

## Short Research Article

# Antibacterial and Antifungal Activities of a Medicinal Soap made from Ginger (*Zingiber officinale*) and Clove (*Syzygium aromaticum*) Essential Oils

Comment [D1]: Change as "Evaluation of Antibacterial and Antifungal Activities of a Medicinal Soap made from *Zingiber officinale*, *Syzygium aromaticum* and Essential Oils"

### ABSTRACT

**Aims:** The objective of this study is to evaluate the antibacterial and sensory properties of a formulated medicinal soap.

**Study design:** Extraction of essential oils, purchase of vegetable oils and caustic soda, soap formulation, testing of its antibacterial and antimicrobial activities, evaluation of its acceptability.

Comment [D2]: To be changed as antifungal

**Place and Duration of Study:** Research unit of biochemistry of medicinal plant, food and nutritional sciences, Department of Biochemistry, Faculty of Science, University of Dschang from October 2019 to July 2020.

**Methodology:** Cold saponification method was used for the production of soap using the following ingredients: Palm kernel oil, olive oil, coconut oil, Palm oleic, caustic soda, essential oils from ginger roots and cloves buds. The formulated soap was used for the evaluation of its anti-bacterial and anti-fungal activities as well as its acceptability using the 9-points hedonic scale. The soap was tested on the following microorganisms: *Pseudomonas aeruginosa* PA01, *Staphylococcus aureus* 56, *Staphylococcus aureus* 18, *Staphylococcus aureus* ATCC 25923, *Candida albican*, *Candida tropicalis*.

**Results:** Results showed that the produced soap has bactericidal activities against *staphylococcus aureus* 18 and *staphylococcus aureus* ATCC 25923 and a bacteriostatic activity against *staphylococcus aureus* 56. Its activity against fungi was very low. The results of the sensory analysis of this product showed that it can be accepted by consumer since the lowest score was 6.55 in a scale of 9.

**Conclusion:** Results showed that the produced soap has bactericidal activities against *staphylococcus aureus* 18 and *staphylococcus aureus* ATCC 25923 and a bacteriostatic activity against *staphylococcus aureus* 56. It was accepted with a score of at least 6.55/9 by the participants.

**Keywords:** anti-bacterial activities, anti-fungal bacteria, minimal bacterial concentration, minimal inhibitor concentration, 9-point hedonic scale

## 1. INTRODUCTION

The cosmetic market in Cameroon and the rest of sub-Saharan Africa has flourished considerably over the past decade. The Cameroonian cosmetic market alone is estimated to be around 150 billion FCFA. Nowadays both sexes are paying more attention to their grooming habits. The absence of cosmetic surgery in Cameroon and sub-Saharan Africa leads to millions of people that rely on cosmetics to perfect their looks. Furthermore, the average consumers of cosmetic products such as soap are in the range of 18-45 years old and make at least one purchase per month. About six (6) millions of the female population in Cameroon suffers from acne and rapid ageing rate that becomes high due to stress [1]. That can be addressed using natural plants extracts in products like soap and other cosmetic products. Amongst the plants, ginger and cloves have been demonstrated to have good antimicrobial and antibacterial activities.

Ginger (*Zingiber officinale*) is a flowering plant whose rhizomes are widely used as a spice and in traditional medicine. It is an herbaceous perennial which grows annual pseudo stems about one-meter-tall bearing narrow leaf blades [2]. Cloves (*Syzygium aromaticum*) are the aromatic flower buds of a tree in the family Myrtaceae, they are native to the Maluku islands in Indonesia and are commonly used as a spice [3]. Ginger and cloves have several uses on the skin due to their anti-microbial, anti-inflammatory, antiseptic property and antioxidant property which implies the inhibition of harmful free radicals that cause skin damage [4]. Furthermore, the Ginger roots have been important for the regeneration of skin tissues over years. Also, it has benefits inside the body but using it directly on the skin increases skin's radiance and decrease inflammation that may contribute to conditions such as psoriasis and acne[5].

As far as clove is concerned, its essential oil helps in reducing the sagginess of the skin and prevents the appearance of fine lines and wrinkles. It is a powerful anti-aging ingredient used in most cosmetics. It removes the dead skin cells and helps in blood circulation, which indirectly ensures a youthful and radiant looking [6]. Clove has an amazing aroma that helps in lowering down the nerves and reducing stress too. Aromatherapy is closely associated with beautiful and glowing skin. In order to contribute to the salvation of skin related problems in Cameroon this study was engaged with the aim to evaluate the antibacterial and sensory properties of a formulated medicinal soap.

## 2. MATERIAL AND METHODS

### 2.1 Material

#### 2.1.1 Ingredients for soap production

Caustic soda, water, virgin olive oil, coconut oil, refined palm oil and palm kernel oil were purchased in October 2019 at Santa Lucia Supermarket, Douala, Cameroon. The Ginger roots and cloves used for the essential oil extraction were purchased at Sandaga market, Littoral Region, Douala, Cameroon in October 2019. The caustic soda was obtained from the laboratory.

#### 2.1.2 Microorganisms

Bacteria and fungi were used in this study. The antibacterial and antifungal tests were carried out on 4 bacteria and 2 fungi consisting of:

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<https://www.coastsvdneybotanicals.com/collections/introducing-our-new-power-players>

Comment [D8]: <https://www.pwcollections.com/products/alkaline-bar>

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- ❖ Two clinical isolates and a Gram-positive bacterium strain of (*Staphylococcus aureus* 56, *S. aureus* 18 and *S. aureus* ATCC 25923).
- ❖ One clinical isolate of a Gram-negative bacterium (*Pseudomonas aeruginosa* PA01).
- ❖ Two clinical candida isolates (*Candida albicans* and *Candida tropicalis*)

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The clinical isolates were from obtained from the Microbiology and Antimicrobial Substances Unit (UMSA) of the Department of Biochemistry, University of Dschang. These microorganisms were chosen based on their availability and because they are major agents involved in skin infections [7].

### **2.1.3 Culture medium**

Four culture media were used in the evaluation of in vitro antimicrobial activity of soap:

- ❖ Mueller Hinton Agar (MHA, Hi Media Laboratories, India), used for the maintenance of bacterial strains and isolates
- ❖ Mueller Hinton Broth (MHB, Conda, Madrid, Spain), used for the determination of Minimal Inhibitory Concentrations (MIC) and Bactericidal (MCB)
- ❖ Sabouraud Dextrose Agar (SDA), for the maintenance and cultivation of fungal isolates
- ❖ Sabouraud Dextrose broth (SDB): for the determination of minimal inhibitory concentrations.

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## **2.2 Methods**

### **2.2.1 Extraction of essential oils**

The essential oils of *Syzygium aromaticum* Buds and *Zingiber officinale* roots were extracted as described by [8]. The plant materials were ground using a blender (Moulinex) and subjected to hydrodistillation using a Clevenger- type apparatus. The essential oil obtained was dried using Sodium sulfate ( $\text{Na}_2\text{SO}_4$ ) crystals and stored as  $-20^\circ\text{C}$  in vials; sealed with Teflon caps and protected from light before use.

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### **2.2.2 Soap preparation**

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124.8 g of caustic soda was added to 298.2 g of water. The mixture was stirred for 15 min, in a container cooled down with ice. 852 g of oil was slowly added in the caustic solution at a temperature of  $300^\circ\text{C}$  with constant stirring. The stirring process was done for 30 min using a spatula and the essential oils (5 ml each were added) 10 min before stopping the stirring process. The final paste added in molds to obtain the desired form. The soaps were kept for two weeks before been used for the determination of the antibacterial and antifungal activities, and for the sensory analysis test.

**Table 1. Ingredients used in soap formulation**

<b>Ingredients</b>	<b>Quantity (g)</b>
Olive oil	426 (g)
Supermont water	298.2
Refined palm oil	85 (g)
Palm Kernel oil	85 (g)
Coconutoil	256 (g)
Caustic soda	124.8 (g)
Ginger essence	5 ml
Cloves essence	5 ml

### **2.2.3 Evaluation of the antibacterial activity of the soap**

The antibacterial activity of the soap was evaluated against strains and clinical isolates of bacteria by the micro dilution method in liquid medium. This was done in 96-well microplates [9].

### **2.2.4 Preparation of bacterial inoculate**

Bacterial inoculate were prepared as described by the Clinical and Laboratory Standards Institute [9]. The organisms were activated on Mueller Hinton Agar and incubated for 18 hours at 37 °C. From these cultures, three to four colonies of microorganism were removed and introduced into 10 ml of sterile distilled water and then adjusted to obtain a turbidity comparable to the point 0.5 of the Mc Farland scale, which corresponds to 1.5 × 10<sup>8</sup> colony-forming units per ml (CFU / ml).

### **2.2.5 Determination of minimal inhibitory and Bactericidal concentrations**

In each of the wells of a microplate (96 wells), 100 µl of culture broth was introduced. The soap sample was prepared in DMSO (150 µl) and then diluted in the culture broth (Mueller Hinton) to reach a concentration of 256 mg / ml. 100 µl of soap was introduced into the upper wells. Subsequently, a series of 8 successive dilutions following a geometric progression of reason 2 was carried out. The contents of each well (100 µl) was diluted by adding 100 µl of inoculum diluted 100-fold in culture broth, corresponding to 1.5 × 10<sup>8</sup> CFU / ml (bacteria) [10, 11]. The reference antibiotic (Ciprofloxacin) was evaluated under the same conditions as soap at concentrations ranging from 128 to 0.0625 µg / ml. Plates were incubated for 24 hours at 37 °C.

For revealing bacterial growth, 50 µl of p-iodonitrotetrazolium chloride solution (INT, 0.2 mg / ml) was added to each well and the plates were incubated at 35 °C for 30 min. Viable microorganisms reduced INT (colorless) to pink. The minimum inhibitory concentration (MIC) was considered to be the lowest soap concentration that prevents the emergence of INT pink staining, thereby reflecting inhibition of microbial growth [12].

### **2.2.6 Determination of bactericidal minimum concentrations**

To determine the minimum bactericidal concentration (MBC), 10 µl of the content of each well which showed no growth of the microorganism at the end of the incubation was incorporated Mueller Hinton Agar, and poured into 90 mm inch Petrie's dishes. The incubation was done at 37 ° C for 24 h (bacteria). The minimum bactericidal concentration (MBC) was recorded as the lowest concentration of soap that prevented the growth of the microorganism at the end of incubation. The calculation of the MBC / MIC ratio helped to determine the bactericidal effect (MBC / MIC <4) of the soap [10].

### **2.2.7 Determination of minimum inhibitory concentrations (MICs) of fungi**

The method of micro-dilution in a liquid medium was used for this purpose according to the protocol described by the National Committee for Clinical Laboratory Standard [13]. In a 96-well plate, 100 µl of Sabouraud broth dextrose was introduced. 100 µl of stock solutions of the soap to be tested was introduced into the first wells of each column and successive serial dilutions of factor 2 made in the other wells while maintaining the volume at 100 µl. A volume of 100 µl of inoculum was introduced again into each well. The plates containing 200 µl of final solution per well, were incubated at 35 °C for 48 hours. For each substance, 3 repetitions were performed. Positive controls were performed in wells containing the culture medium, the culture medium and methanol mixture (1: 1 V / V) and the microorganism. The negative controls were made in wells containing the culture medium and the soap. After the appropriate incubation period, fungal growth was revealed by observing the turbidity at the bottom of the wells and comparing it to that of the negative controls. Minimum inhibitory concentrations (MIC) were defined as the lowest concentrations of soap for which we did not have visible growth with the naked eye (no turbidity).

## **3. RESULTS AND DISCUSSION**

### **3.1 Results**

#### **3.1.1 Antibacterial and antifungal activities**

The antibacterial and antifungal activities of the formulated medicinal soap are presented in table 2. It can be observed that a minimal inhibitory concentration of 128 µg/ml was recorded with staphylococcus aureus 56 while staphylococcus aureus 18 and staphylococcus ATCC 25923 exhibited MIC values of 256 µg/ml. As far as fungi are concerned, a MIC value of 2048 µg/ml was registered with candida albican and candida tropicalis.

For the minimal bactericide concentration, staphylococcus Aureus 18 presented an MBC value of 256 µg/ml while staphylococcus aureus 56 and staphylococcus ATCC 25923 presented an MBC of 1024 µg/ml. The MBC value of fungi was higher than 2048 µg/ml, same with the MIC and MBC value of pseudomonas aeruginosa PA01. The ratios MBC/MIC were respectively 8, 1 and 4 for staphylococcus aureus 56, 18 and ATCC 25923.

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**Table 2: Antibacterial and antifungal activity of the formulated soap**

<b>Microorganims</b>	<b>MIC</b>	<b>MBC</b>	<b>MBC/MIC</b>
<i>Pseudomonas aeruginosa</i> PA01	/	/	/

<i>Staphylococcus aureus</i> 56	128 µg/ml	1024 µg/ml	8
<i>Staphylococcus aureus</i> 18	256 µg/ml	256 µg/ml	1
<i>Staphylococcus aureus</i> ATCC 25923	256 µg/ml	1024 µg/ml	4
<i>Candida albican</i>	2048 µg/ml	/	/
<i>Candida tropicalis</i>	2048 µg/ml	/	/

-: >2048 µg/ml

### 3.1.29-Point hedonic scale analysis

The acceptability scores of the sensory characteristics of the formulated soap are presented in figure X. Color presented a score of 7.9, odor 6.6, texture 6.55, foaming capacity 7.3 and overall acceptability 7.6 all in a scale of nine.

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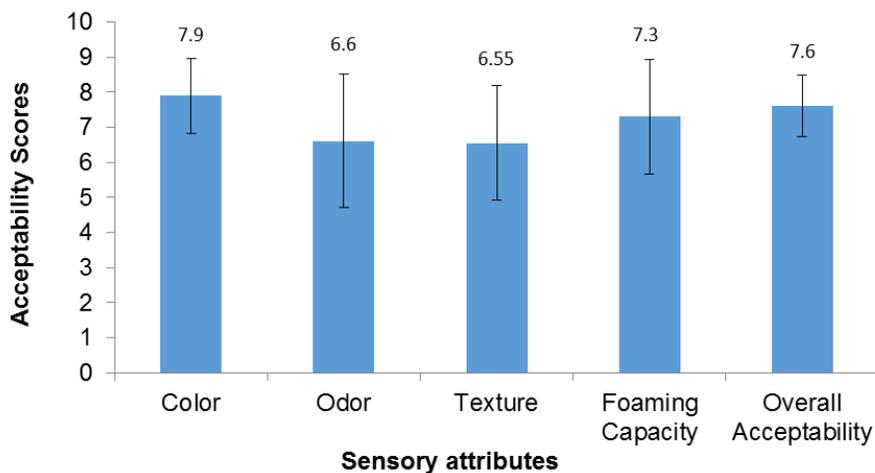


Figure 1. 9-Point hedonic scale analysis results of the acceptability of the soap by consumers

### 3.2 Discussion

Soaps are generally use for cleaning purposes or for the removal or destruction of germs. Among the soaps used, those with antibacterial properties are gradually gaining interest due to their capacity to protect and preserve the skin. The result of the evaluation of the antibacterial activity of the formulated soap showed MIC value of 128µg/ml for

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staphylococcus aureus 56 and 256µg/ml for staphylococcus aureus 18 and staphylococcus ATCC 25923. Based on this value, we can stipulate that the formulated soap has a moderated antibacterial activity against the micro-organisms mentioned. According to kuete[14], the antibacterial activity of a plant extract is considered significant when MIC values are below 100µg/ml, moderate when MIC value fall within 100 and 625 µg/ml and weak when MIC value is higher than 625µg/ml. This suggest that the soap was not efficient against candida albican, candida tropicalis and pseudomonas aeruginosa PA01. The low or weak antibacterial activity registered in this study compared to plant extract as presented as kuete[14] can be attributed to the fact that the essential oils used (cloves and ginger essential oils) were dissolved with other ingredients for the production of soap therefore reducing their concentration. The ratio MBC/MIC indicated bactericidal activity against staphylococcus aureus 18 (R=1 which is < 4) and staphylococcus ATCC 25923(R= 4) and a bacteriostatic activity against staphylococcus aureus 56 (R=8). According to Tchinda *et al.*[15], ratios MBC/MIC > 4 indicate a bacteriostatic activity but when ≤ 4 indicate bactericidal activities. The interesting antibacterial activities observed can be attributed to the bioactives found in ginger and cloves essential oils. Zainol *et al.*[16] showed that clove essential oil has good antibacterial activity. Similar activity of ginger essential oil was recorded by Nader *et al.*[17]. Monoterpenes and sesqueterpernes present in this essential oil could be responsible for these activities.

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The results obtained in this study showing that formulated soap can have antibacterial activities are in accordance with the report of Riaz *et al.*[18] who demonstrated the antibacterial activity of soaps against daily encountered bacteria. Similar observations were made by Abbas *et al.*[19] with different soaps available in local market of Pakistan. Similar report was also released by Obi [20] who showed that some medicated soaps have good antibacterial activities against selected human pathogens.

The result of the 9-points hedonic scale analysis showed that the sensory attributes analyzed were accepted with a minimal score of 6.55/9 showing that this soap can be accepted by consumers pending some test on their effect on the skin. At our knowledge, there is almost no report on the evaluation of the acceptability of formulated medicinal soaps.

#### 4. CONCLUSION

This study was conducted in other to evaluate the antibacterial and sensory properties of a formulated medicinal soap. Results showed that the produced soap has bactericidal activities against staphylococcus aureus 18 and staphylococcus aureus ATCC 25923 and a bacteriostatic activity against staphylococcus aureus 56. It has very weak activity against fungi. The sensory analysis of this product exhibited that it can be accepted by consumer since the lowest call was 6.55 in a scale of 9.

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