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ANTIBACTERIAL ACTIVITY OF SOAP MADE FROM ESSENTIAL OILS AND COMMERCIAL SOAP SOLD IN THE LEBANESE MARKET

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ABSTRACT

Background: Thousands of people are dying as a result of infections caused by bacteria. Among the main routes of germ transmission are the hands, making hand hygiene very important in preventing the spread of pathogens and bacterial infection. Hand washing with soap and water is considered to be a simple and effective measure. Old soap manufacturers have long had traditional uses in the Lebanese community.
Methods: The purpose of this study is to compare the antibacterial activity of oil-based soaps with commercial soap sold in the Lebanese market. Different types of herbal soap and antiseptics have been used in this study. Four bacterial strains were used: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Enterobacter* Spp. The antibacterial activities of these soaps were determined by the diffusion method of disks in agar medium.
Results and Discussion: Statistical analysis of zones of inhibition showed that *S. epidermidis, P. aeruginosa* and *Enterobacter* Spp. were sensitive only to traditional oil-based soaps, which are Sage, Rose Mary and Cedar. In addition, *S. aureus* showed sensitivity to soaps comprised of essential oils as well as antibacterial synthetic soap, Dettol and Lifebuoy. On the other hand, *E. coli* showed resistance to all soaps. Soaps comprised of natural essential oils have shown antibacterial activity superior to so-called "Antibacterial" soaps.
Conclusion: Based on this study, we can say that the use of soaps with essential oils might be the best option due to their organic origin as well as their antibacterial proved activities.

Keywords: soaps, antibacterial activity, essential oils

INTRODUCTION

Hand hygiene has always been the main focus in halting the spread of infectious agents. The use of antibacterial soap has been routinely used in hospitals, at home, and at schools to prevent infections. Soap can be defined as a chemical compound resulting from the interaction of fatty acids, oils and salt (Friedman & Wolf, s.d.). In the treatment of skin diseases, hand washing with soap causes cooling, drying, hydration, crust, and squamous elimination **(Becker, 1974)**. Larson et al. and Toshima et al. have reported that soap containing antibacterial active ingredients such as Triclosan has a higher rate of removing bacteria **(Larson, Eke, Wilder, & Laughon, 1987 ; Toshima et al., 2001)**. Furthermore, Osborne and Grube previously reported that antibacterial soaps could eliminate 65% to 85% of human skin bacteria **(Osborne & Grube, s.d.)**. When washing with soap is done properly, it could reduce the *Propionibacterium acnes* bacterium and effectively prevent secondary infections in the skin infected by this bacterium **(Kuehl, Fyfe, & Shear, 2003)**. Previous experiments have shown that antibacterial chemicals used in household products are unable to kill germs that cause dangerous diseases. Furthermore, antibacterial soaps were banned in the US market on Friday, September 2, 2016 in a final ruling by the Food and Drug Administration (FDA), which declared that the manufacturers had failed to prove that antibacterial soap was more effective than ordinary soap. Woolcock J., director of the FDA's Center for Evaluation and Research, said that some antimicrobial soaps are not at all beneficial to health: "Consumers may think that antibacterial washes are more effective in preventing the spread of germs, but we have no scientific evidence that they are better than regular soap and water". In fact, some data suggests that antibacterial ingredients may do more harm than good in the long run **(Fischer, 2016)**. Soap manufacturers have failed to demonstrate the safety of "long-term daily use" or the fact that antimicrobial soap is more effective than ordinary soap and water in preventing disease and the spread of certain infections. They published results of 19 different antibacterial chemicals, including triclosan and triclocarban, claiming that they were not effective at killing pathogenic bacteria. UK firm Unilever has announced that it will phase out both chemicals by the end of the year, replacing them with "natural and nature-inspired" antimicrobials. However, scientists have said that these measures have not gone far enough to protect customers (*Through their use in toothpastes, mouthwashes, soaps, deodorants and cleaning products, anti-bacterial ingredients help maintain healthy bodies and clean homes.*, 2018).
Scientific developments have shown that the medicinal properties of plants have been of great interest because of their low toxicity, pharmacological activities, and economic viability. One of the additives naturally obtained from these plants is the essential oil. Plant extracts and essential oils have been reported to possess antifungal, antibacterial, and antiviral properties and have been examined on a global scale as potential sources of novel antimicrobial compounds, food preservation agents, and alternatives for treatment. Recently, many studies have deeply investigated the features of essential oils showing remarkable antibacterial effects **(Chouhan, Sharma, & Guleria, 2017 ; Martínez, Betancourt, Alonso-González, & Jauregui, 1996 ; Swamy, Akhtar, & Sinniah, 2016).** Several studies were carried out by applying biological tests, such as the diffusion of wells, the diffusion of disks, and the determination of the minimum inhibitory concentration (MIC), which were shown to be beneficial in studying the effectiveness of different essential oils against several pathogens. Rosmarinus Officinalis oil was tested against gram-positive and gram-negative bacterial strains, showing antibacterial activity especially on gram-positive strains of bacteria. Furthermore, palm oil (the commercial palm kernel oil) has shown a notable bactericidal effect against *Staphylococcus aureus* and *Streptococcus spp*. As a result, essential oils where proven to be a powerful tool for reducing bacterial resistance **(Chouhan et al., 2017)**.
Ever since the fourteenth century, Lebanon has been internationally recognized by its expertise in manufacturing and fabricating soap containing essential oils and specifically olive oil. Over time, this industry has been greatly developed and many other types of oils were introduced to soap synthesis. To date, no studies exist focusing on whether the essential oil-based Lebanese soap has antibacterial effects or not. The starting point of our study was based on the idea that essential oils have antibacterial activities known and described in literature, as well as the question of whether these oils kept this activity during the manufacturing process. In addition, we analyzed the possibility of quantitative and qualitative effects of oils against several bacteria when added to soap.
In order to achieve these findings, a total number of 6 different brands of the most commonly used soap were randomly purchased from stores and pharmacies. These soaps were tested for antibacterial activity by the diffusion method in agar medium (disk method). In parallel, three types of organic/oil-based soaps were purchased from local stores and subsequently submitted to the same test.

MATERIALS AND METHODS

Materials and Reagents

Different types of herbal and antiseptic soap samples were purchased from local markets (Tripoli, Lebanon), taking notes of content and expiration dates of all soaps. The soaps used in the experiment were: Dettol™, Lux®, Lifebuoy®,

Palmolive™, Johnson®, traditional soap (Saifan™) and local Lebanese soaps made from essential oils (Sage, Rose Mary and Cedar). The ingredients, utilization, and manufacturer of each soap type are presented in Table 1. Chapman, Mueller-Hinton agar mediums were purchased from (Bio-Rad, France), and antibiotics and neutral disks were purchased from (Sigma-Aldrich, Germany).

Table 1 Table showing the active ingredient, use, and manufacturer of each type of soap

Brand	Active ingredient	Indication/Utilization	Manufacturer
Dettol™	Chloroxylenol l0.5% Trichrolocarbanilide 0.5%	Bactericidal	Soap and chemicals industrial & tradind CO (United Arab Emirates)
Lifebuoy®	Trichrolocarbanilide 0.06% triclosan 0.02%	Bactericidal	Unilever Mashrek home care S.A.E (Egypt)
Lux®	Etidronic acid, Titanium dioxide, Disodium Distyryl biphenyl disulfonate Sodium Tallowate,	Soothing soap Beauty soap	Unilever Mashreq home care A.S.E (Egypt)
Johnson®	Sodium Cocoate, Glycerin, Sodium Stearate, Sorbitol, Disodium Dilinoleate, Stearic Acid, Propylene Glycol, Potassium Tallowate, Sodium Chloride, Fragrance, Pentasodium Pentetate, Tetrasodium Etidronate	Beauty soap	Green Planet Industries (Dubai)
Palmolive™	Ammonium C12-15 Pareth Sulfate Lauramidopropylamine oxide	Polyvalent	Colgate-Palmolive Arabia L.T.D (SaudiArabia)
Saifan	Olive oil	Polyvalent	SaidSaifan Est (Lebanon)
Sage	Olive oil Sage oil Coconut oil Palm oil	Polyvalent	Bader Hassoun Eco village (Lebanon)
Rose Mary	Olive oil Mary rose oil Coconut oil	Polyvalent	Bader Hassoun Eco village (Lebanon)
Cedar	Olive oil	Polyvalent	Bader Hassoun Eco village (Lebanon)

Isolation of bacteria

The used bacteria in this study are: *Staphylococcus aureus*, *Staphylococcus epidermidis, Escherichia coli, Pseudomonas aeruginosa, and Enterobacter*. The choice of bacterial strains was based on the possibility of hand contamination. All strains were collected from Maritime-Jbeil Hospital except *Staphylococcus epidermidis*, which was recovered from a hand-surface harvest using sterile wet cotton sticks. The collected samples were then cultured on Chapman Agar medium and incubated at 37 °C for 24 hours. Various tests were performed on the obtained colonies in order to identify the collected strains, taking into account the biochemical characteristics of the bacteria, and to verify the strains already obtained from the hospital.

Determination of morphological characteristics

A smear was prepared by placing normal saline on a clean sterile glass slide; a sterilized wire loop was used to pick the colony for emulsification on the slide. The smear was heat fixed by passing the slide through a flame three times. It was then covered with crystal violet to stain the bacteria and allowed to stand for one minute, then washed with distilled water without blotting. This was followed by covering the smear with logul's iodine solution and leaving it to stand for one minute. It was then washed with distilled water, flushed with acetone alcohol for decolorization for three seconds, and washed immediately to prevent excessive decolorization. Following that, the slide was flooded with safranin (counter stain) and left for one minute after which it was washed with distilled water and allowed to dry. The dried slides were viewed under the microscope using the immersion oil objective (x100).

Biochemical tests

The following biochemical tests were carried out: oxidase, catalase, lactose, mannitol, citrate, gas urease, coagulase, DNase and indole tests using standard procedures described in Barrow and Felthham, (Barrow & Feltham, 1993) and Chessbrough **(Cheesbrough, 2006)**.

Antimicrobial Susceptibility Testing

In vitro antimicrobial susceptibility tests were performed on Mueller-Hinton agar by using Kirby-Bauer disks diffusion technique (Bauer, Kirby, Sherris, & Turck, 1966). The tested antibiotics were Amoxicillin (AMC, 30 µg), Ampicillin (AMP, 10 µg), Erythromycin (E, 15 µg), Gentamicin (CN, 10 µg), Chloramphenicol (C, 30 µg), Ciprofloxacin (CIP, 5 µg), Cefoxitin (FOX, 30 µg), Norfloxacin (NOR, 10 µg), Nalidixic acid (NA, 30 µg), Trimethoprim-sulfamethoxazole (SXT, 25 µg), and Tetracycline (TE, 30 µg). Morphologically identical bacterial colonies (4 to 6) from overnight culture were suspended in 5 ml nutrient broth and incubated for 4 hours at 37°C. Broth culture turbidity was equilibrated to reach 0.5 McFarland standards. Then after, the surface of Mueller-Hinton agar plate was inoculated with the culture using a sterile swab. The antibiotic disks were applied on the surface of the inoculated agar. 18–24 hours after incubation, the diameter of the inhibition zone around the disks was measured and interpreted as sensitive, intermediate, or resistant according to Clinical and Laboratory Standards Institute **(*Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; 24th informational supplement (M100S)*, 2016)**.

Soap dilution and disk preparation

With the help of a sharp sterile knife, the soaps were scraped off. 1 g of each soap sample was weighed and dissolved in a volume of 25 ml of sterile distilled water separately. The prepared solution is used for the preparation of the disks (concentration of 0.04 g/ml). Bleach (Clorox) diluted 1/10 in distilled water was used as a positive control. Six mm size filter paper disks were prepared in a Petri dish post sterilization in an autoclave at 121 °C for 15 minutes. Each sterile disk was soaked separately in the different dilutions of soaps prepared and in diluted bleach. They were then incubated at room temperature for a half-hour period to ensure complete saturation.

Disk diffusion method

The measurement of the antibacterial activity of soap was made by the disk diffusion method (Aulet de Saab, de Castillo, de Ruiz Holgado, & de Nader, 2001). The inoculum of each bacterial strain was prepared with 5 ml of saline (NaCl). One to two colonies were added to the prepared solution. The prepared

bacterial suspension was then cultured on Muller Hinton medium. Sterile filter paper disks prepared from the different soap samples were transferred directly to Muller Hinton using sterile forceps. All petri dishes were incubated at 37 °C for 24 hours. After the incubation period, the inhibition zones around the disks were identified and measured. The zone of inhibition was determined by measuring the diameter in millimeters of the area at which the soap inhibits the growth of bacteria.

Statistical Analysis

The statistical significance was determined using a two-tailed *t*-test. A *p* value less than 0.05 was considered significant for all comparisons. Bars represent standard deviations, all experiments were repeated in triplicates (Fig. 1A, 1B, 1C, and 1D).

RESULTS

Bacterial isolation and identification

Following the sample collection, bacterial cultures were done on Champan Agar medium and incubated at 37 °C for 24 hours.
In order to identify the isolated bacterial strains, different biochemical tests were used and the results are shown in Table **2**.

Table 2 Table showing the biochemical characteristics of each strain as positive (+), negative (-) results and not applicable (N/A)

TEST	Bacterial strains				
	S. aureus	*S. epidermidis*	*E. coli*	*P. aeruginosa*	*Enterobacter Spp.*
Oxidase	N/A	-	N/A	+	-
Catalase	+	+	N/A	-	+
Lactose	N/A	N/A	+	-	+
Mannitol	+	-	N/A	N/A	NA
Citrate	N/A	N/A	N/A	N/A	+
Gaz	N/A	N/A	N/A	+	NA
Urease	N/A	N/A	-	N/A	-
Coagulase	+	N/A	N/A	N/A	NA
DNase	+	N/A	N/A	N/A	NA
Indole	N/A	N/A	+	N/A	-
Gram	+	+	-	-	-

Antimicrobial Susceptibility Testing

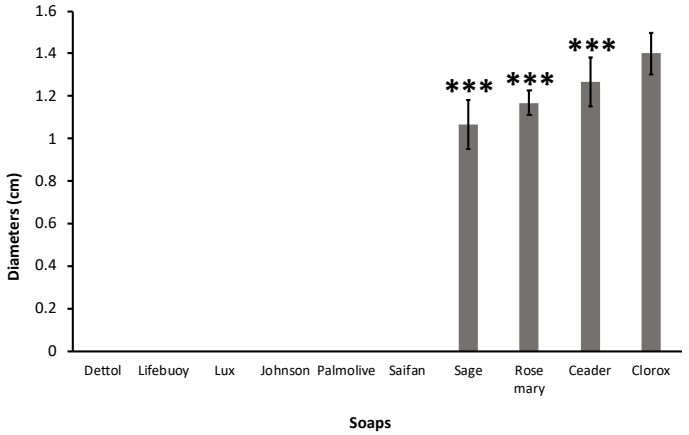
The isolates were resistant to amoxillin, ampicillin, tetracyclin, erythromycin and rimethoprim-sulfamethoxazole. There were also intermediate levels of resistances to cefoxitin, gentamicin, and nalidixic acid, Table **3**.

Table 3 Antibiotic susceptibility pattern of *S. aureus, S. epidermidis, E. coli, P. aeruginosa, and Enterobacter*; S sensitive, I: intermediate, R: resistance. (SXT* = trimethoprim-sulfamethoxazole)

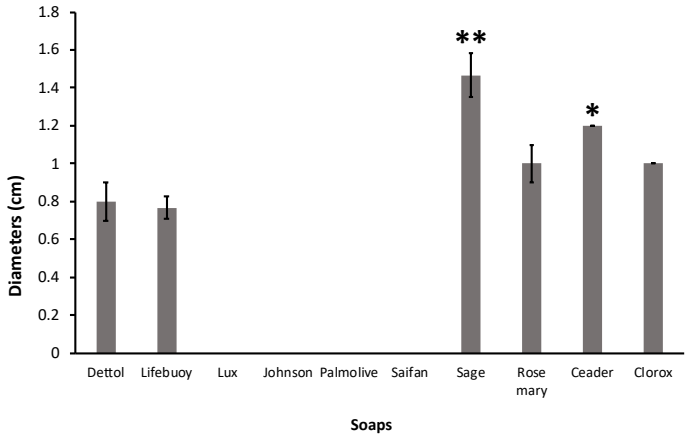
Antibiotics	*S. aureus*	*S. epidermidis*	*E. coli*	*P. aeruginosa*	*Enterobacter spp.*
Amoxicillin	R	R	R	S	S
Ampicillin	R	R	R	R	R
Erythromycin	R	R	I	R	R
Gentamicin	R	S	R	I	S
Chloramphenicol	I	R	I	I	R
Ciprofloxacin	I	I	R	S	S
Cefoxitin	R	I	I	S	S
Norfloxacin	S	S	S	R	S
Nalidixic acid	I	S	R	I	
SXT*	R	R	R	R	R
Tetracycline	R	R	R	R	R

Antibacterial activities

The analysis of Figure **1A** shows that *Staphylococcus aureus* is sensitive on all soaps except Lux, Johnson, Palmolive, and Saifan. Noting that the diameter of the sage soap inhibition zone is more remarkable than other soaps (*p*-value < 0.01). In contrast, *Staphylococcus epidermidis* showed bacterial resistance to all types of soaps except those based on essential oils as shown in Figure **1B**.
Similarly, *Pseudomonas aeruginosa* and *Enterobacter* showed a significant sensitivity only to soaps made in the eco-village of Bader Hassoun (*p*-value < 0.001) as shown in Figure **1C, 1D**. In contrast, the *Escherichia coli* strain showed bacterial resistance against all types of used soaps in this study.



A



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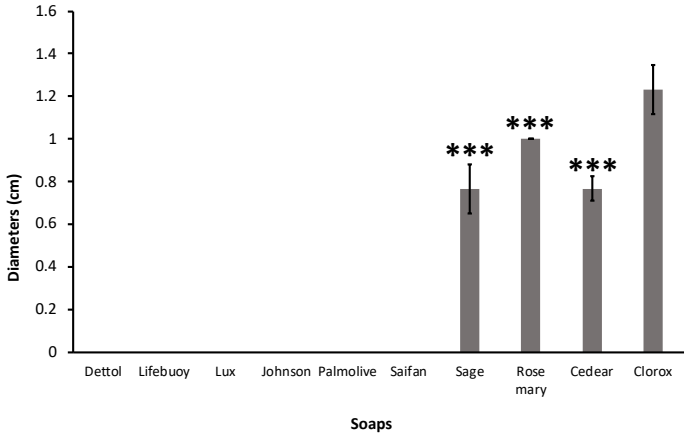

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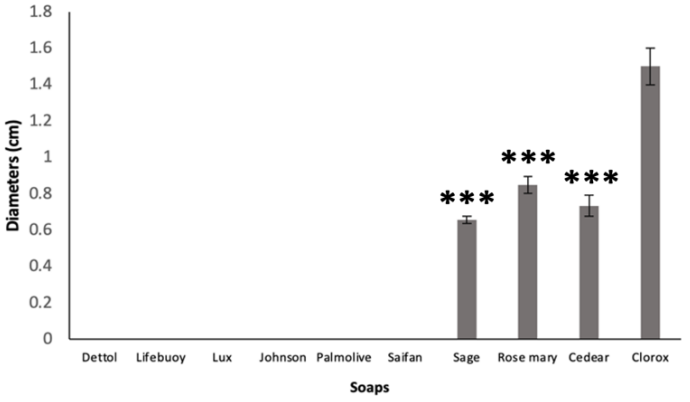

D

Figure 1 Diagrams showing the diameter of the inhibition zones of **A)** *Staphylococcus aureus*, **B)** *Staphylococcus epidermidis*, **C)** *Pseudomonas aeruginosa*, and **D)** *Enterobacter spp.* according to the different tested soaps. (* *p*-value < 0.05, ** *p*-value < 0.01, *** *p*-value < 0.001).

DISCUSSION

Soaps are cleaning agents generally used for the cleaning and elimination of germs. Hand hygiene and the antibacterial activity of soaps were always an interesting point of research and it is known that antibacterial soaps are highly marketed as having bactericidal effects. However, several studies have been done showing otherwise. Due to these facts, the purpose of this study was to compare the antibacterial activity of soaps made from organic and essential oils with so-called antibacterial soaps, marketed and sold in the Lebanese market. To achieve this objective, a semi-quantitative study was carried out using the method of diffusion of the immersed disks in dilutions of different types of soap.

The analysis of the obtained results in this study showed that the tested soaps have antibacterial activities against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa, and Enterobacter*. These bacteria showed a resistant antibiogram profile [Table 3] meaning that they can lead to serious infections for the host patients if not eliminated or treated. *Staphylococcus epidermidis, Pseudomonas aeroginosa, and Enterobacter* were significantly sensitive only to soaps which are Sage, Rose Mary and Cedar based [Figues 1B, 1C, and 1D]. However, *Staphylococcus aureus* showed sensitivity to soaps with essential oils as well as the antibacterial soaps Dettol and Lifebuoy [Figure 1A]. On the other hand, the strain of *Escherichia coli* tested showed resistance to all soaps prompting us to test another strain which also gave the same result. Nevertheless, these two strains were multi-resistant, showing multi-resistance in an antibiogram since they were isolated in a hospital setting because there was a difficulty in isolating them for a limited time during the practical part. It is therefore necessary to study other strains of *Escherichia coli* that are not multi-resistant.

Apart from the antibacterial activity of the soaps tested, it has been abundantly established in previous studies that their prolonged use should be avoided due to the toxicity of their agents (Joubert, Hundt, & Du Toit, 1978 ; Kirsner & Froelich, 1998 ; Ley, Pischel, & Parsonnet, 2017 ; Steinberg et al., 1999).

Due to that fact, essential oil-based soaps which show greater antibacterial activity would be more effective and better considered in our daily lives, to prevent the transmission of pathogens when used in hand washing. Generally, essential oils have a very important characteristic that enables their antibacterial activity which is hydrophobicity. This hydrophobicity in essential oils enables them to separate the lipids in the bacterial membrane and mitochondria causing disruptions in structures and rendering them more permeable (Viuda-Martos, Ruiz-Navajas, Fernández-López, & Pérez-Álvarez, 2008)**.**

By analyzing the natural based ingredients of each of the soaps (Sage, Rose Mary and Cedar) we can distinguish the olive oil as the main component. This natural compound is known worldwide to have antibacterial effects against various numbers of pathogenic bacterial strains such as: *Salmonella* Typhimurium, *Staphylococcus aureus* and *Pseudomonas aeruginosa* **(Guo et al., 2020)**. Several studies have focused on the phenol extracts from olive oil suggesting a possible antibacterial mechanism which includes reductions in intracellular ATP concentrations, depolarization of the cell membrane, a decrease in the bacterial protein content, and cytoplasmic leakage (Guo et al., 2020 ; Medina, de Castro, Romero, & Brenes, 2006). Studies on sage oil show its important antibacterial effects against the *Staphylococci* bacteria responsible for wound infections (Orchard & van Vuuren, 2017 ; Sienkiewicz et al., 2015)**.** Staphylococci mutants in dental plaque **(Beheshti-Rouy et al., 2015)** and *Pseudomonas aeruginosa* **(Podlewski et al., 2017)**. However, literature is currently lacking on a clear view of the antibacterial mechanisms of this oil. Coconut oil was also studied since it contains an acid called Lauric acid which possesses great antibacterial effects against *Pseudomonas aeroginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus pneumonia* compared to usually used antibiotics (Ciprofloxacin) in addition to noted antifungal activity against *Aspergillus fumigates* and *Candida Albican* in comparison with an efficient antifungal agent (ketoconazole). This oil has been found to exhibit antibacterial activity by causing changes and leakage in the bacterial cell walls by forming surface depressions (Widianingrum, Noviandi, & Salasia, 2019). Looking to the effects of rosemary essential oil, research studies have shown their importance as antibacterial substances against *Pseudomonas aeruginosa* strain (Araby & El-Tablawy, 2016) and *Staphylococcus aureus* (Honório et al., 2015 ; Orchard & van Vuuren, 2017)**.** This oil generally has variations in its antibacterial properties and antimicrobial compounds based on many factors such as the time of harvest, the plant's developmental stage, extraction methods, regional and environmental conditions, as well as the methodologies used to evaluate these properties (Burt, 2004 ; Celiktas et al., 2007 ; Okoh, Sadimenko, & Afolayan, 2010 ; Zaouali, Bouzaine, & Boussaid, 2010). This oil has been shown to have five major active components which are borneol, camphor, β-pinene, 1,8-cienole, and α-pinene **(Jiang et al., 2011)** and studies have shown that, since this oil's chemical components differ depending on many factors (as stated above), the difference in components could contribute to a difference in the antibacterial activity (Ojeda-Sana, van Baren, Elechosa, Juárez, & Moreno, 2013). Nevertheless, other studies

showed that isolated components are not as effective against microorganisms as the rosemary essential oil, which is most often comprised of more than 20 different components (**Jiang et al., 2011**).

CONCLUSION

In conclusion, soaps comprised of essential oils have shown greater antibacterial activity than synthetic soaps in the Lebanese market. These soaps are a blend of several essential oils. The difference between the antibacterial activities is due to the different active components of the oils responsible for these effects. The use of these essential oils in the industry of hand soap can be marketed as an antibacterial soap for personal hygiene. Nevertheless, further research is needed regarding the fact of whether soaps comprised from essential oils could be used as a replacement to antibacterial soaps/sanitizing hand gels in hospitals and patient care settings.

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