American Journal of Essential Oils and Natural Products

Available online at www.essencejournal.com

AJEONP

American Journal of Essential Oils and Natural Products

AkiNik

ISSN: 2321-9114
AJEONP 2021; 9(1): 19-27

Received: 18-04-2021
Accepted: 21-06-2021

Kameni Georgette Njila
Department of Microbiology, Laboratory of Microbiology, University of Yaounde I, Yaounde, Cameroon

Emilia Lyonga Mbamyah
a) Department of Microbiology, Parasitology, Haematology and Infectious Diseases, Faculty of Medicine and Biomedical Sciences, University of Yaounde I, Yaounde, Cameroon
b) Center for the Study and Control of Communicable diseases, FMBS University of Yaounde I, Yaounde, Cameroon

Moni Ndedi Esther Del Florence
Department of Microbiology, Laboratory of Microbiology, University of Yaounde I, Yaounde, Cameroon

George Mondinde Ikomey
Center for the Study and Control of Communicable diseases, FMBS University of Yaounde I, Yaounde, Cameroon

Ngongang Tchami Dimitri
Department of Microbiology, Laboratory of Microbiology, University of Yaounde I, Yaounde, Cameroon

Martha Tongo Mesembe
Center for the Study and Control of Communicable diseases, FMBS University of Yaounde I, Yaounde, Cameroon

Nyegue Maximilienne Ascension
Department of Microbiology, Laboratory of Microbiology, University of Yaounde I, Yaounde, Cameroon

Corresponding Author:
Kameni Georgette Njila
Department of Microbiology, Laboratory of Microbiology, University of Yaounde I, Yaounde, Cameroon

Formulation of antiseptics based on essential oils from: *Cananga odorata, Cinnamomum zeylanicum, Citrus sinensis, Eugenia caryophyllata* and *Mentha piperita*

Kameni Georgette Njila, Emilia Lyonga Mbamyah, Moni Ndedi Esther Del Florence, George Mondinde Ikomey, Ngongang Tchami Dimitri, Martha Tongo Mesembe and Nyegue Maximilienne Ascension

Abstract
The COVID-19 pandemic is a stark reminder that the easiest way to stop the spread of hand germs is by washing the hands. The unavailability of hand washing facilities has led to the alternative use of hand antiseptics. The present study was carried out to formulate hand antiseptics with antimicrobial and fragrance properties based on combinations of essential oils (EOs). EOs were obtained by steam distillation of five plants using the Clevenger apparatus. These EOs were combined C_1, C_2 and C_3 according to the Kaloustian method. The bioactivity of EOs and their combinations was tested by Agar disc diffusion and micro dilution methods against reference strains of bacteria and fungi. Hand sanitizers containing combinations of EOs as antimicrobials and fragrance agents were formulated following the guideline recommended by the World Health Organization in 2019 with slight modifications and sensory analysis evaluated by a panel of 50 trained individuals. The EOs obtained were 1.1 % for *Eugenia caryophyllata*, 0.55 % for *Citrus sinensis*, 0.33 % for *Cinnamomum zeylanicum*, 0.26 % for *Cananga odorata* and 0.052 % for *Mentha piperita*. The inhibition effect of EOs on solid medium was exhibited with inhibition diameters ranging from 0 ± 0 mm for *Citrus sinensis* against *Escherichia coli* to 15 ± 1.5 mm for *Eugenia caryophyllata* against *Staphylococcus aureus*. The highest MIC was *Mentha piperita* 25 mg/mL against *Candida albicans* meanwhile the lowest MIC was 0.390 mg/mL for *Cinnamomum zeylanicum* and *Eugenia caryophyllata* against all tested bacteria. The most active combination was C_1 with MIC of 0.390 mg/mL on all six strains. The EO combinations were 16 times more active than the hand sanitizer tested. EOs extracted gave good antimicrobial and antifungal activities. Their fragrance was acceptable and thus can be used in hand sanitizers. This study has shown that the addition of EOs to the standard antiseptics potentiates its action against microbes which may have developed resistance to the standard formula.

Keywords Medicinal plants, essential oils, combinations, antimicrobial activity, formulation, hand sanitizers

1. Introduction
Handshakes, sneezing into our palms, manipulations of phones, door handles, table surfaces, exchange of money and writing materials are some of our daily practices with risk of microbial contamination. We use our hands on daily basis to interact with our environment neglecting the fact that it is an arena saturated with microorganisms. Most communicable disease especially those of Public Health challenge currently faced worldwide and locally like the COVID-19 pandemic and cholera epidemic are pathologies incriminating poor hand hygiene as a principal mechanism of spread [1]. Experience from the World Health Organization has shown that improving hand hygiene strategies can reduce healthcare-associated infection, antimicrobial resistance and potentially prevent an estimated 165,000 deaths from diarrheal diseases each year [2]. Although hand washing with clean water and soap is the standard method for keeping germs away the unavailability of hand washing facilities and basic necessities hinders respect of hand hygiene. There is urgent need for innovative solutions to fit different context, like making alcohol-based antiseptic hand rub both available and affordable. Hand sanitizers can be used as a good alternative when water and soap are not available [3]. They are very effective at keeping microorganisms at bay. Community based epidemiologic studies have shown beneficial effects of hand sanitizers, they have been effective in reducing gastrointestinal illnesses in households [4].

However, they could be more dangerous than being effective depending on the chemicals used which are sometimes harmful to humans. Although, they have been a mainstay in hygiene for decades now strains of antibiotic resistant bacteria show signs of overcoming these hand cleansing agents as well [5]. Much attention is being given throughout the world to minimize the use of synthetic antimicrobial and fragrance agents in hand sanitizers. Given the incidence of increased resistance by bacteria to antibiotics, adverse effects of some antibacterial agents currently used in antiseptics and financial considerations in developing countries, there is a need for alternative options that are safe, effective and economical. This has led to a great interest in identifying natural safe antimicrobial and fragrant compounds from various natural sources.

Substitution of the usual chemical antimicrobials and synthetic fragrance for EOs will be an approach of choice by virtue of the fact that the later have been the subject of numerous scientific studies revealing among others, anti-oxidants, anti-inflammatory, anti-sporicidal, antifungal and antibacterial properties [6-7]. Today they are used in developed countries in aromatherapy and as alternatives to drugs [8-9]. It has been known for decades that, many essential oils are used as topical antiseptics [10]. So it is now interesting to use them in Africa especially in Cameroon as potential source of active ingredients in some cosmetics and pharmaceutical industries. This information was considered interesting more especially since the Cameroonian flora is endowed with aromatic plants rich in EOs [10-11].

Cananga odorata belonging to the Annonaceae family, is cultivated in gardens, parks and close to habitats due to its fruits and pleasant sweet smell. Its essential oil is greatly appreciated in cosmetics to generate products of high value. It has been investigated covering the antibacterial, antifungal and cytotoxic activities [12].

Cinnamomum zeylanicum is a small ever green tree belonging to the Lauraceae family and its essential oil is well known due to its antimicrobial activity against bacteria and fungi. To date, several antimicrobial activities of cinnamon and its oils have been reported in various studies; Matan *et al.* reported the effects of cinnamon oils on different bacterial (*Pediococcus halophilus* and *Staphylococcus aureus*), fungi (*Aspergillus flavus, Mucor plumbeus, Penicillium roqueforti* and *Eurotium sp*), and yeast species (*Candida lipolytica, Pichia membranifaciens, Debaryomyces hansenii* and *Zygosaccharomyces rouxii*) [13].

Citrus sinensis belongs to the Rutaceae family. In Cameroon, fruits of the genus *Citrus* are used in pharmacopoeia to fight against diseases like diabetes, cough, influenza and also deficiency like avitaminosis. Hella *et al.* in 2011 studied the antibacterial and antioxidant properties of essential oils of species *Citrus limonum, Citrus aurantium, Citrus sinensis.*

Eugenia caryopyllata of the Myrtaceae family till date is considered as a plant of high economic importance worldwide. Buds of *Eugenia caryophyllata* have shown *in vitro* antibacterial and antifungal activity [14]. The bactericidal mechanism of action of *Eugenia caryophyllata* buds essential oils on *Bacillus subtilis, Mycobacterium phlei* and *Mycobacterium fortuitum* has been proven [15].

Lastly, *Mentha piperita* of the Lamiaceae family before everything is known for its pretty and fresh menthol taste used in the kitchen and in cosmetic industries. *Mentha piperita* essential oil is highly effective against *Staphylococcus aureus, Bacillus cereus, Enterococcus faecium, Klebsiella pneumoniae* and *Escherichia coli* [16].

The choice of essential oils from these plants in this scientific approach is justified not only by their low irritation potential but also by their antimicrobial, deodorizing, refreshing and relaxing virtues. Many of these natural products have an adequate chemical composition and have the advantage of being significantly less toxic than other synthetic disinfectants for a comparable result [17]. Thus, in this study, we formulated antiseptics (hand sanitizers) based on essential oils from five plant species namely *Eugenia caryophyllata*, *Cinnamomum zeylanicum, Mentha piperita, Citrus sinensis* and *Cananga odorata*.

2. Materials and Methods

2.1 Plant Material

Healthy and mature plants without any visible disease symptoms were carefully chosen for sampling. Dried buds of *Eugenia caryophyllata* and fresh leaves of *Mentha piperita* were bought at Mfoundi market, Yaoundé (Center Region, Cameroon), flowers of *Cananga odorata* were harvested in the Melen locality, Yaoundé (Center Region, Cameroon) in October 2021, leaves of *Cinnamomum zeylanicum* were harvested in Bagante (West Region, Cameroon) in October 2021 and Zests of *Citrus sinensis* were bought at the Acacia market, Yaoundé (Center Region, Cameroon). Their identifications were carried out in the Botanic Laboratory of the Faculty of Science of the University of Yaoundé I and later confirmed at the Cameroon National Herbarium (Yaoundé), where voucher specimens were deposited as *Eugenia caryophyllata* (Myrtaceae) Ref: TSN: 506167, *Mentha piperita* (Lamiacae) Ref: 25t45/SRF-Cam, *Cananga odorata* (Annonaceae) Ref: 42250/HNC, *Citrus sinensis* (Rutaceae) Ref: 25859/SRF/Cam and *Cinnamomum zeylanicum* (Lamiaceae) Ref: 66993/HNC.

2.2 Bacterial and Fungal strains

Bacterial and fungal reference strains were obtained from the National Public Health laboratory by the National Antimicrobial Surveillance program and given to the Centre for the Study and Control of Communicable Diseases of the Faculty of Medicine and Biomedical Science, The University of Yaoundé I. They were made up of five bacteria and one fungi strain: *Staphylococcus aureus* (CIP 7625), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (CIP 7610), *Klebsiella pneumoniae* (ATCC 700603), *Salmonella typhi* (2008365501) and a pathogenic yeast *Candida albicans* (ATCC 60193).

2.3 Essential oil extraction

The plant material was subjected to steam distillation using a Clevenger apparatus for 3 to 4 hours (three replicates). The EOs were collected by decantation and finally dried over anhydrous sodium sulfate and kept at 4 °C in opaque bottles [18]. The extraction yields were calculated as the ratio of the mass of EO to the mass of the starting plant material and expressed as a percentage [18].

2.4 Preparation of various combinations of essential oils

EOs were combined based on their individual antibacterial/ antifungal activity and olfactic properties according to the Kaloustians method [19]. It consisted of mixing in a proportionate manner Eos so as to obtain an equilibrated result and an original fragrance. This was to limit the use of chemical fragrance and hydrogen peroxide and also, to optimize the antimicrobial activity and bring aroma to products. Three combinations were made from these five

plant EOs in the proportions C_1: (2/1/1/1/1), C_2: (1/2/1/1/1) and C_3: (2/2/1/1/1) Table I.

Table I: Proportions of EOs in various combinations

Plants (volume (ul)	C_1	C_2	C_3
Plant A	60	10	35
Plant B	10	60	35
Plant C	10	10	10
Plant D	10	10	10
Plant E	10	10	10
Total	100ul	100ul	100ul

2.5 Antimicrobial assays

2.5.1 Agar disk diffusion test

A standard disk diffusion method by CLSI was used with modifications [20]. In each experiment, bacteria were cultured at 37 °C for 24 hours, while fungi were cultured at 37°C for 48 hours. The microbial inoculum of each reference strain was prepared from fresh colonies grown on Muller Hinton for *Pseudomonas aeruginosa*, Eosine methylene blue for *Salmonella typhi*, *Klebsiella pneumoniae* and *Escherichia coli*, Mannitol salt for *Staphylococcus aureus* and Sabouraud supplemented with chloramphenicol for *Candida albicans* agar plates. In each experiment, microorganisms were cultured and prepared to a turbidity equivalent to 0.5 McFarland concentration of 1.5 x 10^8 CFU/mL. Then 100 µL of the suspension was spread on the test plate (Muller Hinton Agar) for bacteria strains and Sabouraud dextrose agar supplemented with chloramphenicol for *Candida albicans*. Sterile filter paper disks of 6mm in diameter (Schleicher, Germany) were impregnated with 10 µL of Eos diluted in Tween 20 (Merck, Germany), working concentration (100 mg/mL) and placed on the surface of the test plate. Gentamicin® (2mg/mL) and Ketoconazole® (20 mg/ml) were use as the antimicrobial reference control. Bactol hand sanitizer (Biopharma, Cameroon) was used as the reference antiseptic. Plates were subsequently incubated at 37 °C for 24/48 hours according to conditions suitable for the growth of microorganism. The absence of bacterial/fungal growth around each disc expressed an antimicrobial activity illustrated by a translucent halo of the same color than the sterile medium. This diameter expressed in millimeter (mm) was measured using a slide caliper or a scale [20]. All tests were performed in triplicate.

2.5.2 Determination of the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC)

The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the antimicrobial agents were determined using the micro dilution reference method recommended by the Clinical Laboratory Standards Institute (CLSI) with slight modification [20]. Muller Hinton (100 ul) was dispensed into a 96-well micro titer plates. A serial double dilution of geometric ratio of 2 of the EOs was realized into the broth in the wells over a concentration range of 25 mg/mL to 0.0488 mg/ml and 0.25 mg/ml to 0.000488 mg/ml for Gentamicin® and ketoconazole®. Overnight cultures of each reference strain were prepared at 1.5 x10^8 CFU/mL and added. Positive control (Muller Hinton broth + inoculum) and negative control wells (EO + Muller Hinton broth) were included in each test. All tests were done in triplicate and incubated at 37ºC for 24 hours. The Minimal Inhibitory Concentration (MIC) was defined as the lowest concentration of samples at which the microorganism did not demonstrate growth [21]. The MBC and MFC (Minimal Fungicidal Concentration) were assessed by subculture as follows: 50 µL of the content of wells (unrevealed) corresponding to concentrations ≥ MIC were transferred unto 150 µL of fresh Muller Hinton broth. The plates were incubated at 37 ºC for 24 hours. Alamar blue (Bio-rad, USA) (0.5%) 40ul, was used to reveal bacterial and fungal growth in each well. The MBC and MFC were considered as the lowest concentration of each antimicrobial substance that did not allow any noticeable color change from blue to pink.

2.6 Sensory analysis of EO combinations

Sensory analysis being a subjective method that evaluates a product through the use of human senses. Sensory benefits have great impact on consumer product choice. The sensory characteristics of cosmetic products are very important in the process of consumer choice, acceptance and loyalty. In this case, the objective was to determine if the incorporation of essential oils in hand sanitizer formulas is perceived as pleasant based on a scale of preference. The sensorial attributes evaluated were fragrance (aroma) referring to the scent, note of freshness and acceptance that can be detected through olfaction (sense of smell). Essential oil combinations (C_1, C_2 and C_3) were presented to a trained panel of 50 individuals following the experimental plan. The objective which was to evaluate the acceptance, note of freshness and fragrance of combinations was explained to them. The evaluators or panelist were asked to respond on questionnaires provided. This information was then collected and tabulated to assess the products that were used in the study. The evaluation was carried out with the collaboration of 50 trained panelists. Each of the panelists received 3 formulations (Essential oil combinations). For the sensory evaluation, 3 containers each containing one gram of the formulations were given to each panelist along with spoons of approximately 0.5 g, so that each participant could evaluate the same amount. Participants applied the formulations to the anterior sides of their right and left forearms. The evaluations were triadic and balanced, and the evaluated attributes were fragrance, note of freshness and acceptance assessed by participants through 3 short aspirations made with the mouth closed to allow the volatiles to enter the olfactory cavity of the panelist. Evaluations took place at 3 different points in time:

1. Time 0 - “Primary Aroma”: corresponds to the first olfactory perception when the bottle is opened
2. Time 1 - “Aroma on skin”: corresponds to the olfactory perception when the product is applied to the skin.
3. Time 2 - “Aroma on skin at 5 minutes”: corresponds to the olfactory perception after 5 minutes has elapsed since initial application to skin.

To assess the panelist’s levels of acceptance of the formulations, we used a 2-point dyadic scale considering 1 as the lowest score and 2 as the highest. While for the note of freshness a triadic scale was used considering 1 as moderate, 2 as intense and 3 as very intense. For the evaluation, after each review and signing of the Informed Consent Form panelist was given a form with the instructions to follow and the time was controlled by the sensory panel facilitator. Each of the 50 panelists evaluated 3 parameters of 3 formulations resulting in a total of 450 data points obtained for determining the preferred formulations [22]. Excel spreadsheet was used to analyze the samples.

2.7 Formulation of hydro alcoholic solutions and gels.

EO combinations and other components were used to formulate hydro alcoholic gels and solutions following the protocol recommended by the WHO guideline 2019 with slight modifications [22]. They included alcohol (ethanol 96 %), glycerol 99 %, distilled water, gelling agent (carbopol) and hydrogen peroxide 3 %. To prepare hydro alcoholic solutions, the alcohol (833.3ml) for the formula was poured into a large bottle or tank up to the graduated mark. Hydrogen peroxide (41.7ml) was added using a measuring cylinder. Glycerol (14.5 ml) was then added. A quantity of alcohol and H_2O_2 was removed and replaced by essential oil (<0.5ml) combinations. The bottle tank was then topped up to the 1-litre mark with sterile distilled water. The lid was placed on the bottle as soon as possible after preparation, in order to prevent evaporation. The solution was mixed by shaking gently using a paddle and poured into 100ml spray bottles [23]. Meanwhile to prepare hydro alcoholic gels, under magnetic agitation, and in a beaker 1g of carbopol (Pharma Adda, India) was dispersed into 86 ml of ethanol for 15 minutes. Then 0.7 ml of NaoH was added so as to increase the gel pH and maintain a neutral value. After, 1.47 ml of glycerol and (<0.5ml) of essential oil combinations were added. The lid was placed on the bottle as soon as possible after preparation, in order to prevent evaporation. The solution was mixed by shaking gently using a paddle [23]. The prepared gels and solutions were evaluated for pH and homogeneity. The pH of the formulation was determined using litmus paper ((Annalab, India). The measurement was performed at the 1st, 15th and 30th day after preparation to detect any pH fluctuations with time.

2.8 Organoleptic control

This technique was used to study the organoleptic properties of formulated antiseptic gels and solutions. Four parameters were tested; texture, aspect, fragrance and color. The panelists gave their response on questionnaires.

2.9 Statistical analysis

The data of sensory analysis and sensitivity test were recorded, analyzed on Microsoft Excel 2016 spreadsheet and Graph pad prism 8 was used to plot graphs.

3. Results

3.1 Extraction of EOs

The five EOs were extracted and weighed, the extraction yield (%) was determined by dividing essential oil mass by the mass of the plant material used. Values obtained are shown in table II. *Eugenia caryophyllata* had the highest yield equivalent to 1.1 % meanwhile the lowest yield 0.052 % was for *Mentha piperita*.

Table 2: EOs extraction yield

Plants	Plant parts	Yield %
Eugenia caryophyllata	Terminal buds	1.1
Citrus sinensis	Zests	0.55
Cinnamomum zeylanicum	Leaves	0.33
Cananga odorata	Flowers	0.26
Mentha piperita	Leaves	0.052

3.2 Antimicrobial Assays

The susceptibility pattern of the tested microorganisms to the EOs are indicated in Table III. The presence of inhibition zones after incubation revealed the activity of the EOs on the bacteria and fungi tested. It was observed that in the aromatogram method inhibition zones ranged from 0 ± 0 to 15 ± 3 mm for the EOs, 14 ± 1 mm to 24.27 ± 0.75 mm for the reference antibiotic and 0 ± 0 mm to 13,57± 0.98 mm for the reference hand sanitizer (Bactol). The most active EO was *Eugenia caryophyllata* with inhibition diameters of 15 ± 1.5 mm and 15 ± 3 mm against *Staphylococcus aureus* and *Candida albicans* respectively. The least active Eo was *Citrus sinensis* which did not exhibit any activity on all strains used.
Concerning inhibition parameters, the results are shown in Table IV, Gentamicin® showed activity on all the strains used with MICs ranging from 0.001 mg/mL to 0.00039 mg/mL while ketoconazole MIC was 0.0007 mg/ml on *Candida albicans*. All the Eos tested exhibited an activity with MICs ranging from 0.390 to 1.562 mg/ml while that of bactol hand sanitizers ranged from 6.25 mg/ml to 25 mg/ml showing that essential oils were16 times more active than bactol. Also, inhibition parameters of essential oil combinations on microbial growth showed that essential oil combinations present more interesting activity than that of essential oils tested individually. Majority of the combinations showed a bactericidal effect characterized by an MBC/MIC ratio ranging from 1 to 4 for bacteria and *Candida albicans*. Their MICs ranged from 0.390 to 0.781 mg/mL.

Table 3: Inhibition diameters of EOs, gentamicin, ketoconazole and bactol on reference strains

Eos/Strains	*E. coli*	*S. typhi*	*S. aureus*	*C. albicans*	*P. aeruginosa*	*K. pneumonia*
E. caryophyllata	14 ± 2.3	7,33 ± 1.5	15 ± 1.5	15 ± 3	10,97 ± 0.57	8,67± 1.7
C. zeylanicum	13,07±0.4	10 ± 1	12,9 ± 1.65	14,33 ± 1.04	7,33 ± 1.15	7,53 ± 0.5
C. odorata	6,33 ± 1.2	0 ± 0	11,17±0.76	0 ± 0	0 ± 0	0 ± 0
C. sinensis	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
M. piperita	10,2 ± 0.72	0 ± 0	9,33 ± 0.57	0 ± 0	0 ± 0	0 ± 0
Gentamicin®	14 ± 1	20 ± 1	24,27 ± 0.75	Nt	19,33 ± 2.08	14,23 ± 0.68
Ketoconazole®	Nt	Nt	Nt	19,67 ± 1.52	Nt	Nt
Bactol	13,57± 0.98	0 ± 0	11,67±0.58	12,5 ± 0.5	6,17 ± 0.28	0 ± 0

Legend: Nt: not tested

Table 4: Inhibition parameters of EOs, Ketoconazole®, Gentamicin® and bactol on strains

Microbial strains	Inhibition parameters (mg/mL)	Essential oils and combinations							Reference antimicrobials (mg/mL)			
		Eugenia caryophyllata	*Cinnamomum Zeylanicum*	*Mentha piperita*	*Cananga odorata*	*Citrus sinensis*	C1	C2	C3	Gentam-icin	Bactol	Ketoco-nazol
S. aureus	MIC	0.390	0.390	0.781	1.562	12.5	0.390	0.390	0.390	0.097	6.25	/
	MBC	0.781	0.390	3.125	>25	25	0.390	0.390	0.781	0.097	>25	/
	MBC/MIC	2	1	4	Ud	2	1	1	2	1	Ud	/
P. aeruginosa	MIC	0.390	0.390	6.25	25	25	0.390	0.390	0.390	0.195	6.25	/
	MBC	0.781	0.390	6.25	>25	>25	0.390	0.390	0.781	0.195	>25	/
	MBC/MIC	2	1	1	Ud	Ud	1	1	2	1	Ud	/

K. pneumoniae	MIC	0.390	0.390	3.125	12.5	25	0.390	0.390	0.390	0.0390	6.25	/
	MBC	1.562	0.390	3.125	>25	>25	0.781	1.562	1.562	0.1562	>25	/
	MBC/MIC	4	1	1	Ud	Ud	2	4	4	4	Ud	/
S. typhi	MIC	0.390	0.390	6.25	12.5	25	0.390	0.390	0.390	0.0390	25	/
	MBC	3.125	3.125	>25	>25	>25	0.390	1.562	1.562	0.0390	>25	/
	MBC/MIC	8	8	Ud	Ud	Ud	1	4	4	1	Ud	/
E. coli	MIC	0.390	0.390	6.25	3.125	25	0.390	0.390	0.390	0.0195	6.25	/
	MBC	0.781	0.390	6.25	>25	>25	0.390	0.390	0.781	0.0195	>25	/
	MBC/MIC	2	1	1	Ud	Ud	1	1	2	1	Ud	/
C. albicans	MIC	1.562	1.562	25	25	25	0.390	0.195	0.781	/	25	0.0781
	MBC	6.25	3.125	>25	>25	>25	1.562	0.390	3.125	/	>25	0.0781
	MBC/MIC	4	2	Ud	Ud	Ud	4	2	4	/	Ud	1

Legend: Ud: Undetermined; (/): not tested; >25: MIC greater than 25

3.3 Sensory perception of essential oil combinations by a trained panel

The results obtained after evaluation are illustrated in figure 1, 2 and 3. Figure 1 shows the various perceptions gotten from the EO combinations. According to the responses of consumers, the majority of the EO combinations had a scent/fragrance similar to that of cosmetic perfumes, cooking ingredient and chewing gum. Figure 2 indicates the grading of freshness attributed to the different EO combinations by the panel. This varies from one consumer to another. Nevertheless, the chart shows that C_1, C_2, and C_3 presented an intense grade of freshness. C_2 was the most appreciated in terms of fragrance with 60% followed by C_1 25% and lastly C_3 with 15% acceptability this result is clearly shown in figure 3.

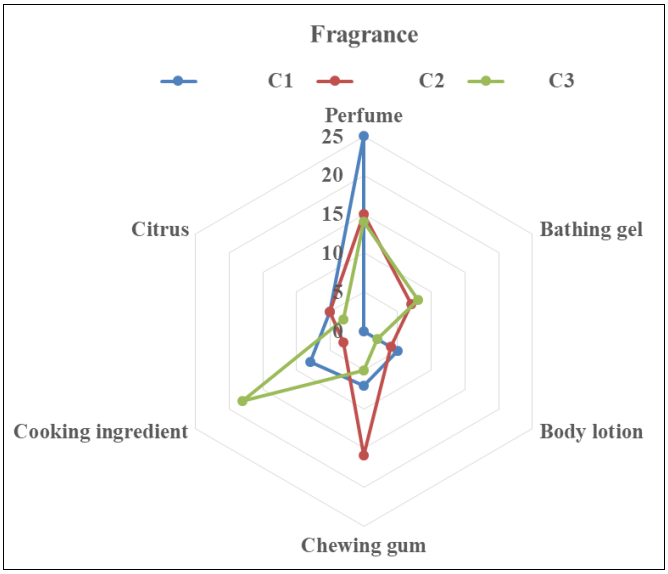

Fig 1: Perception of the fragrance of each EO combinations

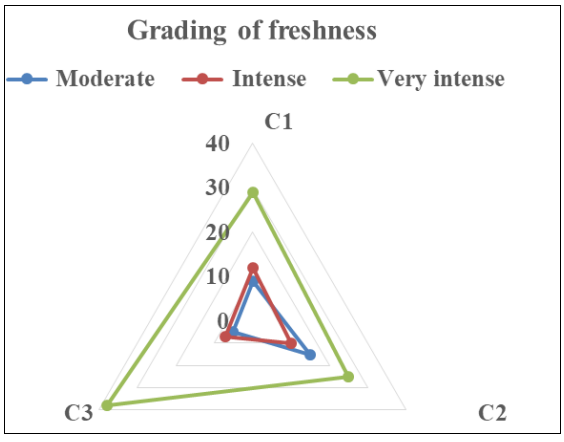

Fig 2*:* Grading of freshness for each EO combinations

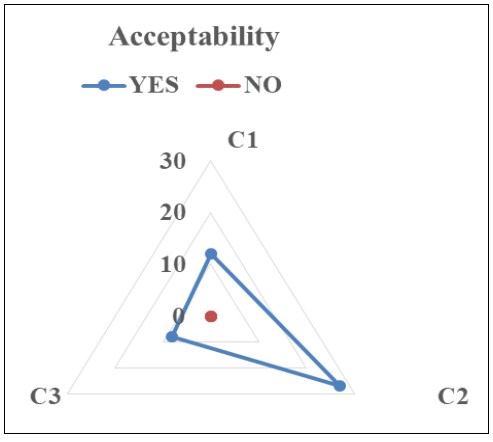

Fig 3: Grading of the acceptability of EO combinations

3.4 Organoleptic control and evaluation of product acceptability

75% of persons preferred the gel form of the antiseptic product while 15% preferred the semi liquid form and 10%. The liquid form (figure 4). In terms of fragrance 55% preferred Formula 2, 10% Formula 3 and 35% Formula 1 (figure 5).

Table 5: Evaluated organoleptic properties of hydro alcoholic solution (F1, F2 and F7)

Parameters	Results
Aspect	Clear liquid
Scent/fragrance	Cosmetic product
Color	Transparent
Texture	Liquid

Table 6: Evaluated organoleptic properties of hydro alcoholic gels (F2, F3, F5, F6, F8 and F9)

Parameters	Results
Aspect	Clear semi liquid
Scent/fragrance	Cosmetic product
Color	Transparent
Texture	Gel

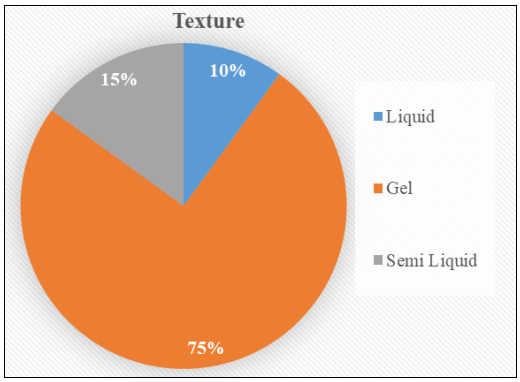

Fig 4: Texture evaluation of formulated hydro alcoholic solutions and gels

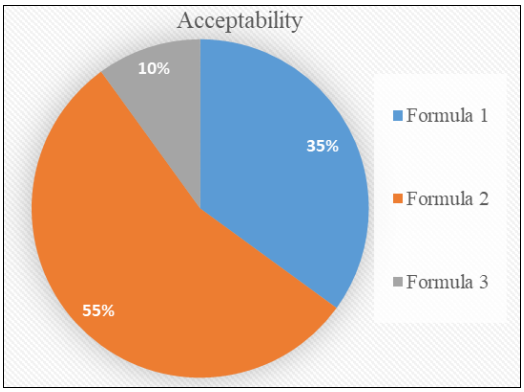

Fig 5: Fragrance acceptability of formulated hydro alcoholic solutions and gels

4. Discussion

4.1 Extraction of Essential oils

The Yield of extraction for *Eugenia caryophyllata* (%) is, lower than that reported by Nyegue *et al.*, 2015 [14]; where the yield was 5.9%. This could be due to the conservation time of the clove buds. Nurdjannah and Bermawie, 2000 [24]; Alma *et al.*, 2007 [25] 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 [26].

The yield of *Mentha piperita* essential oil was lower than those reported by Benayad, 2008 [27]; Derwich *et al.*, 2010 [28]; Mohammedi, 2010 [29] and Nyegue, 2015 [14] whose values were respectively 1.7%, 1.02%, 1.01% and 0.2% but higher than that reported by Ngongang *et al.*, 2018 [30] which was 0.047%. This difference can be explained by the period and the area of harvesting; Brunetton, 1993 [31] and the treatment of samples before the extraction of essential oil Pitarevic *et al.*, 1985 [32] and also due to plant parts used Arslan *et al.*, 2004 [33]. In our investigation only fresh leaves were used, while dried aerial parts were used in reported study.

The extraction yield of *Citrus sinensis* essential oil was lower than that reported by Olugbenga *et al.*, (2018) [34] whose values ranged from (0.57-3.24%) and the difference could be due to extraction temperature and time. Studies revealed that yield of essential oils were mainly influenced by different extraction temperature, time and power, Hui, 2012 [35]. This can also be due to the difference in quantity of plant material used.

The results of extraction for *Cinnamomum zeylanicum* was higher than that reported by Bouwen, (2020) [36]; where the yield was 0.14%. This difference can be explained by the period and the area of harvesting, Brunetton, 1993 [31] and the treatment of samples before the extraction of essential oil, Pitarevic *et al.*, 1985 [32] or due to the quantity of plant material used Arslan *et al.*, 2004 [33].

The yield of *Cananga odorata* oil was slightly higher than that reported by Ndjekou, 2020 [37], where the yield was 0.22% and this slight difference can be due to the conservation time of the flowers of *Cananga odorata*. Our sample was harvested in the Melen locality very early in the morning and hydro distillation was done immediately.

This result shows that the proportion of EOs present in a plant varies not only from one botanical family to another but also from one specie to another [29]. Equally, the variation in content depends on several parameters like the plant degree of maturity, interaction with its environment (climate, soil type) and the season of harvest [38].

4.2 Antimicrobial Assays

The highest inhibition diameter 15 ± 3 mm for *Eugenia caryophyllata* against *Candida albicans* is similar to that obtained by Ngongang *et al.*, 2018 [30], where the inhibition diameter was 16.5 ± 1.44 mm. The MIC obtained against *Pseudomonas aeruginosa* 0.390 mg/ml corroborate with that obtained by Kengne *et al.*, 2019 [7], which was 0.390 mg/ml. Also, results obtained showed that *Eugenia caryophyllata* had an activity on *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Candida albicans*, *Escherichia coli* and *Pseudomonas aeruginosa* thus, reaffirming studies reported by Bouwen, 2020 [36]; Ngongang *et al.*, 2018 [30]; and Nyegue *et al.*, 2015 [14]. This activity of *Eugenia caryophyllata* essential oil could be due to the presence of high proportions of eugenol in its composition which is a phenolic compound and known for its high antimicrobial activity in general [7]. Studies have reported that *Eugenia caryophyllata* essential oil consist mainly of eugenol (70%), eugenol acetate (16.2%) and caryophyllene (5.2%) [14, 7].

The inhibition diameters 9.33 ± 0.57 mm and 0 ± 0 mm of *Mentha piperita* against *Staphylococcus aureus* and *Pseudomonas aeruginosa* respectively differs that reported by Kengne *et al.*, 2019 [7], which were 22.66 ± 1.52 mm and 13.66 ± 1.52 mm respectively. This could be explained by the difference in the method used, in our investigation the aromatogram method was used, while the micro atmosphere method was used in the reported study. The MIC of 6.25 mg/ml obtained against *Pseudomonas aeruginosa* corroborate the result reported by Kengne *et al.*, 2019 [7]. *Mentha piperita* had a bactericidal action on *Escherichia coli*, *Klebsiella*

pneumoniae, *Pseudomonas aeruginosa* and *Staphylococcus aureus* with lowest MIC of 0.781 mg/ml. The efficiency of the essential oil of *Mentha piperita* can be attributed to the synergetic action of monoterpenes hydrocarbons such as pinene-type well-known compounds having antimicrobial potentials [47].and phenol components (menthol, menthol <neo>, carvacrol methyl ether) which may interfere with cell wall enzymes [48-49].

The MIC and inhibition diameter of *Cananga odorata* against *Staphylococcus aureus* 25 mg/ml and 11.17 ± 0.76 mm respectively differ those reported by Kengne *et al*., 2019 [7], which was > 25 mg/ml for the MIC and 0 ± 0 mm for the inhibition diameter this could be due to the difference in concentration of the stock solution of essential used. In this study stock solutions were prepared at a concentration of 100 mg/ml while stock solutions were prepared at a concentration of 50 mg/ml in the reported study. The MICs of *Cananga odorata* ranged from 1.562 mg/ml to 25 mg/ml for all tested strains, this activity could be due to the presence of compounds known to possess antimicrobial properties in their chemical composition they include linalool, geranyl acetate, gemacrene-D, beta caryophyllene, benzyl acetate, geraniol, methyl p-cresol, methyl benzoate, geranyl acetate, farnasene and benzyl benzoate [7].

The inhibition diameter of *Cinnamomum zeylanicum* against *Candida albicans* 14.33 ± 1.04 mm corroborates with those reported by Bouwen, 2020, which showed that *Cinnamomum zeylanicum* had an activity on clinical isolates of Candida isolates. The activity of *Cinnamomum zeylanicum* EO against *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* confirms the study reported by Naveed *et al*., 2013 [51] and Al Marriri, 2014 [52]. Analyses of chemical composition has revealed the presence of cinnamaldehyde, eugenol and minor components such as cuminaldehyde and terpinene which are compounds with antibacterial and antifungal activity [53].

The difference in results could be explained by the fact that, the activity of an essential oil is strictly linked to its chemical composition which in turns depends on parameters like the plant part used, period and place of harvest, extraction method, climate, geographical zone and the screening method used [52].

With regard to the activity of the EO combinations the MICs ranged from 0.195-3.125 mg/ml with a bactericidal action, this shows that they are more efficient than the essential oils taken individually. This efficiency could be due to the synergitic action of various classes of antimicrobial compounds brought by each plant. These essential oils combination at suitable concentration could be an alternative to the use of synthetic antimicrobial agents.

4.2 Formulation of antiseptic and sensory analysis

According to the responses of consumers, the majority of the Eo combinations had a scent/fragrance which referred to that of cosmetic perfumes, cooking ingredient and chewing gum. Nevertheless, it should be noted that small differences in olfactory receptor genes, which are extremely common in humans, can affect the way each person perceives scent. These genetic differences mean that when two people smell the same compound, one person may detect a floral scent while another smells nothing at all.

5. Conclusions

Alcohol based hand sanitizers have been a mainstay in hygiene for decades but now strains of antibiotic resistant bacteria show signs of overcoming these hand washing agents as well. The use of EOs in combination as an active ingredient in hand sanitizers would reinforce the alcohol action, expand its spectra, reduce the use of chemical compounds, irritation effect, germs spread and propagation of diseases. These EOs and their combinations might be considered as promising candidates for the development of natural antimicrobials for the control of microbial contaminants.

6. Acknowledgments

Authors are grateful to the Cameroon National Herbarium for the identification of plants and the Centre for the Study and Control of Communicable Diseases for their technical support.

7. Declaration

The authors declare that there is no conflict of interests regarding the publication of this paper.

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