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Antibacterial Activities of Soaps Formulated from *Carapa procera* Oil

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Authors' contributions

This work was carried out in collaboration among all authors. Author GKKS wrote the protocol, carried out the manipulations and wrote the first draft of the manuscript. Authors YYG and BL designed the study and carried out the analyses of the results. Authors BIAV and AAM supported the manipulations. Authors CKO and DAJ managed the design and analyses of the study. All the authors read and approved the final manuscript.

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ABSTRACT

Aims: The *Carapa procera* species is used of traditional for its antimicrobial properties especially for the skin. Its oil is used for various applications including the production of soaps used for personal hygiene and other skin conditions. The purpose of this study is to assess the antibacterial properties of soaps formulated from the oil of this species.

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Place and Duration of Study: The units of organic biological chemistry and structural organic chemistry of the UFR Structural Sciences of Matter and Technology (Université Félix Houphouet-Boigny) for the manufacture of soaps and the bacteriology unit of the Institut Pasteur de Côte d'Ivoire for bioassays between January 2019 and February 2020.

Methodology: The antibacterial evaluation was carried out by the disc method on nutrient agars with determination of bacterial parameters (MIC and MBC). Discs of usuals antibiotics (Tetracycline, Rifampicin and Ampicillin) were used as a control to assess this activity as well as a commercially soap.

Results: The inhibition diameters obtained from these different test soaps vary from 16.00 - 17.15 mm; 15.75 - 17.70 mm and 20.33 - 24.00 mm respectively on growth of the bacterial strains *E. coli, P. aeruginosa* and *S. aureus*. Unlike unscented soaps, scented soaps have the best inhibition diameter zones values. Moreover, the best MBC values (25 mg/mL) are obtained with the 2 soaps in fresh or aged state on the *S. aureus* strain against 50 mg/mL on the 2 other strains. In addition, with the exception of the *E. coli* strain which is resistant to Tetracycline, the 2 other strains are sensitive to all the antibiotics tested. In addition, this study also shows variability in the effect of soaps on these targets.

Conclusion: Soaps formulated from *Carapa procera* oil do indeed possess antibacterial properties that could justify its usage against dermatoses in traditional medicine.

Keywords: Carapa procera; oil; soaps; antibacterial activity.

1. INTRODUCTION

One of the main uses of fats remains oleochemistry with the production of detergents and other cosmetic products [1,2]. This is the case of the oil from the *Carapa procera* species which is used of the traditional for the production of soap known as "soft soap" used for corporal hygiene and other skin conditions [3,4].

Thus, most soaps are considered as quick and effective cleaning agents against certain pathogens or not, which constitute the human environment. Indeed, these pathogens agents of great diversity are found in soils, air, waste water or not, and on the animal and human body. Some of them are of paramount importance in the medical environment through their infectious action in both humans and animals [5,6]. Thus, hand washing and personal hygiene are essential for the maintenance of health and in part to prevent cross-contamination and infection by opportunistic germs [7,8,9]. It is therefore important and inappropriate to develop soaps with proven microbial activity to control these pathogens. With this in mind, *Carapa procera* oil has been used for the development of soaps. This oil has already proven these properties both for its physical-chemical characteristics for soap production and for its antioxidant and antifungal properties [10,11].

Thus, the objective of this work is to verify the antibacterial activity of different types of soaps derived from the formulation of *Carapa procera* oil on three (3) bacterial strains including *Staphylococcus aureus*, *Eschierica coli* and *Pseudomonas auruginosa* known for their cutaneous mucosal pathogenicity. Indeed, these bacteria are susceptible to superficial or deep infections in which one of pathways into the body remains the skin [12,13].

2. MATERIALS AND METHODS

2.1 Materials

The biological material consists of strains of bacteria including *Pseudomonas aeruginosa* ATCC4723, *Escherichia coli* ATCC4502 and *Staphylococcus aureus* ATCC4525. These strains were supplied by the Bacteriology Unit of the Institute Pasteur of Côte d'Ivoire (IPCI). In addition, the media used for the biological tests are the chapman medium for *Staphylococcus aureus*, the EMB (Eosine Methylene Blue) medium for *Escherichia coli*, the cetrimide medium (N-cetyl-N,N,N-trimethylammonium Bromide) for *Pseudomonas aeruginosa* and the Mueller-Hinton agar and broth media.

Furthermore, eight (8) formulated soaps and one commercially soap were tested together and also 3 usuals antibiotics (Tetracycline, Rifampicin and Ampicillin). As for the technical equipment, it is made up of material commonly found in a laboratory.

2.2 Methods

The soaps used for these tests were prepared according to manufacturing standards. Thus, one of the soaps was prepared solely from oil of the species *Carapa procera* and the other from a mixture of *Carapa procera* oil, palm kernel, coconut and palm stearin. To these two (2) types of soaps are added or not for subsequent preparations of the perfume.

These test soaps have been formulated following the same procedure.

Indeed, the *Carapa procera* oil or the mixture with other oils has been heated. Then to each preparation was added taking into account the saponification index [11] of the alkali (Potassium hydroxide). The mixture was heated again in a stainless-steel container at a temperature of 100 °C for 30 min. The end of each reaction was checked and confirmed by the free alkali controls. The soaps obtained were coded as follows, taking into account the period of use for the tests and whether or not perfume was added. Thus: -Soap formulated solely with *Carapa procera* oil: soap 1 (S_1), for immediate use 'S1 fresh' (S_1F) then for use after 180 days and after exposure to the open air 'S1 aged' (S_1A). In addition, when perfume has been added to the manufacturing, it is rated S_1 scented fresh (S_1FS) and S_1 scented aged (S_1AS). -As for mixing *Carapa procera* oil with other oils and components, we note soap 2 (S_2), S_2 fresh (S_2F), S_2 aged (S_2A), S_2 scented fresh (S_2FS), S_2 scented aged (S_2AS).

Subsequently, antimicrobial tests were carried out with the different soaps formulated according to the diffusion disc method. These soaps in the form of 6 mm discs at a concentration of 200 mg/mL were impregnated on the surface of an agar medium chosen according to the strains and beforehand prepared and sterilized in autoclave for 1 hour, in petri dishes which were previously placed in an oven at 25 °C for 15 min. The inoculation was done by flooding with a 24-hour inoculum of each strain with a density between 0.12 and 0.15 ($1\text{-}5.10^6$ CFU/mL).The whole was incubated at 37 °C for 18-24 hours.

The control soap was also tested under the same conditions and by the same method as well as the usuals antibiotics including Tetracycline 30 µg, Rifampicin 30 µg and Ampicillin 10 µg. Thus, the inhibition diameter zone obtained made it possible to assess the sensitivity of soaps and antibiotics, while the activity was evaluated by antibacterial parameters including the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC). They were determined respectively in liquid medium by turbidity with Mueller-Hinton broth and in agar medium with Mueller-Hinton agar.

The determination of the MIC, which corresponds to the concentration in the tube where no turbidity is observed, was made for each strain tested using a series of 8 hemolysis tubes (T_1-T_8). In tubes T_1 to T_7, 1 mL of inoculum was introduced. To these different contents of tubes T_1 to T_6, 1 mL of each soap extract of concentration (mg/mL) of 200, 100, 50, 25, 12.5 and 6.75 was added. As for the contents of tubes T_7 and T_8, they constitute respectively the bacterial growth control tube and the sterility control tube of the medium. Following incorporation, all these tubes were incubated at 37 °C for 24 hours. For the MBC values, they were determined after reading the MIC value, then the liquid broth was inoculated on agar medium in a petri dish following dilutions of 10°, 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}.

2.3 Statistical Analyses

Statistica 8.0 software was used for the analysis of variance and comparison of means with the Newman-Keuls test at the significance level (P < 5 %). The various interactions between the parameters were evaluated for significance by Factorial ANOVA.

3. RESULTS AND DISCUSSION

3.1 Results

Antibacterial activity reveals inhibition diameters zone of 16.00 ±1.15 to 17.15 ±1.04 mm on the growth of *E. coli* and 15.75 ±0.45 to 17.70 ±1.30 mm on that of *P. aeruginosa*. For *S. aureus*, inhibition diameters zone ranges from 20.33 ±1.25 to 24.00 ±0.57 mm. On the same microbial strain, the inhibition diameters zones are statistically identical at $P < 0.05$. They form a homogeneous group according to the Newman-Keuls threshold test ($\alpha = 5$ %).The control soap present inhibition diameters zone of 7 mm, 6 mm and 8.5 mm on the growth of *E. coli, P. aeruginosa* and *S. aureus*, respectively (Table 1).

In addition, the MIC values for fresh or aged S_1 soaps and fresh or aged S_2 soaps are 25 mg/mL on *E. coli* and *P. aeruginosa* and 12.5 mg/mL on *S. aureus*. As for fresh or aged scented soaps, MIC values could not be determined within the established concentration range. The same applies to the control soap with a MIC and MBC greater than 200 mg/mL on all strains. The BMC values were 50 mg/mL for *E. coli* and *P. aeruginosa*, while of *S. aureus* was 25 mg/mL (Table 2).

With regard to sensitivity to the usuals antibiotics, only the *E. coli* strain is resistant to tetracycline with an inhibition diameter of 6 mm (Table 3).

Regarding factor analysis between bacterial strains, it showed a highly significant strain effect. This is synonymous with the variability of the soap effect depending on the bacterial target. (Table 4)

Moreover, the Newman-Keuhls homogeneity test allowed to classify these strains into 2 groups by seeing inhibition diameter zone, group 1 is composed of *E. coli* and *P. aeruginosa* and the more sensitive group 2 is composed of *S. aureus* (Table 4).

3.2 Discussion

The work carried out revealed that the zones of inhibition diameters observed with the formulated soaps are lower than those of all the antibiotics tested on the in vitro growth of strains of *E. coli* and *P. aeruginosa* with the exception of Rifampicin and Tetracycline which have zones greater inhibition diameters on *S. aureus*. According to Biyiti et al., 2004 [14], an extract is considered active if the diameter of the inhibition zone is greater than or equal to 10 mm. This reflects that the strain *E. coli* whose inhibition diameter zone of 6 mm is therefore resistant to Tetracycline and that the other antibiotics (Rifampicin and Ampicillin) and formulated soaps are active on these strains. However, soaps formulated from *Carapa procera* oil (alone or in combination with other oils) have shown variable inhibitory effects on these strains (*E. coli, S. aureus, P. aeruginosa*).

Furthermore, the different inhibition diameters show that *S. aureus* is the most sensitive bacterium to the tested soaps. This sensitivity

Table 1. Different inhibition diameters of soaps in mg/mL on bacterial strains

Soap types	*E. coli*	*P. earoginosa*	*S. aureus*
Control soap	$7.00±1.50^a$	$6.00±1.00^a$	$8.50±0.75^a$
Soap S_1 fresh (S_1F)	$16.00±1.15^b$	$16.10±1.25^b$	$21.22±2.20^b$
Soap S_1 aged (S_1A)	$16.25±0.50^b$	$15.75±0.45^b$	$20.70±1.30^b$
Soap S_2 fresh (S_2F)	$17.00±1.50^b$	$16.700±0.60^b$	$22.00±2.33^b$
Soap S_2 aged (S_2A)	$16.70±1.00^b$	$16.50±1.50^b$	$20.33±1.25^b$
Soap S_1 scented fresh (S_1FS)	$16.50±2.50^b$	$16.65±0.50^b$	$22.50±2.50^b$
Soap S_1 scented aged (S_1AS)	$16.50±2.00^b$	$16.00±0.50^b$	$21.66±2.00^b$
Soap S_2 scented fresh (S_2FS)	$17.15±1.04^b$	$17.70±1.30^b$	$24.00±0.57^b$
Soap S_2 scented aged (S_2AS)	$16.80±1.30^b$	$16.50±1.50^b$	$23.00±3.10^b$

**(a, b) Numbers followed by the same number in the column are identical*

Table 2. Antibacterial parameters (MIC and BMC) of different soaps (mg/mL)

Soap types	*E. coli*		*P. aeruginosa*		*S. aureus*	
	CMI	CMB	CMI	CMB	CMI	CMB
Control soap	> 200	> 200	> 200	> 200	> 200	> 200
S_1F	25	50	25	50	12.5	25
S_1A	25	50	25	50	12.5	25
S_1FS	nd	nd	nd	nd	nd	nd
S_1AS	nd	nd	nd	nd	nd	nd
S_2F	25	50	25	50	12.5	12.5
S_2A	25	50	25	50	12.5	12.5
S_2FS	nd	nd	nd	nd	nd	nd
S_2AS	nd	nd	nd	nd	nd	nd

**nd: not determined*

Table 3. Diameter of the inhibition zones of the usual antibiotics (mm)

Antibiotics	*S. aureus*	*E. coli*	*P. aeruginosa*
Rifampicin	28 (S)	15 (S)	14 (S)
Tetracycline	26 (S)	6 (R)	10 (S)
Ampicillin	14 (S)	14 (S)	12 (S)

**R : Resistant; S: Sensitive*

Table 4. Effect of formulated soaps on bacterial strains

Souches bactériennes	Inhibit diameters zone (mm)	Standard deviation	Coefficient of variation	Effect strains
Pseudomonas aeroginosa	16.56[a]	0.38	2.29 %	0.000 (**)
Escherichia coli	16.67[a]	0.59	3.54 %	0.000 (**)
Staphylococcus aureus	21.77[b]	1.22	5.60 %	0.000 (**)

**Means followed by the same letters (single or double) in the same column are not significantly different at the 5% threshold according to the Newman-Keuls test; - significant effect (*); highly significant effect (**).*

increased when it was added to the manufacture of the perfume. This degree of sensitivity on these strains confirms the work of Obi et al., 2014 and of Aliyu et al. 2009 [15,16]. However, the soaps formulated in this study are more sensitive on *E. coli* and *P. aeruginosa* compared to the result of works of Obi et al., 2014 and of Ike, 2016 [15,17]. These authors used soaps whose antimicrobial principles contain chemical compounds such as trichlocarban, triclosan and potassium tetraiodomercurate or potassium mercuric iodide. These compounds are less effective than those contained in the extracts used to make the soaps in our study. Indeed, the work of Gbamélé et al., 2020 [11] showed that *Carapa procera* oil would contain alkaloids, terpenoids, flavonoids and sterols (molecules with proven antimicrobial properties) [18,19,20]. These molecules could act synergistically on microbial metabolism at the bacterial membrane, by chemo-osmotic and ion leakage (K^+) and by action with certain proteins of the microbial membrane such as the Na/K^+ ATPase pump [21].

In effect This inhibitory effect is more pronounced on *S. aureus* than *E. coli* and *P. aeroginosa*. This shows that this function is related to the type of germ, hence the strain-effect determined by factor analysis and the characteristics of each soap. This action would be linked to the structure of the bacterial wall on the one hand and on the other hand the intrinsic composition of the soap while knowing that the strain *S. aureus* is a $Gram^+$ bacterium (BG^+) and that *E. coli* and *P. aeruginosa* are $Gram^-$ bacteria (BG^-). This made it possible to classify the bacteria tested into 2 groups according to the Newman-Keuhls homogeneity test. Morever according Ugbogu, 2006 [22], fatty acids such as lauric and myristic acids present in palm kernel oil contribute to strengthen the inhibitory effect on microorganisms.

In addition, the comparison of the MIC of the soaps obtained in this study with that of Touré et al. 2017 reveals that whatever the type of soap formulated, the MIC values are better than theirs (25 mg/mL and 12.5 mg/mL versus 31.25 mg/mL) [23].

4. CONCLUSION

The study of the antibacterial properties of soaps formulated from *Carapa procera* oil has shown that these soaps have a proven antibacterial effect. This could justify its use in traditional environment against skin infections.

Moreover, these properties are preserved in view of the activities of the soaps (fresh or aged) and its more active when perfume is added. It would be important to conduct tests on patients in order to confirm the antimicrobial efficacy of these soaps.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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