RI	*A. citratum*	*A. daniellii*	*P. capense*	*M. myristica*
Monoterpenes		**99.3 ± 0.1**	**93.3 ± 2.4**	**56.3 ± 4.3**	**79.3 ± 2.8**
Monoterpene hydrocarbons		**0.3 ± 0.2**	**3.2 ± 1.1**	**52.3 ± 4.4**	**74.6 ± 3.0**
Ethylether	689	-	-	-	0.9
α-Thujene	921	-	-	-	1.1 ± 0.2
α-Pinene	927	-	0.5	8.1 ± 0.9	3.1 ± 0.4
Sabinene	968	-	-	4.7 ± 1.3	-
β-Pinene	971	-	2.2 ± 0.3	36.5 ± 1.9	-
Myrcene	990	-	-	-	1.0 ± 0.2
β-Myrcene	995	0.2±0.0	-	0.7 ± 0.1	2.0 ± 0.2
α-Phellandrene	1002	-	-	-	61.5 ± 5.1
δ-3-Carene	1006	-	-	1.4 ± 0.1	-
p-Cymene	1020	-	-	-	1 ± 0.1
Limonene	1024	-	1.3 ± 0.2	1.5 ± 0.5	5 ± 0.6
1,8-Cineole	1027	0.3	-	-	-
Oxygen-containing monoterpenes		**99.0 ± 0.1**	**90.1 ± 3.4**	**4 ± 1.5**	**4.7** ± 1.0
Eucalyptol	1026	-	**48.8 ± 9.9**	-	-
Linalool	1100	1.3 ± 0.2	2.6 ± 0.7	1.3 ± 0.4	2.8 ± 0.2
Linalyl propionate	1164	-	0.7	-	-
4-Terpineol	1174	-	2.9 ± 1.9	1.5 ± 1.2	-
α-Terpineol	1188	-	**21.7 ± 2.6**	-	1 ± 0.1
Sabinol	1200	-	**-**	-	1.1 ± 0.3
Geraniol	1257	**96.8 ± 0.3**	**10.5 ± 1.2**	**-**	**-**
Geranial	1274	0.3 ± 0.05	0.4	-	-
Bornyl acetate	1288	-	-	1.8 ± 0.3	-
Geranyl acetate	1388	0.5 ± 0.1	3.0 ± 1.0	-	-
Sesquiterpenes		**-**	**3.8 ± 2.1**	**41.2 ± .2**	**18.9** ± 3.3
Sesquiterpene hydrocarbons		**-**	**2.6 ± 1.1**	**34.3 ± 4.2**	**8.3** ± 2.6
α-Cubebene	1357	-	-	0.5 ± 0.05	-
α-Copaene	1380	-	-	0.6 ± 0.1	-
β-Cubebene	1393	-	-	2 ± 0.6	-
Trans-β-caryophyllene	1424	-	1.6 ± 0.5	6.5 ± 1	-
Santalen	1425	-	-	-	1.3 ± 0.3
β-Selimene	1460	-	-	1.1 ± 0.1	-
α-Amorphene	1482	-	-	0.9 ± 0.0	-
Germacrene D	1488	-	-	7.4 ± 1.7	-
α-Amorphene	1500	-	-	2 ± 0.7	0.5
α-Muurolene	1506	-	-	-	1.0 ± 0.3
γ –Cadinene	1522	-	1.5 ± 0.2	-	1.9 ± 0.4
Naphthalene	1524	-	-	10.7 ± 0.4	-
δ-Cadinene	1532	-	0.4	1.5 ± 0.5	4.2 ± 1.0
Germacrene B	1570	-	0.7	2.4 ± 0.05	-
Oxygen-containing sesquiterpenes		**-**	**1.7 ± 0.4**	**6.9 ± 4.1**	**10.6 ± 0.8**
Octabicyclooctanol	1350	-	0.4	-	-
Germacradienol	1588	-	-	-	7.9 ± 0.6
Caryophyllene oxide	1598	-	1.2 ± 0.2	1.2 ± 0.2	-
Guaicol	1609	-	-	2.4 ± 0.8	-
t-Muurolol	1659	-	0.7	-	1.4 ± 0.1
α-Amorphene	1661	-	-	1.5	-
t-Muurolol	1672	-	-	0.9 ± 0.4	1.3 ± 0.2
Azulene methanol	1684	-	-	1.2 ± 0.6	-

RI: Retention indice.

0.9%), naphthalene (8.3 ± 3.5%) and sabinene (4.8 ± 1.1%). Twenty compounds were identified in the essential oil of *M. myristica* belonging to monoterpenes (84.8±6.3%) especially monoterpene hydrocarbons (81.8±8.2%). The main compounds are α-phellandrene (61.5±5.1%), germacradienol (7.9 ± 0.6%) and δ-cadinene (4.2 ± 1.1%).

Antimicrobial activity of essential oils

In this study, the antibacterial and antifungal activities of essential oils from the studied spices were evaluated using broth micro dilution method against pathogenic microorganisms including yeasts, dermatophytes, Gram-positive and Gram-negative bacteria. The results summarized in Tables 2 to 4 showed that essential oils were active against the tested microorganisms with MIC values varying from 8 to 4096 µg/ml. Essential oil of *A. citratum* was active against all the tested bacteria (100%), while essential oils of *A. daniellii, P. capense* and *M. myristica* were active against only thirteen of the seventeen tested bacteria (76.5%) (Table 2). Interestingly, the antibacterial activity of *A. citratum* was significant particularly against *S. aureus* MSSA01, *B. subtilis* and *E. coli* S2 (MIC = 8 µg/ml and MBC = 32 µg/ml). *E. coli* EC136 and *Klebsiella spp* (clinical isolates) were the most resistant bacteria to the essential oils. The bactericidal effect of essential oils was observed with *A. citratum*, *A. daniellii*, *P. capense* and *M. myristica* essential oils on 11/17 (64.70%), 8/17 (47.05%), 7/17 (41.17%) and 6/17 (35.29%) of tested bacteria, respectively.

The results also showed that essential oils were active against the tested fungi with MIC values ranging between 32 and 4096 µg/ml (Table 3). Essential oils of *A. citratum*, *P. capense*, *A. daniellii* and *M. myristica* were active against all the fungal species (100%), while essential oil of *A. citratum* was the most active. *C. albicans* ATCC 9002 was the most sensitive yeast (MIC = 256 - 512 µg/ml), whereas *C. lipolithica* (MIC = 512 - 4096 µg/ml) was the most resistant yeast. The essential oils from the studied spices displayed different degrees of antifungal activity against *C. albicans* and *Cryptococcus neoformans* resistant isolates with MIC values ranging between 128 and 4096 µg/ml (Table 4).

The essential oils of *A. citratum*: 23/28 (82.14%) were the most active followed in a decreasing order by those of *A. daniellii*: 13/28 (46.42%), *P. capense*: 6/28 (21.42%) and *M. myristica*: 6/28 (21.42%). The combination of essential oils of *A. citratum* and *A. daniellii* (1:1) was active against 100% of the tested isolates, while the combination of essential oils of *P. capense* and *M. myritica* (1:1) was only active against 3/28 (10.71%) of the tested isolates (Table 4). Moreover, the antifungal activity of the combination of essential oils of *A. citratum* and *A. daniellii* (1:1) was greater than that of these essential oils used alone. However, the combination of essential oils of *P. capense* and *M. myritica* (1:1) reduced their antifungal activities compared to those of these essential oils used alone. In general, the MFC and MBC values are fourfold lesser than the MIC values on the corresponding microorganism; suggesting that the tested essential oils have fungicidal / bactericidal effects.

***In vivo* antibacterial activity**

The therapeutic effect of a cream based on essential of *A. citratum*, which was found to be the most active essential oil, was evaluated against dermatosis induced with a Methicillin-Resistant *S. aureus* (MRSA) in rats. Animals infected with *S. aureus* showed visible inflammation 24 h post infection, characterized by the redness and swelling of the skin at the sites of inoculation. The percentage of inflammation reduces progressively during the treatment untill it reaches at 0% after 15 days of treatment in male and female rats (Figure 1).

In male rats, the epithelialization times were 9 days with 1.25 and 5% of cream and uninfected group and 12 days for the other groups (Figure 2). In female rats, the epithelialization times were 6 days for the groups treated with 1.25 and 5% of cream; 9 days for the untreated group and 12 days for the other groups (Figure 2). These results indicate that rats treated with 1.25 and 5% of cream and Baneocin exhibited shorter epithelialization times than controls (untreated, uninfected and blank groups) and those treated with 2.5% of cream.

The results also show that treatment significantly ($p < 0.05$) reduced the number of Colony Forming Units (CFU) of bacteria at the infection site (Figure 3). In male rats, the number of CFU of bacteria at the infection site comparable with those of uninfected group were noted with 5, 2.5 and 1.25% of cream based on essential oil from *A. citratum* and Baneocin after 14 days of treatment (Figure 3). In female rats, the number of CFU of bacteria at the infection site in the groups treated with 5% of cream based on essential of *A. citratum* and Baneocin were lower than those obtained in the uninfected group. These results suggest that 5% of cream based on essential of *A. citratum* and Baneocin may be used to treat methicillin resistant *S. aureus* dermatosis induced in rats. Cream without essential oil (blank treatment) has a number of CFU of bacteria at the infection site comparable to that of untreated group. Organ-to-body weight ratio, an index often used in toxicological evaluations, was not significantly altered by the treatments (Table 5).

DISCUSSION

Differences in the extraction yields were noted between

Table 2. Antibacterial activities (MIC and MBC in µg/ml) of essential oils from the tested spices.

Bacteria	*A. citratum*			*A. daniellii*			*P. capense*			*M. myristica*			AMOX	
	MIC	MBC	R	MIC	MBC	R	MIC	MBC	R	MIC	MBC	R	MIC	MBC
S. aureus ATCC25923	4096	>4096	-	8	>4096	-	32	>4096	-	4096	>4096	-	1	2
S. aureus ATCC25923	1024	>4096	-	>4096	-	-	2048	>4096	-	512	>4096	-	0.5	2
S. aureus	32	32	1	128	512	4	2048	4096	2	2048	>4096	-	2	2
S. aureus MSSA01	8	32	4	32	128	4	256	256	1	512	4096	8	128	128
S. aureus MRSA03	64	512	8	512	512	1	1024	2048	2	1024	2048	2	32	32
S. aureus MRSA04	32	256	8	32	64	2	512	2048	4	512	>4096	-	32	32
S. aureus ST120	2048	>4096	-	2048	>4096	-	>4096	>4096	-	>4096	-	-	0.50	1
B. subtilis	8	32	4	512	4096	8	8	64	4	1024	4096	4	4	4
E. coli ATCC10536	1024	>4096	-	1024	>4096	-	1024	>4096	-	512	>4096	-	0.5	1
E. coli EC136	2048	>4096	-	>4096	-	-	>4096	-	-	>4096	-	-	4	4
E. coli S2(1)	8	32	4	8	64	8	2048	>4096	-	8	128	16	256	256
Entero-aggregative E. coli	256	512	2	1024	>4096	-	512	>4096	-	>4096	-	-	16	16
E. adecarboxylate	1024	2048	2	>4096	-	-	>4096	-	-	1024	>4096	-	32	32
E. aerogenes	512	2048	4	512	>4096	-	512	>4096	-	2048	2048	1	2	4
P. aeruginosa ATCC27853	32	256	8	32	256	8	32	512	16	32	1024	32	4	4
Klebsiella spp	512	>4096	-	>4096	-	-	>4096	-	-	>4096	-	-	32	32
Shigella flexneri	256	1024	4	512	2048	4	1024	1024	1	512	>4096	-	16	16

MIC: Minimum inhibitory concentrations; MBC: Minimum bactericidal concentrations; R = MBC/MIC; AMOX: amoxicilline.

Table 3. Antifungal activities (MIC and MFC) of essential oils from the tested spices.

Yeast	*A. citratum*			*A. daniellii*			*P. capense*			*M. myristica*			Reference*		
	MIC	MFC	R	MIC	MFC	R	MIC	MFC	R	MIC	MFC	R	MIC	MFC	R
C. albicans ATCC 9002	256	256	1	512	512	1	512	512	1	512	512	1	0.50	1	2
C. albicans ATCC 1663	1024	1024	1	1024	1024	1	512	512	1	1024	1024	1	2	2	1
C. albicans IS1	256	512	2	512	1024	2	512	1024	2	256	1024	4	4	4	1
C. parapsilosis	512	1024	2	512	1024	1	512	1024	2	512	1024	2	0.50	1	2
C. parapsilosis ATCC 22019	1024	1024	1	1024	1024	1	512	1024	2	1024	1024	1	1	1	1
C. krusei ATCC 6258	512	1024	2	2048	2048	1	1024	1024	1	1024	1024	1	0.50	1	2
C. krusei	1024	1024	1	256	1024	4	1024	1024	1	512	1024	1	32	32	1
C. tropicalis ATCC 750	1024	2048	2	2048	2048	1	1024	1024	1	1024	1024	1	0.50	1	2
C. lipolithica	2048	2048	1	4096	4096	1	512	1024	2	1024	1024	1	0.50	1	2
C. haemophilus	256	1024	4	4096	4096	1	512	1024	2	512	1024	1	0.50	1	2
Dermatophytes															
M. gypseum E1420	256	4096	16	4096	4096	1	512	512	1	2048	4096	2	0.50	1	2

Table 3. Cont.

T. violaceum	32	1024	32	128	256	2	512	512	1	2048	4096	2	1	1	1				

MIC: Minimum Inhibitory Concentrations; MFC: Minimum Fungicidal Concentrations; - : not determined; R = MFC/MIC; *Nystatin for yeast and griseofulvin for dermatophytes

Table 4. Antifungal activities (MIC and MFC in µg/ml) of essential oils from the tested spices against *Candida albicans* and *Cryptococcus neoformans* resistant isolates.

Yeasts	*A. citratum*			*A. danielli*			*P. capense*			*M. myristica*			*Ac/Ad*			*Pc/Mm*			*Nystatin*		
	MIC	MFC	R	MIC	MFC	R	MIC	MFC	R	MIC	MFC	R	MIC	MFC	R	MIC	MFC	R	MIC	MFC	R
Amphotericin B and nystatin resistant *C. albicans*																					
Ca Da11	128	512	4	1024	1024	1	>4096	-		>4096	-	-	4096	4096	1	>4096	-	-	16	32	2
Ca E01	512	512	1	1024	1024	1	>4096	-	-	>4096	-	-	4096	4096	1	>4096	-	-	32	32	1
Ca F021	1024	1024	1	2048	2048	1	>4096	-	-	1024	1024	1	4096	4096	1	2048	-	-	32	128	4
Ca F045	1024	1024	1	2048	2048	1	>4096	-		>4096	-	-	4096	4096	1	>4096	-	-	32	128	4
Ca K42	2048	2048	1	2048	2048	1	>4096		-	2048	2048	1	4096	4096	1	>4096	-	-	32	32	1
Ca D10	4096	4096	1	4096	4096	1	4096	4096	1	4096	4096	1	2048	4096	2	2048	2048	1	16	64	4
Ca F066	4096	>4096	-	2048	-	-	4096	-	-	>4096	-	-	4096	4096	1	>4096	-	-	-	-	-
Ca F005	4096	4096	1	>4096	-	-	>4096	-	-	>4096	-	-	4096	4096-	1	>4096	-	-	256	256	1
Ca F017	4096	4096	1	>4096	-	-	>4096	-	-	>4096	-	-	4096	4096	1	>4096			32	64	2
Ca F057	4096	4096	1	4096	4096	1	2048	2048	1	>4096	-	-	2048	4096	2	>4096	-	-	128	128	1
Ca K14	512	512	1	1024	2048	2	>4096	-	-	>4096	-	-	512	1024	2	>4096	-	-	64	64	1
Ca F015	1024	1024	1	2048	2048	1	>4096	-	-	>4096	-	-	2048	2048	1	>4096	-		64	64	1
Ca F023	>4096	-	-	>4096	-	-	>4096	-	-	>4096	-		4096	4096	1	>4096	-	-	256	256	1
Ca F040	>4096	-	-	>4096	-	-	>4096	-	-	>4096	-		2048	4096	2	>4096	-	-	256	256	1
Ca F002	>4096	-	-	>4096	-	-	>4096	-	-	>4096	-		4096	4096	1	>4096	-	-	128	256	2
Ca F041	512	512	1	1024	1024	1	4096	4096	1	>4096	-	-	2048	2048	1	>4096	-	-	32	128	4
Ca K072	2048	2048	1	>4096	-	-	>4096	-	-	>4096	-	-	4096	4096	1	>4096	-	-	128	256	2
Ca F049	2048	-	-	4096	-	-	>4096	-	-	>4096	-	-	2048	2048	1	>4096	-		128	256	2
Ca F026	2048	4096	2	>4096	-	-	>4096	-	-	>4096		-	4096	4096	1	>4096	-	-	256	256	1
Ca K22	4096	-	-	>4096	-	-	>4096		-	>4096	-	-	4096	>4096	-	>4096	-	-	128	256	2
Nystatin resistant *C. neoformans*																					
CN	4096	4096	1	>4096	-	-	4096	-	-	4096	-	-	2048	>4096	-	2048	-	-	256	256	1
CN 169	4096	4096	1	>4096	-	-	>4096	-	-	>4096	-	-	4096	4096	1	>4096	-	-	256	256	1
CN 047	1024	4096	4	>4096	--	-	>4096	-	-	>4096	-	-	4096	4096	1	>4096	-	-	128	256	2
CN 091	4096	4096	1	>4096	-	-	>4096	-	-	>4096	-	-	4096	>4096	-	>4096	-	-	256	256	1
CN 046	>4096	-	-	>4096	-	-	>4096	-	-	4096	-	-	512	512	1	>4096	-	-	32	32	1
CN 158	4096	-	-	4096	-	-	1024	-	-	4096	4096	1	2048	4096	2	>4096	-	-	128	256	2
CN 173	>4096	-	-	>4096	-	-	>4096	-	-	>4096	-	-	4096	4096	1	>4096	-	-	256	256	1
CN 096	4096	4096	1	>4096	-	-	>4096	-	-	>4096	-	-	4096	4096	1	>4096	-	-	128	128	1

MIC: Minimum Inhibitory Concentrations; MFC: Minimum Fungicidal Concentrations; - : not determined; R = MFC/MIC; Ac/Ad: combination of essential oils of *A. citratum* and *A. daniellii* (1:1), Pc/Mm : combination of essential oils of *P. capense* and *M. myristica* (1:1).

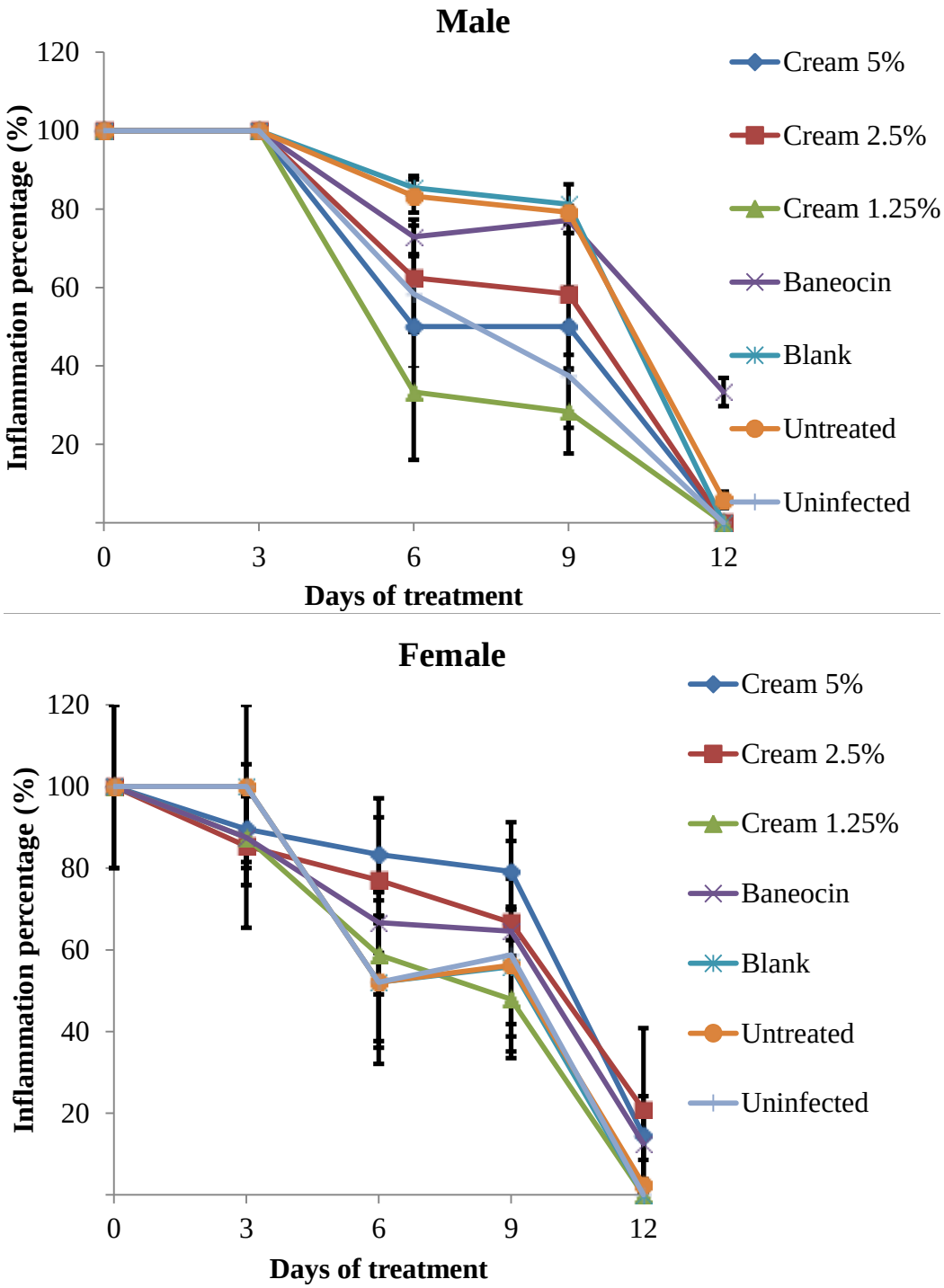

Figure 1. Evolution of the inflammation in male and female rats during the treatment.

the studied essential oils. These differences may be due to the different plant species used. The extraction yield of the essential oil from *A. citratum* seeds is lower than that obtained after 4 h of hydrodistillation from *A. citratum* seeds (2.8%) bought at Kribi in Cameroon (Amvam Zollo et al., 2002). The extraction yield of the essential oil from *A. daniellii* dried seeds is comparable to that obtained from the fresh seeds of this plant species (1.3%) collected from Nigeria (Essiens et al., 2017).

The extraction yield from *P. capense* seed (1.6%) is slightly similar to that obtained in the literature (1.98%) after 3 h of hydrodistillation from dried ground seeds

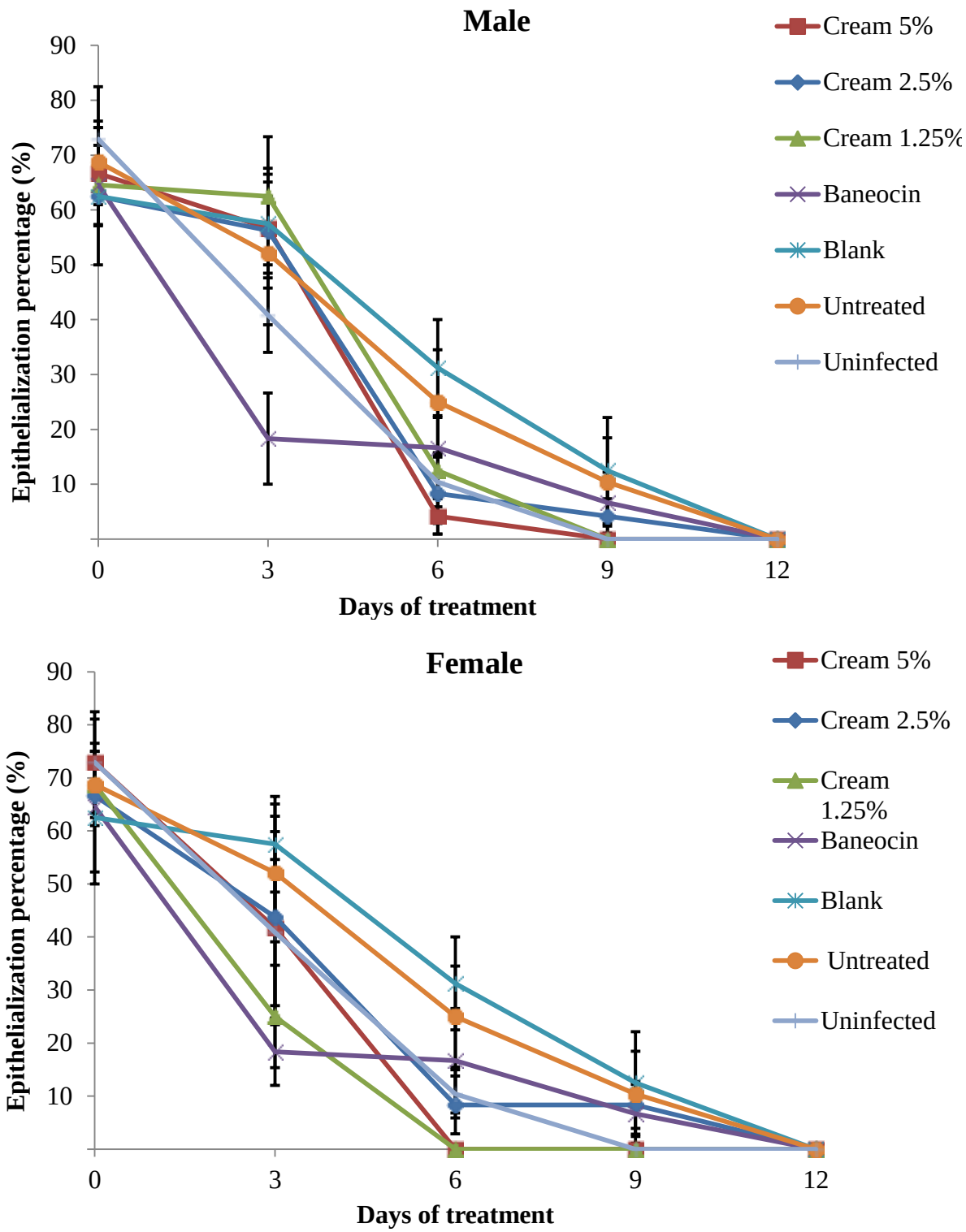

Figure 2. Evolution of epithelialization percentages in male and female rats during the treatment. Bars represent the mean ± SD of three independent experiments carried out.

(Woguem et al., 2013). This result was also slightly similar with that obtained by Tchoumbougnang et al. (2009), after 5 h of hydrodistillation (1.51%). The extraction yield of the essential oil from *M. myristica* seed (2.4%) was higher than that obtained after 7 h of hydrodistillation of this plant species (0.21%), in

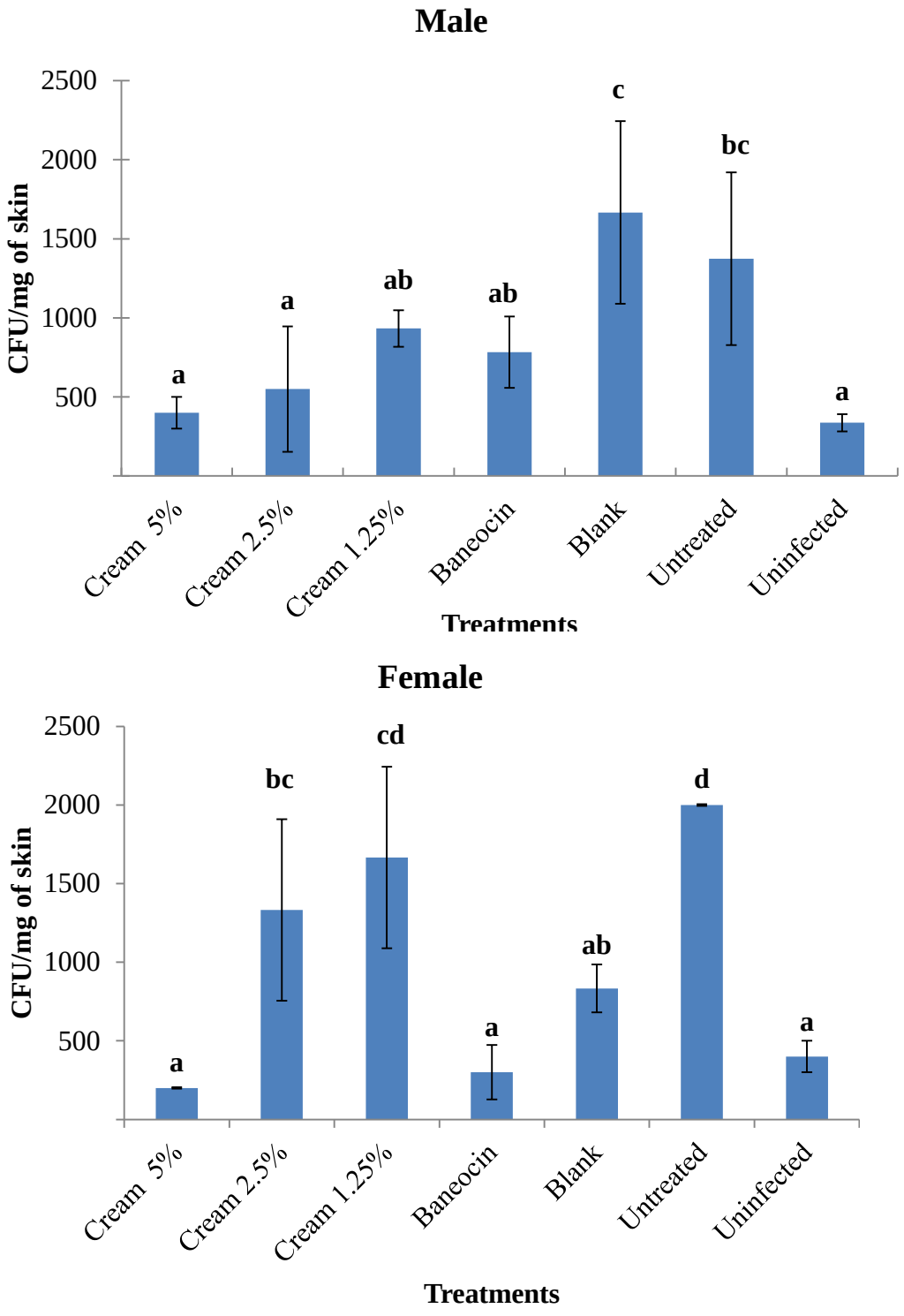

Figure 3. Effect of cosmetic cream based on essential oil of *A. citratum* on bacterial load (CFU/mg) on male and female rats after 14 days of treatment. Bars represent the mean ± SD of three independent experiments. Letters a-d indicate significant differences between samples according to one way ANOVA and Waller Duncan test; $p<0.05$.

Democratic Republic of Congo (Cimanga et al., 2002). However, this result was comparable to those obtained after 5 h of hydrodistillation from the same plant species collected in Kribi-Cameroon (2.72%) and Gore-Chad

Table 5. Relative organ weight in male and female rats at the end of the treatment.

Sex	Treatment	Relative organ weight (g/100 g b.w.)				
		Heart	Lung	Liver	Kidney	Spleen
Male	Cream 5%	0.53 ± 0.05^{a}	0.94 ± 0.14^{a}	5.78 ± 0.62^{a}	0.88 ± 0.14^{a}	0.57 ± 0.3^{a}
	Cream 2.5%	0.62 ± 0.11^{a}	1.03 ± 0.08^{a}	6.52 ± 0.48^{a}	1.00 ± 0.1^{ab}	0.63 ± 0.11^{ab}
	Cream 1.25%	0.52 ± 0.06^{a}	0.99 ± 0.11^{a}	5.99 ± 0.78^{a}	1.05 ± 0.14^{abc}	0.31 ± 0.07^{a}
	Baneocin	0.57 ± 0.08^{a}	1.03 ± 0.17^{a}	6.38 ± 0.13^{a}	1.08 ± 0.1^{abc}	0.46 ± 0.19^{a}
	Blank	0.62 ± 0.06^{a}	1.09 ± 0.15^{a}	7.05 ± 0.84^{a}	1.19 ± 0.1^{bc}	0.98 ± 0.23^{bc}
	Untreated	0.56 ± 0.02^{a}	0.96 ± 0.21^{a}	6.40 ± 0.57^{a}	1.08 ± 0.14^{abc}	1.09 ± 0.19^{cd}
	Uninfected	0.63 ± 0.02^{a}	0.86 ± 0.02^{a}	6.64 ± 0.65^{a}	1.23 ± 0.01^{c}	1.41 ± 0.01^{d}
Female	Cream 5%	0.41± 0.02^{ab}	0.84 ± 0.13^{a}	5.19 ± 0.35^{abc}	0.92 ± 0.05^{a}	0.53 ± 0.1^{abc}
	Cream 2.5%	0.41 ± 0.03^{ab}	0.80 ± 0.06^{a}	4.68 ± 0.8^{abc}	0.90 ± 0.06^{a}	0.46 ± 0.06^{ab}
	Cream 1.25%	0.47 ± 0.03^{abc}	0.98 ± 0.21^{a}	5.00 ± 0.49^{ab}	1.00 ± 0.08^{a}	0.51 ± 0.07^{abc}
	Baneocin	0.32 ±0.23^{a}	0.60 ± 0.43^{a}	3.92 ± 2.89^{a}	0.81± 0.6^{a}	0.41 ± 0.31^{a}
	Blank	0.61 ± 0.08^{c}	1.11 ± 0.36^{a}	6.52 ± 0.71^{bc}	1.21 ± 0.12^{a}	1.00 ± 0.12^{bc}
	Untreated	0.59 ± 0.01^{bc}	1.02 ± 0.02^{a}	7.32 ± 0.81^{c}	1.29 ± 0.15^{a}	1.03 ± 0.59^{c}
	Uninfected	0.57 ± 0.02^{bc}	1.00 ± 0.05^{a}	6.24 ± 0.04^{abc}	1.18 ± 0.03^{a}	0.44 ± 0.13^{a}

Data represent the mean ± SD of three independent experiments. For the same organ and sex, letters a-d indicate significant differences between samples according to one way ANOVA and Waller Duncan test; $p<0.05$.

(1.87%) (Bakarnga-Via et al., 2014). The differences in the extraction yields and those in the literature may be due to the place and period of harvesting of the plant, the variety of plant species used, the duration of extraction, the environmental variations (Zheljazkov et al., 2015).

The findings of the present study showed that the essential oil of *A. citratum* seeds was mainly composed of monoterpene with a predominance of oxygenated monoterpenes (geraniol, 96.80%) and the absence of sesquiterpenes. These results are in agreement with those of Amvam Zollo et al. (2002) who obtained an essential oil consisting mainly of oxygenated monoterpenes with a predominance of geraniol (70%). The results of the chemical composition of essential oils from *A. daniellii* seeds were in agreement with those of the early reports. Indeed, Essien et al. (2017) reported a high content of 1,8-cineole (53.4%), α-terpineol (12.2%) and β-pinene (9.1%) in the essential oil from *A. danielli* seeds whereas the main compounds identified from the essential oils of this plant collected in Sao Tome were 1,8-cineole (25.5-34.4%), β-pinene (14.1-15.2%) and α-terpineol (9.9-12.1%) (Martins et al., 2001). Moreover, Adegoke et al. (1998) recorded 1,8-cineole (59.8%), β-pinene (13.2%) and α-terpineol (9.3%) in high proportions in the same essential oil from Nigeria, while 1,8-cineole (48.9%) was the main compound of *A. danielli* seeds collected in Cameroon (Menut et al., 1991).

The chemical composition of the essential oil of *P. capense* seed was comparable to those reported in the literature (Woguem et al., 2013, Amvam Zollo et al., 1998, Tchounbougnang et al., 2009). Indeed, previous study showed that essential oil obtained from *P. capense* seed is mainly constituted by monoterpenes (64.7%) with predominance of hydrocarbons (56.5%). Major constituents were monoterpene hydrocarbons: α-pinene (8.9%), sabinene (10.0%), β-pinene (33.2%) and sesquiterpene hydrocarbons: α-caryophyllène (6.3%) and germacrene D (3.8%) (Woguem et al., 2013). Other studies also reported that major volatile compounds of fruits from Western Cameroon were monoterpene hydrocarbons: α-pinene (10.5-14.4%), sabinene (14.7-17.4%), β-pinene (46.8-59.3%) and sesquiterpene hydrocarbons: (E)-caryoplyllene (3.4-4.0%) and germacrene D (2.5-5.2%) (Amvam Zollo et al., 1998; Tchoumbougnang et al., 2009).

However, the extraction yields were slightly different with respect to those obtained in this study. The results of the chemical composition of essential oils of *M. myristica* seeds are comparable to those of Bakarnga-Via et al. (2014), who found 67.1% of α-phellandrene and 4.2% of α-pinene in a sample collected from Kribi (Cameroon) and 52.7% of α-phellandrene and 14.9% of limonene in a sample collected from Gore (Chad). Our results are also in agreement with those of Lamaty et al. (1987), who found 48.8% of α-phellandrene in a sample collected in Yaounde (Cameroon); however, the extraction yields were slightly different.

Differences in antimicrobial activity were noted between the studied essential oils. These differences may be due to the different phytoconstituents identified in these essential oils. Indeed, the antimicrobial activities of medicinal plants are correlated with the presence in their extracts of one or more classes of bioactive secondary metabolites (Reuben et al., 2008).

According to established criteria, MIC values in the range of 100- 1000 µg/ml are indications that botanicals

have antimicrobial activities (Simoes et al., 2009). Also antimicrobial activity of edible plant extracts or extracts from edible parts of plants is considered highly active if MIC values are below 100 µg/ml, significantly active if 100 ≤ MIC ≤ 512 µg/ml, moderately active if 512 < MIC ≤ 2048 µg/ml, low activity if MIC > 2048 µg/ml and not active if MIC > 10 000 µg/ml (Tamokou et al., 2017).

Amongst essential oils that showed the highest activities (MICs < 100 µg/ml), there are essential oil of *A. citratum* against *S. aureus*, *S. aureus* MSSA01, *S. aureus* MRSA03, *S. aureus* MRSA04, *B. subtilis*, *E. coli* S2(1), *entero-aggregative E. coli* and *P. aeruginosa* ATCC 27853; essential oil of *A. daniellii* against *S. aureus* ATCC 25923, *S. aureus* MSSA01, *S. aureus* MRSA04, *E. coli* S2(1) and *P. aeruginosa* ATCC 27853; essential oil of *P. capense* against *S. aureus* ATCC 25923, *B. subtilis* and *P. aeruginosa* ATCC 27853 and essential oil of *M. myristica* against *E. coli* S2(1) and *P. aeruginosa* ATCC 27853.

The results of antimicrobial activities of *A. citratum*, *P. capense*, *A. daniellii* and *M. myristica* clearly indicate that the essential oils from these plants have antibacterial and antifungal properties. These data corroborate those of the previous works (Tatsadjieu et al., 2003; Fasoyiro and Adegoke, 2007; Steenkamp et al., 2007; Samie et al., 2010). Collectively, the present study showed that the tested essential oils have antimicrobial activities and are effective against methicillin resistant *S. aureus*, amphotericin B and nystatin resistant *Candida albicans* and *Cryptococcus neoformans*. The overall results of the present investigation confirmed the traditional uses of the studied spices in the treatment of microbial infections. Taking into account the medical importance of the tested microorganisms, this result can be considered as promising in the perspective of developing new antimicrobial agents from plant origin. During the MIC and MMB determination, we have noted that MMC values are in general fourfold lesser than the MIC values on the corresponding microorganism; suggesting that the studied essential oils have a microbicidal effect on the sensitive microorganisms (Mims et al., 1993).

Combinations of antibiotics can lead to synergistic effects especially during the therapy of fungal infections. These combinations have been recognized as being able to delay the emergence of resistant strains of microorganisms (Aiyegoro and Okoh, 2009). The effect of synergy between plant-derived essential oils makes it possible to use essential oils when their efficacy alone is reduced (Nascimento et al., 2000). These observations could explain the evaluation of the antifungal activity of the combination of essential oils of the studied plants, because in addition to substances having direct antifungal activity, it has been demonstrated that within plants, other substances can act as adjuvants by modulating the activity of antifungal agents (Veras et al., 2012). The antifungal activities of the combination of essential oils of *A. citratum* and *A. daniellii* (1:1) were greater than those of these essential oils used alone. However, the combination of essential oils of *P. capense* and *M. myritica* (1:1) reduced their antifungal activities compared to those of these essential oils used alone. The above findings suggest that the combination of essential oils of *A. citratum* and *A. daniellii* (1:1) has synergistic effect, whereas the combination of essential oils of *P. capense* and *M. myritica* (1:1) has an antagonistic effect. The monoterpene and sesquiterpene compounds found in these essential oils would be responsible for the observed effects with respect to certain *C. albicans* and *C. neoformans* resistant isolates.

The results of the therapeutic effect of the cream based on essential oil of *A. citratum* seeds against dermatosis induced with methicillin resistant *S. aureus* in rats revealed that epithelialization time was significantly shorter in animals treated with cream based on essential oil of *A. citratum* compared to negative control groups. Indeed, epithelialization involves the proliferation and migration of epithelial cells through the wound bed (Sanwal and Chaudhary, 2011). Therefore, a shorter epithelialization time could be due to facilitated epithelial cell proliferation and/or increased viability of epithelial cells (Mulisa et al., 2015).

Thus, the shorter epithelialization time in the animals treated with the essential oil reinforce the hypothesis according to which the essential oil of *A. citratum* has a potential application as an antibacterial healing agent. Moreover, the fact that the cream based on essential oil of *A. citratum* significantly reduced the number of Colony Forming Units (CFU) of bacteria at the infection site compared to the negative controls also supports the *in vivo* antibacterial properties of *A. citratum* essential oil. To the best of our knowledge, this is the first report on the therapeutic effect of the essential oil from *A. citratum.*

Conclusion

The overall results of the present investigation indicated that the main compounds identified in the essential oils are geraniol (98%) for *A. citratum*; eucalyptol (48.8%), α-terpineol (21.7%) and geraniol (10.5%) for *A. daniellii*; β-pinene (37.3%), germacrene D (9.8%), trans-β-caryophyllene (8.8%), α-pinene (8.6%), naphthalene (8.3%) and sabinene (4.8%) for *P. capense*; α-phellandrene (61.5%), germacradienol (7.9%) and δ-cadinene (4.2%) for *M. myristica.* The tested essential oils possess antimicrobial activities which could be a function of either the individual or the additive effects of the identified volatile components. The cream based on essential oil of *A. citratum* (5%) can be used in the treatment of dermatosis induced with MRSA subject to further toxicological and pre-clinical studies.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGMENT

The authors are particularly grateful to Prof. Dzoyem Jean Paul for providing resistant isolates of yeasts for the partial realization of the antifungal activity.

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