

**Development of antimicrobial soaps using essential oil of *Schinus terebinthifolius* and
*Piper nigrum***

**Desenvolvimento de sabonetes antimicrobianos utilizando-se óleo essencial de *Schinus
terebinthifolius* e de *Piper nigrum***

**Desarrollo de jabones antimicrobianos utilizando aceite esencial de *Schinus
terebinthifolius* y *Piper nigrum***

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Abstract

A cosmetic product consists of natural or synthetic substances, the purpose of which is to wash, scent, correct and protect the various parts of the human body. Human skin has pores that connect to the oil glands, which produce sebum, an oily liquid that carries dead cells through the hair follicles to the surface of the epidermis, causing the appearance of skin lesions and the growth of microorganisms. In the present work, two antimicrobial soaps were formulated in solid and liquid form using essential oil of *Schinus terebinthifolius* and *Piper nigrum*, analysed and compared with other antimicrobial soaps available on the market. In addition, the microbiological safety of the products developed was tested, according to ANVISA criteria. All products developed in this work have microbiological safety, but the best inhibitory results regarding the growth of the microorganisms *Staphylococcus aureus* and *Escherichia coli* were obtained with the soaps in liquid form.

Keywords: Cosmetics; Liquid soap; Bar soap; Essential oil; Antimicrobial activity.

Resumo

Um produto cosmético é constituído por substâncias naturais ou sintéticas, cujo objetivo é lavar, perfumar, corrigir e proteger as diversas partes do corpo humano. A pele humana tem poros que se conectam às glândulas de óleo, que produzem sebo, um líquido oleoso que carrega células mortas através dos folículos pilosos para a superfície da epiderme, propiciando o aparecimento de lesões cutâneas e crescimento de microrganismos. No presente trabalho, dois sabonetes antimicrobianos foram formulados na forma sólida e líquida utilizando-se óleo essencial de *Schinus terebinthifolius* e de *Piper nigrum*, analisados e comparados com outros sabonetes antimicrobianos disponíveis no mercado. Além disso, a segurança microbiológica dos produtos desenvolvido foi testada, segundo os critérios da ANVISA. Todos os produtos desenvolvidos neste trabalho apresentam segurança microbiológica, mas os melhores resultados inibitórios quanto ao crescimento dos microrganismos *Staphylococcus aureus* e *Escherichia coli* foram obtidos com os sabonetes na forma líquida.

Palavras-chave: Cosméticos; Sabonete líquido; Sabonete em barra; Óleo essencial; Atividade antimicrobiana.

Resumen

Un producto cosmético consiste en sustancias naturales o sintéticas, cuyo propósito es lavar, perfumar, corregir y proteger las diversas partes del cuerpo humano. La piel humana tiene poros que se conectan a las glándulas sebáceas, que producen sebo, un líquido aceitoso que

transporta las células muertas a través de los folículos capilares hasta la superficie de la epidermis, lo que provoca la aparición de lesiones en la piel y el crecimiento de microorganismos. En el presente trabajo, se formularon dos jabones antimicrobianos en forma sólida y líquida utilizando aceite esencial de *Schinus terebinthifolius* y *Piper nigrum*, analizados y comparados con otros jabones antimicrobianos disponibles en el mercado. Además, se probó la seguridad microbiológica de los productos desarrollados, de acuerdo con los criterios de ANVISA. Todos los productos desarrollados en este trabajo tienen seguridad microbiológica, pero los mejores resultados inhibitorios con respecto al crecimiento de los microorganismos *Staphylococcus aureus* y *Escherichia coli* se obtuvieron con los jabones en forma líquida.

Palabras clave: Cosméticos; Jabón líquido; Jabón en barra; Aceite esencial; Actividad antimicrobiana.

1. Introduction

Bacterial resistance to antimicrobial agents has increased in recent years due to many factors, including the general increase in antibiotic prescriptions. Gram-negative bacteria such as *Escherichia coli*, commonly found in the intestinal tract, and Gram-positive bacteria such as *Staphylococcus aureus*, are known to be frequently found on the skin and in the nasal passages. Thus, the use of antiseptic agents for the correct cleaning of human skin is extremely important.

According to RDC nº 07 of February 2015 in Brazil (ANVISA, 2015), cosmetics, perfumes and personal hygiene products are “preparations made up of natural or synthetic substances, for external use in the different parts of the human body, skin, capillary system, nails, lips, genitals external, teeth and mucous membranes of the oral cavity, with the exclusive or main objective of cleaning, perfuming, altering their appearance and or correcting body odours and either protecting or maintaining them in good condition”.

Within the skin care line, disinfectants or antiseptics must have high germicidal efficacy, that is, have a rapid effect, broad antimicrobial spectrum, prolonged action, present chemical stability, in addition to being odourless or having a pleasant odour (Alterthum & Carvalhal, 1999). In general, microorganisms grow at a pH close to 7, mainly in the range of 6.6 to 7.5, although there are some that develop at pH 4 (Gomes, Silva-Mann, Mattos & Rabbani, 2013).

Herbal Cosmetics are products formulated using one or more herbal ingredients to provide cosmetic benefits and are known as Cosmeceuticals. The demand of these products is increasing due to their lack of side effects. Cosmeceuticals are cosmetic products intended to improve the health and beauty of the skin by providing a specific result (Joshi & Pawar, 2015). In June 2006, the Decree No. 5.813 (Brasil, 2006) was signed, approving the National Policy on Medicinal Plants and Herbal Medicines, whose main objective is to guarantee to society safe access and correct handling of both herbal medicines and medicinal plants. The measure also aims to promote the sustainable use of biodiversity and the development of the sector's industry (Brasil, 2006).

Essential oils are secondary metabolites produced by plants (roots, floral parts, leaves, fruits, stem), can be extracted by hidrodistillation or cold pressing and there are strong evidences of their antinflammatory, antioxidant and antimicrobial activity. The essential oils have a complex chemical composition and vary the amount of different organic compounds classes (Pérez-Recalde, Arias & Hermida, 2018).

Among the various sources of natural products, *Schinus terebinthifolius* (commonly known as pink pepper) is a species belonging to the Anacardiaceae family, also known as aroeira – da-praia, aroeira, aroeira-Vermelha, pink pepper, cambuí, among other names (Lorenzii & Matos, 2008). It is native to South America and can be found in Europe, Asia and other regions of America, with increasing pharmacological use being considered by popular medicine as astringent, antidiarrheal, anti-inflammatory, depurative, diuretic and febrifugal (Paiva & Aloufa, 2009) . The aroeira fruits have from 5.50 to 8.41% of essential oil, which has a predominant chemical composition of monoterpenes, the most abundant being δ -3-carene, limonene, α -felandrene, α -pinene, mircene and o-cimene, followed by the *trans*-caryophyllene sesquiterpenes, γ -mumulene. However, variations in the composition of essential oils can occur depending on the time of hydrodistillation of the fruits for oil extraction, the geographic region in which the fruits were collected and also on their maturation time (Oliveira Junior et al., 2013).

Piper nigrum (commonly known as black pepper), on the other hand, is a species of shrub creeper, belonging to the Piperaceae family, popularly known as black pepper or black pepper, originating in the tropical regions of India (Bomtempo, 2007; Ee, Lim, Rahmani, Shaari. & Bong, 2010; Carnevalli & Araújo, 2015). Black pepper is a plant rich in retinol (vitamin A), ascorbic acid (vitamin C), iron and potassium, among other compounds. In addition, this spice has a wide variety of metabolites that are distributed in different classes of compounds, such as: amides, alkaloids and propenylphenalins, which have important

biological actions, especially anti-inflammatory and antioxidant (Bomtempo, 2007; Ee, Lim, Rahmani, Shaari. & Bong, 2010), analgesic, healing, circulatory, as well as combating the signs of aging caused by free radicals when consumed in appropriate amounts (Carnevali & Araújo, 2015)

Thus, this study aimed to evaluate the antimicrobial activity of the essential oils of *S. terebinthifolius* and *P. nigrum* and use them in the development of bar and liquid soaps, assessing their antiseptic activity in the prepared formulations, as well as their stability, comparing its antibacterial prospecting with other antimicrobial soaps available on the market, in solid and liquid conditions, such as Dettol, Protex, Lifebuoy, Biocrema and Asepxia. In addition, the microbiological safety of the products was also assessed.

2. Methodology

The work was a laboratory research on a quali/quantitative basis (Pereira et al., 2018).

Obtaining essential oils: *S. terebinthifolius* essential oil was purchased from the company Destilaria Bauru, while *P. nigrum* essential oil was purchased from Ferquima Indústria e Comércio Ltda.

Obtaining commercial soaps: The soaps described in their packaging as antibacterial, in common use, used comparatively in this work, were purchased at cosmetic stores in the city of Uberaba-MG, but are widely marketed in the country. These were used in comparative tests of effectiveness as antiseptics. The bar soaps used were Asepxia, Biocrema, Dettol, Lifebuoy and Protex, while the brands of liquid soap used were Dettol, Lifebuoy and Protex.

Evaluation of antimicrobial activity of essential oils: The suspensions of *S. aureus* (ATCC 29213) and *E. coli* (ATCC 35218) were diluted 1:100 based on the amount of Muller-Hinton Agar medium placed in the Petri dishes (CLSI, 2018). Sterile 5 mm diameter filter paper discs impregnated with the essential oils of *S. terebinthifolius* and *P. nigrum* were placed in 9.2 cm diameter Petri dishes. Then the plates were incubated at 37°C for 24 hours. The evaluation of the antimicrobial activity of the formulated soaps was carried out in triplicate.

Preparation of the formulation of the antiseptic bar soap: The formulation of the bar soap was based on a basic composition with the presence of surfactants that provide detergency, as

well as quantity and quality of foam, viscosity agents, overfat, preservatives and ion scavengers following the formula in Table 1. Three soap formulations were prepared in solid form: adding essential oil of *S. terebinthifolius* (SSPR), adding essential oil of *P. nigrum* (SSPP) and adding both essential oils (SSPP/PR).

Table 1. Formulation for bar soap.

Materials	Function	Compositionção%
glicerine	moisturizer	31.,25
lauril eter	bubbly	10.42
propileneglicol	hHumectant	10.42
Essential oil	antibacterial	6.24
citric acid	pH concealer	10.42
destiled water		31.25

Source: Authors (2020).

Preparation of the formulation of an antiseptic liquid soap: For the formulation of liquid soaps, a soap base was used whose components are sodium lauryl ether sulfate, amide, water, sodium chloride, amphoteric acid, EDTA and citric acid. Distilled water and essential oils were added slowly, to avoid previous foam formation. Three soap formulations were prepared in liquid form: adding essential oil of *S. terebinthifolius* (SLPR), adding essential oil of *P. nigrum* (SLPP) and adding both essential oils (SLPP/PR).

Organoleptic parameters analysed: Samples of commercial and formulated soaps were analysed for colour, odour and homogeneity, according to ANVISA (2012).

Physical-Chemical Analysis: for pH analysis, formulations were evaluated on a pH meter; to analyses the viscosity of the formulated liquid soaps, a Brookfield Viscometer was used; to assess the density of the formulated liquid soaps, a clean, dry and calibrated pycnometer with a capacity of 100 mL was obtained. The density analysis was performed according to the guidelines of the Brazilian Pharmacopoeia (Farmacopéia Brasileira, 1988). All tests were done in triplicate.

Evaluation of the stability of the formulations: Based on the combinations made, the organoleptic and physical-chemical parameters were evaluated using the centrifugation test, in which the liquid soaps were subjected to centrifugation at 3,000 rpm for 30 minutes and the formation or not of sediments, phase separation and formation of agglomerates; Foam analysis, in which the foam index was determined using 100 mL beaker, with 5 mL of sample, addition of distilled water to the 50 mL mark, followed by stirring for 10 seconds, the foam formed was measured in cm, with the aid of a ruler, at times 0, 5, 10 and 15 minutes. All tests were done in triplicate.

Evaluation of the antimicrobial activity of commercial soaps: The suspensions of *S. aureus* and *E. coli* were diluted 1: 100 based on the amount of Muller-Hinton Agar medium placed in Petri dishes (CLSI, 2018). Sterile 5 mm diameter filter paper discs impregnated with the samples of the soaps, once diluted in 5mL of distilled water, were placed in 9.2 cm diameter Petri dishes. Then the plates were incubated at 37°C for 24 hours for the bacteria. The evaluation of the antimicrobial activity of essential oils was carried out in triplicate.

Evaluation of antiseptic properties of formulated soaps: The antiseptic activity of formulations containing bioactive essential oils was verified by measuring the zone of inhibition of bacterial growth and compared with each other and with the antibacterial activity of commercially available soaps. The methodology used for this evaluation was the same used for the evaluation of the antiseptic properties of commercial soaps. All tests were done in triplicate.

Evaluation of the antiseptic properties of antibiotic discs: The antiseptic activity of antibiotic discs (Bio-Rad, was performed for some antibiotics commonly used for comparison purposes. The methodology used for this evaluation was the same used for the evaluation of the antiseptic properties of antibiotics). commercial soaps All tests were performed in triplicate.

Evaluation of the microbiological safety of the formulated soaps: for the evaluation of the microbiological safety of the formulated soaps, the research of *Escherichia coli* was carried out, as well as, of *Staphylococcus* and *Pseudomonas*, according to ANVISA (2012). For *E. coli* research, 1 g of sample was aseptically transferred to 9 mL of saline. Then, 1 ml of the solution was transferred to the Petri dish containing Eosin Methyl Blue agar (EMB) and the

dish was incubated at $36 \pm 1^\circ\text{C}$ for 24 hours. After this period, it was observed whether there was a growth of colonies, as well as their characteristics (if present, they are black metallic colonies). When there is growth of suspicious colonies, the biochemical series for the identification of *E. coli* must be followed. For the research of *Staphylococcus* and *Pseudomonas*, 1 g of sample was aseptically transferred to 9 mL of soy-casein broth and this broth was incubated at $36 \pm 1^\circ\text{C}$ for 24 to 48 hours. Then, it was sown in Petri dishes containing Vogel Johnson agar for *Staphylococcus* research, and in cetrimide agar plates for *Pseudomonas* research. The plates were incubated at $36 \pm 1^\circ\text{C}$ for 24 hours. After this period, it was observed whether there was growth and the characteristics of the colonies. For *Staphylococcus*, the presence of yellowish colonies or the appearance of specific characteristics must be confirmed with the catalase and coagulase tests. For *Pseudomonas*, on the other hand, the presence of blue-green colonies must be confirmed by the microorganism by the biochemical series.

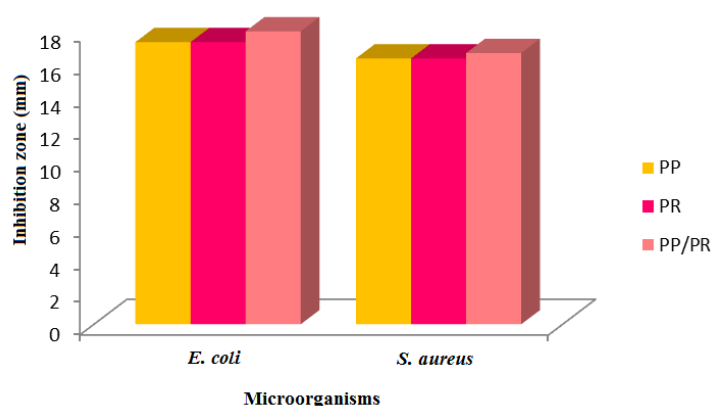
3. Results and Discussion

Antimicrobial activity of essential oils used: The antibacterial activity of essential oils was evaluated. Figure 1 shows the inhibitory zones presented by the essential oils used in this study.

The inhibition halos are symmetrical, proportional, with significant and similar sizes around 18 mm. In addition to the compounds that make up essential oils that have antimicrobial activity, it is noted that their volatility and odour can influence the diameter of the inhibitory zones.

Organoleptic Analysis: The organoleptic characteristics of the commercialized soaps and the formulations developed in this work were evaluated. Colour, odour, and general appearance were evaluated, and the brands evaluated were: Asepxia, Biocrema, Dettol, Lifebuoy and Protex. In all evaluated samples, the properties were shown to be adequate, as shown in Tables 2 and 3.

Figure 1. Antibacterial activity of essential oils.



Legend: PP - black pepper essential oil; PR - essential oil of pink pepper; PP / PR - both essential oils.
 Source: Authors (2020).

Table 2. Organoleptic characteristics of bar soaps.

Sample	Colour	Odour	Aspect
Asepxia	Blue	Characteristic	Solid/light transparency
Biocrema	Ggreen	Characteristic	Solid / opaque
Dettol	White	Characteristic	Solid / opaque
Lifebuoy	Ggreen	Characteristic	Solid / opaque
Protex	Cream	Characteristic	Solid / opaque
SB(PP)	Yyellow	Characteristic	Solid/light transparency
SB(PR)	Rose	Characteristic	Solid/light transparency
SB(PP/PR)	Rose	Characteristic	Solid/light transparency

Legend: SL (PP) - liquid soap with essential oil of black pepper; SL (PR) - liquid soap with essential oil of pink pepper; SL (PP / PR) - liquid soap plus essential oils of black pepper and pink pepper.
 Source: Authors (2020).

Table 3. Organoleptic characteristics of liquid soaps.

Sample	Colour	Odour	Aspect
Dettol	White	Characteristic	Creamy
Lifebuoy	Green	Characteristic	Creamy/Pearly
Protex	Orange	Characteristic	Creamy/Pearly
SL(PP)	Yellow	Characteristic	Creamy
SL(PR)	Rose	Characteristic	Creamy
SL(PP/PR)	Rose	Characteristic	Creamy

Legend: SL (PP) - liquid soap with essential oil of black pepper; SL (PR) - liquid soap with essential oil of pink pepper; SL (PP / PR) - liquid soap plus essential oils of black pepper and pink pepper. Source: Authors (2020).

The organoleptic parameters observed were within the standards and comparable among all soaps, which showed color, odor and characteristic aspects, differing according to each brand or formulation. None of the samples showed visual alteration, nor was any solid matter found in any formulation. All brands had an odor compatible with the product and its composition.

Physical-Chemical Analysis: Table 4 shows the results obtained for pH, viscosity and density, relative to soaps in liquid form. Among the physical-chemical characteristics, pH is the fundamental analysis, since the pH of the skin, especially the skin of the face, where there is a higher concentration of infections due to the presence of numerous microorganisms, is a more neutral pH and requires the formulation of cosmetic product has a pH compatible to the region thus helping to maintain your health and protect the skin region. The soap that has both essential oils (SLPP/PR) showed a pH equal to six, closer to the skin's pH and close to the pH values of the tested commercial soaps. This is important because, with a pH close to neutral, the cells of the epidermis are not attacked by the product.

Table 4. Physical-chemical parameters of liquid soaps.

Sample	pH	viscosity (cP)	relative density (g/mL)
Dettol	5.2	3400	1.003
Lifebuoy	5.3	2500	1.061
Protex	5.7	2300	1.034
SL(PP)	5.1	4000	1.086
SL(PR)	5.5	4200	1.092
SL(PP/PR)	6	3300	1.099

Legend: SL (PP) - liquid soap with essential oil of black pepper; SL (PR) - liquid soap with essential oil of pink pepper; SL (PP / PR) - liquid soap plus essential oils of black pepper and pink pepper. Source: Authors (2020).

The liquid soap containing only the essential oil of pink pepper (SLPR) showed higher viscosity (4200 cP). However, it should be noted that all values are within the range established by ANVISA for liquid components and gels, which varies from 2000 to 7000 cP. The Protex brand liquid soap presented the lowest viscosity (2300 cP), thus, a more fluid flow product, i.e., its flow is faster. The soaps that showed intermediate viscosity were Dettol, Lifebuoy. The soaps containing only black pepper essential oil (SLPP) and the mixture of both oils (SLPP / PR), presented viscosity of 3400, 2500, 4000 and 3300 cP, respectively.

A small variation in viscosity values was observed in Table 4. It is noted that the soap with higher viscosity, flows through the packaging with more difficulty and slowly, while the soaps that presented lower viscosity, flowed through the packaging. This fact is linked to waste due to accelerated flow. Regarding viscosity, all formulated liquid soaps present values like or higher than those of the analysed brands, showing better options than the soaps found in the national market.

The Relative density is the amount of mass per volume of a substance. The liquid soaps formulated in this work showed the highest density values among the analysed samples. Dettol soap had the lowest density (1,003 g/mL), while the soap containing the mixture of essential oils (SL PP/PR), the highest density among the samples analysed (1,099 g/mL). However, the relative densities of liquid soaps did not differ from ideality, and were similar, remaining within the range of 0.9 - 1.1 g/mL.

Evaluation of the stability of the formulations: After the centrifugation test, none of the samples showed any change, such as agglomerates, phase separation, or precipitation. All

samples remained stable during the test, so the results were satisfactory for all samples analysed, commercial or formulated in this work. Table 5 shows the foam indices during fifteen minutes of analysis.

Table 5. Índice de espuma das amostras dos sabonetes líquidos.

time (min)	Samples					
	Dettol	Lifebuoy	Protex	SL(PP)	SL(PR)	SL(PP/PR)
0'	7.5	8.5	8.5	10.0	9.5	9.5
5'	6.0	7.0	7.5	8.5	8.0	8.0
10'	5.5	6.0	6.0	7.0	7.5	7.0
15'	4.0	4.5	5.5	5.5	6.0	5.5

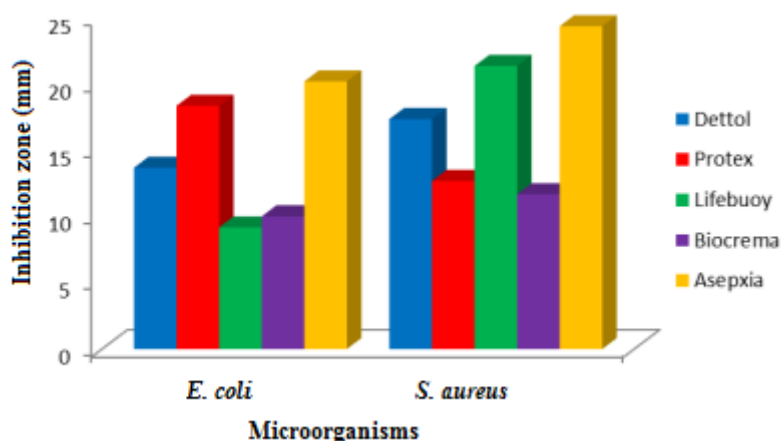
Legend: SL (PP) - liquid soap with essential oil of black pepper; SL (PR) - liquid soap with essential oil of pink pepper; SL (PP / PR) - liquid soap plus essential oils of black pepper and pink pepper. Source: Authors (2020).

It is observed that the foam index decreases over time for all samples, ranging from 0.5 to 1.5 cm. The soap with the essential oil of black pepper (SLPP) showed the largest amount of foam in the zero time, but at the end of the fifteen minutes, the greatest loss was obtained among all the analysed samples (4.5 cm).

Evaluation of the antimicrobial activity of commercial bar soaps: data on the antimicrobial activity of bar soaps from the brands Asepxia, Biocrema, Dettol, Lifebuoy and Protex are shown in Figure 2.

In general, *S. aureus* was more sensitive to the soaps tested. However, the Assepxia soap showed a maximum inhibition zone of 24.33mm in diameter. Despite presenting smaller inhibition zones, the other brands also showed good inhibition results for the growth of *S. aureus*, such as Lifebuoy - 21.33mm, Dettol - 17.33mm, Protex - 12.67mm and Biocrema - 11.67mm. For *E. coli*, the inhibition zones were not as significant as for *S. aureus*. The Assepxia brand soap showed the best result with a 20.16mm of inhibition zone, followed by Protex soaps with 18.33mm, Dettol with 13.67mm, Biocrema with 10mm and Lifebuoy with 9.17mm.

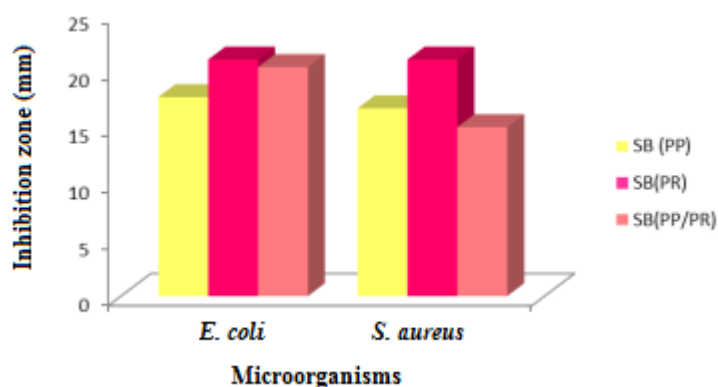
Figure 2. Antibacterial activity of commercially purchased bar soaps.



Source: Authors (2020).

Evaluation of antiseptic properties of bar formulations: to evaluate the antibacterial activity of formulated bar soaps, the same methodology used to evaluate the antimicrobial activity of commercially available antibacterial bar soaps was used. Figure 3 shows the values of the zones of inhibition (in mm) for bar soaps.

Figure 3. Antibacterial activity of formulated bar soaps.



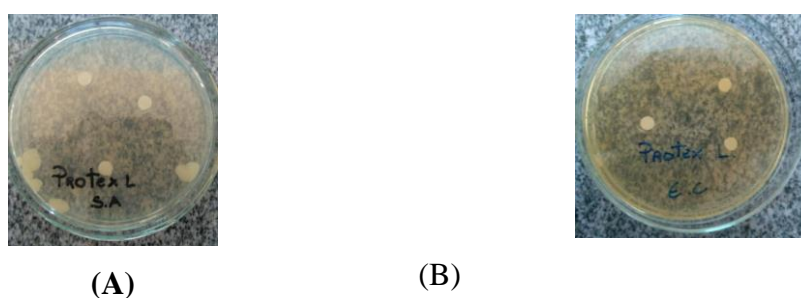
Source: Authors (2020).

It is observed that for both microorganisms, the soap containing only the essential oil of pink pepper (SSPR) presented the greatest zone of inhibition for the strain of *S. aureus* (21 mm) and for the strain of *E. coli* (21 mm). The other samples had lower values, 17.67 mm for *S. aureus* and 20.33 mm for *E. coli* for soaps containing only black pepper oil (SSPP) and for soap with both oils (SSPP / PR) respectively, using the *E. coli* strain. Taking into account *S.*

aureus, the soaps with black pepper (SSPP) and with both oils (SSPP / PR), showed inhibition zones of 16.67 mm and 15 mm, respectively.

Evaluation of antiseptic activities of commercially acquired liquid soaps: commercially acquired liquid soaps showed large inhibitory zones, greater than 25 mm. The liquid Protex soap proved to be the most effective and inhibited 100% of the growth of the colonies of both studied microorganisms, as shown in Figure 4. Among the commercial liquid soaps that did not obtain results as satisfactory as the Protex soap, stands out the Dettol Liquid brand. Although this soap did not have the best zones of inhibition, it still presented significant and acceptable zones of approximately 28.67 mm for *S. aureus* and 25.33 mm for *E. coli*, as shown in Figure 5.

Figure 4. Liquid Protex soap against *S. aureus* (A) and against *E. coli* (B).



Source: Authors (2020).

Figure 5. Dettol liquid soap against *S. aureus* (A) and against *E. coli* (B).



Source: Authors (2020).

The liquid soap of the brand Lifebuoy showed intermediate zones of inhibition, of approximately, 28.33 mm against *S. aureus* and 31.67 mm against *E. coli*.

Evaluation of antiseptic activities of formulated liquid soaps: for liquid soaps formulated with bioactive essential oils, large inhibitory zones were also expected, which would match

the areas presented by commercially acquired liquid soaps, since the isolated essential oils showed antimicrobial activity considerable. Figure 6 shows the Petri dish used in the bioassay of antimicrobial activity of the liquid formulation containing black pepper essential oil (SLPP) against *S. aureus*.

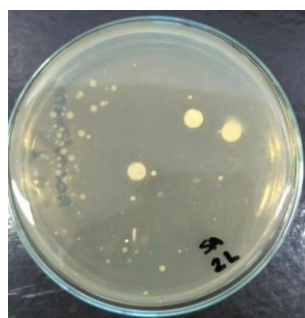
Figure 6. Black pepper essential oil soap against *S. aureus*.



Source: Authors (2020).

Similar areas are observed with diameters of approximately 32.67 mm, these being the lowest values observed for the formulation containing black pepper essential oil. The formulation containing pink pepper essential oil showed an intermediate inhibition zone of approximately 26.67 mm in diameter, which is considered good, large and desirable. When both essential oils were used together in the formulation of liquid soap, the result obtained from antibacterial activity was equivalent to the brand Protex Liquid, inhibiting, practically, 100% of the growth of *S. aureus* as shown in Figure 7.

Figure 7. Soap formulated with both essential oils against *S. aureus*.

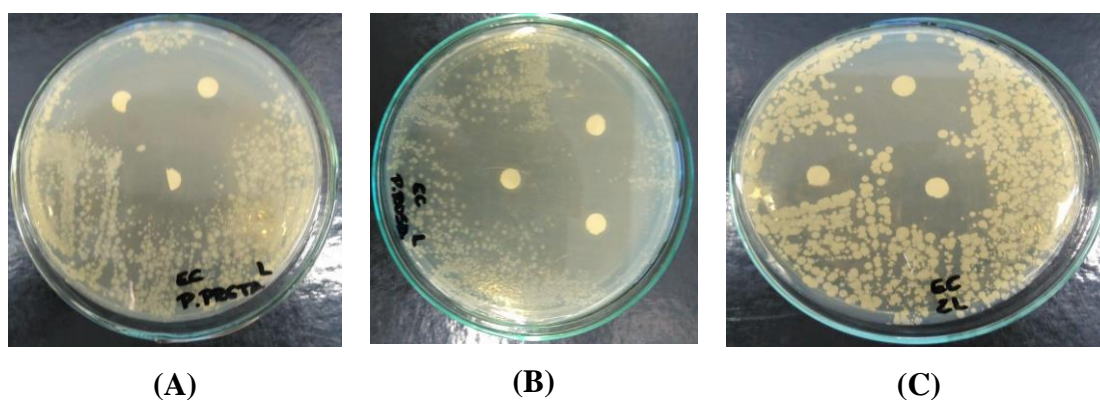


Source: Authors (2020).

The *E. coli* bacteria, proved to be more resistant to the effects of formulated liquid soaps, as well as to the effects of bar soaps. These showed satisfactory zones of inhibition,

however, with smaller diameters, when compared to the Protex Liquid brand against the same microorganism. Figure 8 shows the diameters of the zones of inhibition for formulations containing only the essential oil of black pepper (A), only the essential oil of pink pepper (B) and the formulation with both essential oils (C), with diameters approximately 32.67 mm, 30 mm and 29.33 mm, respectively.

Figure 8. Black Pepper essential oil soap (A), pink pepper essential oil (B) and the formulation with both essential oils (C).



Source: Authors (2020).

Evaluation of the antiseptic properties of antibiotic discs: antibiotics impregnated in discs used in antibiograms were: clarithromycin (CLA 15), ciprofloxacin (CIP 05), cefaclor (CFC 30), ceftriaxone (CRO 30), ampicillin (AMP 10), penicillin G (PEN 10), vancomycin (VAN 30) and chloramphenicol (CLO 30), with a concentration of 5 µg / disc. The data obtained for commercial soaps, formulated soaps and antibiotic disks are shown in Table 6.

The bar soaps formulated in this work had an antibacterial action comparable to commercial bar soaps with better antibacterial action, such as Asepxia and Lifebuoy. The antibacterial action against *S. aureus* of the black pepper bar soap had an antibacterial action comparable to the antibiotic Vancomycin. The Asepxia soap had an action comparable to the antibiotic Chloramphenicol. When the antibacterial action against *E. coli* is compared, the antibacterial action of the pink pepper bar soap, the bar soap containing both essential oils and the Asepxia soap had an action comparable to the antibiotic Cefaclor.

Comparing the liquid soaps, the liquid soap containing both essential oils and the Protex soap had the best results, having no antibiotics, of those tested, with comparable action. All formulated liquid soaps and Lifebuoy liquid soap had antibacterial action comparable to the antibiotics ceftriaxone and ciprofloxacin.

Table 6. Inhibition zones of commercial, formulated soaps and antibiotic disks.

		<i>S. aureus</i>	<i>E. coli</i>
	Asepxia	24.33	20.16
	Biocrema	11.67	10.00
Dettol	bar	17.33	13.67
	liquid	28.67	25.33
Lifebuoy	bar	21.33	9.00
	liquid	28.33	31.67
Protex	bar	12.67	18.33
	liquid	100%	100%
PP	bar	16.67	18.00
	liquid	32.67	32.67
PR	bar	21.00	21.00
	liquid	26.67	30.00
PP/PR	bar	15.00	20.33
	liquid	100%	29.33
	CLA15		19.00
	CIP 05		33.00
	CFC 30		21.00
	CRO 30		29.50
	AMP 10	-	-
	PEN 10	-	-
	CLO 30	23.50	
	VAN 30	20.00	

Legend: PP - black pepper essential oil, PR - pink pepper essential oil, PP / PR - black pepper and pink pepper essential oil, clarithromycin (CLA 15), ciprofloxacin (CIP 05), cefaclor (CFC 30), ceftriaxone (CRO 30), ampicillin (AMP 10), penicillin G (PEN 10), vancomycin (VAN 30) and chloramphenicol (CLO 30). Source: Authors (2020).

Thus, it is evident that the soaps in pink pepper bar and with both essential oils, as well as all formulated liquid soaps are proving to be new, promising and innovative options for the segment of antimicrobial soaps.

Evaluation of the microbiological safety of formulated soaps: according to the microbiological safety assessment of the products studied, there was in *E. coli*, just as there was in *Staphylococcus* and *Pseudomonas* growth. In this way, it is possible to conclude that

the essential oils used are effective antimicrobial additives, as none of the products developed showed growth of pathogenic microorganisms in the research carried out. Therefore, the products developed in this work are in accordance with RDC 07/2015 (ANVISA, 2015) which deals with the microbiological control of cosmetic products.

Following Lima et al. (2020), any cosmetic products are subject to microbial contamination. However, the lack of hygiene in the development and the low stability of the constituents of the formulation contribute to the contamination of the products and cause visible changes.

4. Conclusions

The present work brought the development of liquid and bar formulations of antimicrobial soaps, using the essential oils of black pepper, pink pepper and both, in order to prevent skin infections aggravated by microorganisms, emphasize the importance of their use and thus guide through the data the importance of phytocosmetics. The products developed in this work are in accordance with Resolution 07/2015, which deals with the microbiological control of cosmetic products. Thus, it is concluded that the soaps in pink pepper bar and with both essential oils, as well as all formulated liquid soaps are new, promising and innovative products for the segment of antimicrobial soaps.

In future studies other essential oils will be used to test the same properties.

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