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Preparation, Characterization, and Antibacterial Efficacy of Green Aromatic Soap

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Abstract

In this work, preparation of fragrant soap was performed by mixing rose, mint, lemon, or orange essential oil with "Al-Jouf" olive oil (a well-known olive oil brand) in ethanolic-aqueous solution during the saponification process. To avoid volatilization of the essential oils, saponification was conducted in reflux system at 95°C for 1h. The resulting soap was then precipitated using different volumes of 30% sodium chloride aqueous solution, filtered, poured into silicon molds, and left to dry at room temperature till constant weight. The resulting soap was evaluated by 1) reaction efficiency, 2) UV-Visible spectrophotometer, 3) Fourier-Transform Infrared Spectroscopy, and 4) antibacterial efficacy. Results showed that increasing sodium chloride concentration highly increases the reaction efficiency. Colony counting method showed that all soap samples prepared using olive oil/essential oil mixture acquired good antibacterial properties, whereas antibacterial efficacy of soap prepared from olive oil only acquired up to 95.4% reduction of *Staphylococcus aureus* colonies and up to 89.5% reduction of *Escherichia coli* colonies.

Keywords: Soap, saponification, olive oil, essential oils, mint, orange, lemon, rose, UV-Visible spectrophotometer, FTIR, reaction efficiency, antibacterial.

1. Introduction

Soap is used daily for cleanliness and sterilization because of its ability to break down complex molecules such as fats, dyes, germs, and microbes and thwart their work. Soap played and still playing the most important role in infection prevention from the Coronavirus. For sake of cleanliness, freshness, vitality and comfort, the body soap is physically mixed with different proportions of perfumes. Chemically, oils and fats are the esters of organic acids and glycerol. They are termed "triglycerides", as they have three fatty acid molecules attached to each carbon in the glycerol skeleton. The reaction between strong base and oil is called "saponification" [1, 2]. Soaps are one of the skincare products that can be defined as the cleansing agent produced by the saponification reaction between an oils and a fatty acids in presence of strong alkali [3]. Soaps are amphipathic in nature, consisting of polar ionic hydrophilic head molecules and nonpolar hydrophobic tail molecules. In dissolution with water, these soap molecules arrange themselves as spherical aggregates called micelles. These micelles have the water- hating tails (hydrophobic) oriented towards the center and the water-loving heads (hydrophilic) facing outside and interacting with the water molecules. The cleansing action can be explained by the fact that the hydrophobic tails get attached to oil/fat-containing components and simultaneously, the hydrophilic head molecules push them to move upwards to interact with water molecules and thus, the oil-containing components are removed from the surfaces.

When a dirty cloth is put in water containing soap, then the hydrocarbon ends of the soap molecule in the micelle attach to the oil or grease particles present on the surface of dirty cloth. In this way the soap micelles entrap the oily particles by using the

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hydrocarbon ends. The ionic ends of the soap molecules remain attached to the water when the dirty cloth is agitated in soap solution. The oily particles present on its surface gets dispersed in the water due to which the cloth gets clean.

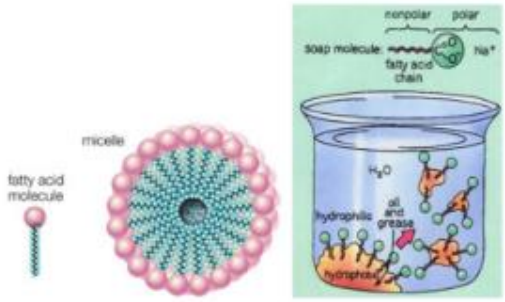

Figure 1: Mechanism of Soap

In the soap production process, various mixtures of oils are used, and in some cases improper combinations of the oils may not undergo the saponification action and remain in the soap as unreacted fatty acids, which causes skin irritation and bleaching effects. Hence, the appropriate selection of the right proportions of the oil mixtures is highly challenging in the product development phase [4].

Olive, palm, castor, and Coconut oils [2, 5-9] as well as waste cooking oil [10, 11] have been used in the preparation of soap. Among these oils, olive oil has got great interest due to its valuable and antibacterial properties [12-15].

Olive oil is a triacylglyceride with oleic and linoleic acid as primary fatty acids and small amount of linolenic acid. It is used extensively in the food industry and is of significant commercial importance [16]. Polyphenols are probably one of the most important groups of minor polar components present in the extra virgin olive oil. The biological importance of polyphenols gives rise from their numerous ascertained biochemical activities, such as the prevention of oxidation reactions to fatty acids. They also contribute to the stability of the oil over time, delaying rancidity. Polyphenols are also capable of preventing and inhibiting radical-type reactions in the human body. The principal subfamilies of polyphenols detectable in the extra virgin olive oil are phenolic acids, phenolic alcohols, secoiridoids, lignans and flavonoids. Olives and its derived-products are capable, within certain limits, to resist against the biotic and abiotic stresses, for instance against pathogen attack, affecting the host-pathogen interaction. Such property is mainly due to the presence of polyphenols, which can also exhibit antimicrobial activity [17, 18]. Recently, the inhibitory effect of extra virgin olive oil polyphenols was demonstrated also against some oral microorganisms, such as oral streptococci, *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, and *Parvimonas micra* [13-15, 17, 19].

Compared with synthetic preservatives, natural extracts have attracted great interest due to their ability to inhibit the growth of food-borne pathogens and not trigger negative safety worries. Some plant extracts had been well documented to be used as potential natural antimicrobial agents or preservatives against *Listeria monocytogenes*, such as fruit and vegetable extracts from mint and pomegranate, spice extracts from cinnamon and clove, and phenolic extracts from legumes processing by-products. Consumers are more willing to accept natural extracts as preservatives than synthetic preservatives due to the nature and relative safety of natural products [18].

Polyphenols have demonstrated a series of biological effects anti-inflammatory, including antibacterial, antioxidants, antiviral, and antiallergic action. Olive oil polyphenol extract is a natural substance obtained from olive oil and contains abundant polyphenolic compounds. Researchers reported that polyphenolic compounds separated from olive mill wastewater have good antibacterial activity against Gram-positive and negative bacteria. A study proved that the extracts of olive leaves and fruits of Olea europaea Linné from Mediterranean countries also have higher effective antimicrobial activity in food matrices. In previous studies, the antibacterial effect of olive oil extract and its action approach against *Cronobacter sakazakii* and *Bacillus cereus* have already been reported by our team [18]. Olive oil polyphenol extract, composed of polyphenols, hydroxytyrosol, tyrosol, and phenolic acids, has attracted much attention on account of its antioxidant properties, which are considered to be the main reason that natural extracts can inhibit the growth of pathogenic microorganisms [12].

Many essential oil isolates exhibit inhibitory properties in challenge tests against microorganisms. These oil isolates contained specific components that can inhibit the growth of certain microorganisms [20].

Manizzella and Henry screened thirty-five oils, five fixed oils, five infused oils, and ninety-five oil combinations for their effect on the growth of five different bacteria. They found that eucalyptus, cinnamon, and origanum oils exhibited the greatest antibacterial activity. Deans and Ritchie [21] tested the inhibitory effect of fifty oils against twenty five genera of bacteria with an agar diffusion assay. They found that the most comprehensively inhibitory oils were angelica, bay, cinnamon, clove, thyme, almond (bitter), marjoram, pimento, geranium, and lovage. Kivanc and Akgul [22] tested the effect of twenty two oils on seven different bacteria. They found that seven of the oils were active against all the bacterial strains screened, but they produced variable zones of inhibition. Kim et al. [23] tested eleven oil constituents on five food borne pathogens (including *Escherichia coli*, *Salmonella typbimzrrium*, and *Ltsteria monocytogenes*) and found that carvacrol, citral, geraniol, and perillaldehyde showed high levels of inhibition [20].

Essential oils have shown promise as antiviral agents against several pathogenic viruses [24]. Scientific literatures [25] proved that plant extracts and herbal products exhibit therapeutic efficacies against influenza, HIV, HSV, hepatitis, and coxsackievirus.

Fragrant soaps were prepared for sake of refreshment and good smell during hand washing or shower. The concept of fragrant soap was extended to produce antimicrobial soap by adding herbal extracts/ herbal essential oil during saponification reaction. Recently, anti-microbial soap was prepared by hydroalcholic extracts of Curcuma longa L., Azadirachta indica A Juss, and Cassia tora L. The prepared soap showed antimicrobial efficacy and was effective in management of Tinea corporis [26] Clove oil-solid soap was prepared using olive, palm, coconut and castor oils. The results showed that the obtained solid soap exhibited inhibition *against Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Escherichia coli* [9].

In the current work, olive oil and/or its mixture with some essential oils were chosen for making aromatic, fragrant, and antibacterial soap. because of 1) and 2) the excellent antibacterial activity of olive oil and essential oils, as mentioned in the Introduction section.

To do so, olive oil alone and/or a mixture of olive oil and any of four essential oils, namely, Mint, Rose, Orange, and Lemon essential oil in small proportions These essential oils were selected due to their pleasant smell and their expected antibacterial activity.

The current work aims at:

1- Utilizing olive oil as a local product in the Kingdom of Saudi Arabia (KSA), Al-Jouf district in making useful products.

2- Green preparation of aromatic soap with fragrance of lemon, orange, mint, and rose to acquire the feel of comfort and refreshment.

3- Making antibacterial soap depending on the antibacterial properties of olive oil and its mixture with the essential oils of lemon, orange, mint, and rose.

2. Experimental

2.1. Materials

Olive oil was purchased from Al Jouf Agricultural Development Co, KSA.

Lemon, Orange, Rose, and Mint essential oils were purchased from Now Foods, USA.

Sodium hydroxide, sodium chloride, and ethanol were analytical grade chemicals.

2.2. Method

2.2.1. Soap preparation

Soap was prepared according to a reported method [27]. The method could be summarized as follows:

1. Six g of sodium hydroxide were weighed in a 100 ml beaker, dissolved in 14 ml water, and allowed to cool to room temperature. After cooling 24 ml of ethanol were added.

2. In another 100 ml beaker 12 g of olive oil were weighed.

3. The alcoholic sodium hydroxide solution was then added dropwise to the olive oil and stirred using magnetic stirrer till complete mixing of oil and sodium hydroxide.

4. Step two was repeated using mixtures of 11.5 g olive oil and 0.5 g essential oil, lemon, orange, mint, or rose. Alcoholic sodium hydroxide solution was added to each olive oil/essential oil mixture.

5. The oil/alcoholic sodium hydroxide mixture was then quantitatively transferred into a 250 ml round flask fitted by reflux condenser where the saponification reaction took place in reflux system for one hour at 95^{o}C.

6. After the saponification reaction, the soap solution was precipitated using 200 or 400 ml of 30% sodium chloride aqueous solution, filtered, and transferred into silicon mold. The soap was then allowed to dry at room temperature.

2.3. Characterization of Soap

Reaction Efficiency (%) was calculated using the following equation:

Reaction Efficiency (%) = $(w2 - w1)/w \times 100$

Where:

W1: weight of empty silicon mold

W2: weight of silicon mold and soap (after drying)

W: weight of reactants (oils and sodium hydroxide).

UV-Visible (UV-Vis) spectrophotometer, UV-1900, Shimadzo supported with LabSolutions UV-Vis software version 1.1 was used to measure the absorbance of the prepared soap after dissolving 0.01 g of the dry soap in 50 ml distilled water. The spectra of prepared soap were recorded at wavelength range from 190 to 350 nm.

Fourier-Transform Infrared Spectroscopy (FTIR) spectra of dry powder soap was performed using Nicolet iS50 FTIR Spectrometer, US supported with OMNIC Spectra software. The data were recorded from 400 to 4000 cm^{-1}.

2.4. Evaluation of Antibacterial Activity in vitro:

2.4.1. Materials

Two bacterial strains were *Escherichia coli* (*E. coli*) ATCC 11229 (Gram-negative) and *Staphylococcus aureus* (*S. aureus*) ATCC 6538 (Gram-positive). These bacterial strains were selected as test cells because they are the most frequent bacteria in wound infection. Fresh inoculants for antibacterial assessment were prepared on nutrient broth at 37°C for 24 hours.

2.4.2. Colony counting method

The antibacterial activity of the finished fabrics against *S. aureus* and *E. coli* was evaluated by using the colony counting method [28] where; a liquid culture was prepared by mixing 0.5 g peptone and 0.3 g beef extract in 100 ml water. 1 cm diameter blended film samples were cut and put into 10 ml of liquid culture, to which 10 µl of microbe culture was inoculated. All samples were incubated for 24 h at 37°C. From each incubated sample, 100 µl of the solution was taken, diluted, and distributed onto an agar plate. All plates were incubated for 24 hours, and the colonies formed were counted.

The percentage bacterial reduction was determined as follows:

Reduction in CFU (colony forming units) % =(C-A)/C x100

Where A is CFU/ml after contact (end test) and C is CFU/ml at zero contact time.

3. Results and discussions

3.1. Saponification Reaction

The chemical reaction between olive oil and sodium hydroxide can be represented by the following equations:

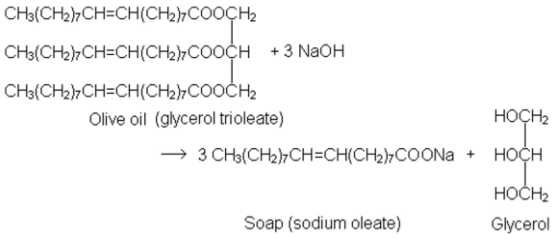

Figure 2: Saponification reaction of olive oil using sodium hydroxide

The sap°nification reaction shows the formation of sodium oleate (soap) because of the reaction of glycerol trioleate (olive oil) with three moles of sodium hydroxide. Glycerol was obtained as a by-product. In the current work, the saponification reaction was performed in alcoholic solution and the produced soap was easily precipitated using aqueous sodium chloride solution. The concentration of sodium chloride showed a significant effect on the soap yield, i.e., reaction efficiency.

3.2. Effect of sodium chloride concentration on Reaction Efficiency (%)

Table 1 show the effect of increasing the concentration of sodium chloride used in the precipitation of the alcoholic soap solution of the reaction efficiency.

As seen, the increase in the volume of 30% sodium chloride from 200 to 400 ml increases the yield of soap and hence the reaction efficiency regardless of the oil used. This could be because of the effect of the electrolyte (NaCl) on the isolation of the fat component of soap stock and hence collection of more soaps from the suspended solution [29, 30].

Table 1 also shows that, upon using 400 ml of 30% NaCl, the highest reaction efficiency was obtained by using olive oil alone or with its mixture with rose essential oil.

The sequence of increasing the reaction efficiency was found to follow the following order:

Olive oil = Olive + Rose > Olive + Mint > Olive + Limon > Olive + Orange

These variations in reaction efficiencies as a result of the oil used could be mainly due to chemical structure of the oil.

Table 1: Effect of increasing sodium chloride concentration on the % reaction efficiency.

Oil	Reaction Efficiency (%)	
	200 ml of 30% NaCl	400 ml of 30% NaCl
Olive	73.26	95.79
Olive + Rose	78.21	96.13
Olive + Mint	70.32	73.99
Olive + Limon	61.17	72.44
Olive + Orange	53.70	64.64

3.3. UV-Visible spectra soap prepared from olive oil or olive oil/essential oil mixture

Figure 3 shows the UV-Vis spectra of 0.02% (w/v) aqueous soap solution prepared from olive oil or olive oil/essential oil mixture. As clear from the figure, the absorbance of soap prepared from olive oil is higher than the absorbance of soap prepared from olive oil/ essential oil mixture. This could be due to the higher content of olive oil (12g) compared by the content of olive oil/essential oil mixture (11.5g +0.5g). It is also shown that soap prepared from and olive oil/ essential oils are characterized by three peaks, 196-212 nm, 232–256 nm, and 280-316 nm which could be attributed to the conjugated double bonds of vitamin A present in olive oil, the terpenes of essential oils used in the olive oil/essential oil mixture (11.5g + 0.5g), the polyphenol compounds of olive and essential oils, double bons of C=C of the oil hydrocarbon chain, C=O of the carboxyl group of the prepared soap[31-33].

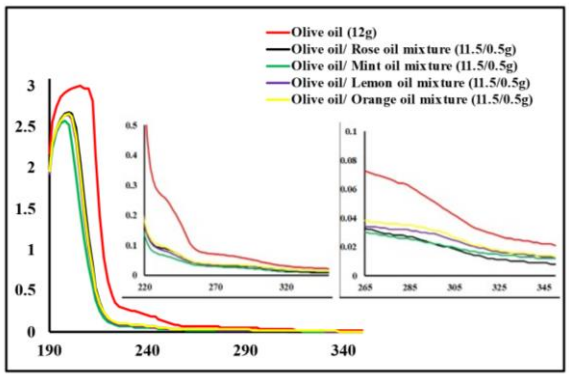

Figure 3: UV-Vis spectra of soap prepared from olive oil or olive oil/ essential oils mixture

3.4. FTIR spectra of soap prepared from olive oil or olive oil/ essential oils mixture

Figure 4 shows FTIR spectra of soap prepared from olive oil or olive oil/essential oil mixture. As shown in figure, different peaks with high and low absorbances were found. The main peaks are:

1- Two strong peaks at about 2917 and 2848 cm^{-1} characteristic to CH stretching of all oils
2- A very strong peak at about 1737 cm-1 characteristic to C=O stretching of the ester or carboxylic acid (soap).
3- The peak at about 1557 cm-1 characteristic to C=C stretching attributed the chemical structure of olive oil and essential oils.
4- The peak at about 1443 cm-1 is characteristic to OH- bending of carboxylic acid which could be due to carboxylic group of the formed soap.
5- The peak at about 1378 cm-1 is characteristic to OH- bending of alcohols which could be due to glycerol by-product of the soap formation process.

The variation in the strength and positions of FTIR peaks assures the different structure of the oils used in the preparation of soap and, consequently the produced soap [34].

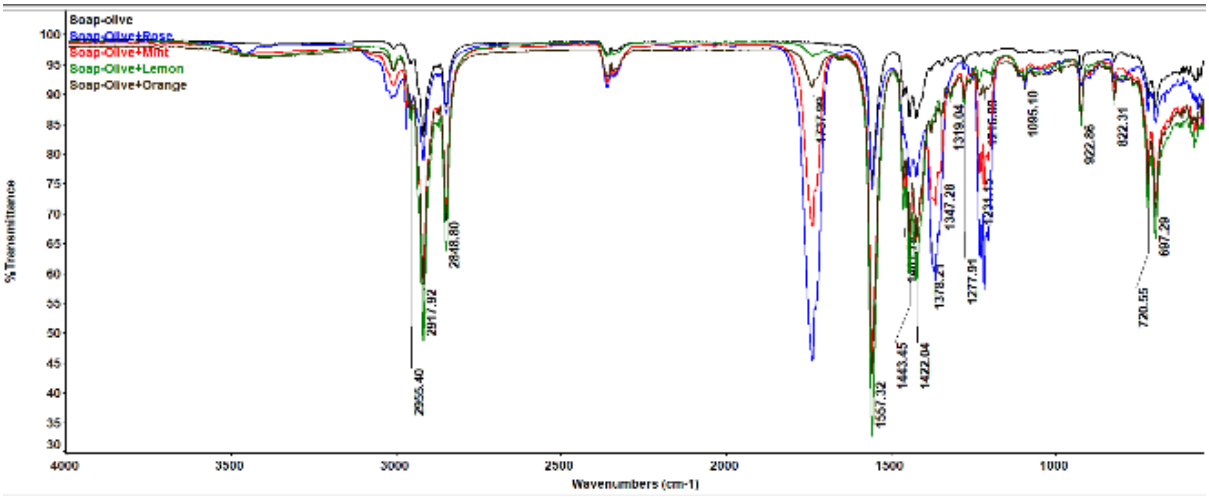

Figure 4: FTIR spectra of soap prepared from olive oil or olive oil/ essential oils mixture

Table 2: Antibacterial Efficacy of soap prepared from olive oil or olive oil/ essential oils mixture 3.5. Antibacterial Efficacy

Sample No	*S. aureus*		*E. coli*	
	Colonies No.	Reduction	Colonies No.	Reduction
	CFU x10^5	%	*CFU x10*^{7}	%
Blank	5.7	0	26.3	0
O/None	0.26	95.44	2.76	89.51
O/M	0.715	87.46	3.062	88.36
O/L	1.1	80.70	7.1	73.00
O/R	0.49	91.40	6.201	76.42
O/O	1.01	82.28	8.071	69.31

3.5. Antibacterial Efficacy

The antibacterial activity of olive oil or olive oil/ essential oils mixture soap samples were

evaluated towards *S. aureus* as gram-positive bacteria and *E. coli* as gram-negative bacteria according to bacterial count method [28]. Table 2 shows the antibacterial properties of soap prepared from olive oil only (O/None) using 12g (olive oil) /0 g essential oil in the soap formulation; olive oil/ mint essential oil (O/M) using 11.5/0.5g; olive oil/ lemon essential oil (O/L) using 11.5/0.5g; olive oil/ rose essential oil (O/R) using, 11.5/0.5g; olive oil/ orange essential oil; using 11.5/0.5g. The results reported in Table 2 indicate that all soap samples acquired high antibacterial properties, regardless of the type of the bacteria. It is also revealed that antibacterial properties acquired against *S. aureus* was higher than that of *E Coli*. It is worthy to mention that using olive oil only in the soap formulation produced soap with antibacterial efficacy higher than using olive oil/essential oil mixture regardless of the type of bacteria. It is also revealed that antibacterial efficacy of soap prepared from olive oil only acquired up to 95.4% reduction of *S. aureus* colonies and up to 89.5% reduction of *E. coli* colonies. The increase in the antibacterial efficacy of the soap produced from olive oil/essential oil mixture depends on both the type of the essential oil used and the type of bacteria. The sequence of increasing the antibacterial efficacy of soap prepared using olive oil/essential oil mixture was found to follow the following order:

1- S. aureus

Olive / Rose > Olive / Mint > Olive / Orange > Olive / Lemon

2- E. coli

Olive / Mint > Olive / Rose > Olive / Lemon > Olive / Orange

4. Conclusion

In the current work, aromatic soap was prepared using olive oil and/or olive oil/ essential oil mixture. Essential oils used were rose, mint, lemon and orange. The prepared soap was precipitated using 30% sodium chloride aqueous solution. The prepared soap was tested for %reaction efficiency, and confirmed by UV-Vis spectrophotometry and FTIR. It was found that the % reaction efficiency is highly affected by the amount of sodium chloride used in precipitation process. Consequently, it could be concluded that 1) soap could be prepared from olive oil and or olive oil/ essential oil mixture with high reaction efficiency, 2) the preparation process was easy and consistent, 3) the drying process of the prepared soap was fast, compared with traditional method, 4) the prepared soap showed nice smell typical to the mixed essential oils, and 5) all soap formulations showed high antibacterial efficacy towards *S. aureus* and *E. coli*.

5. Conflicts of interest

There are no conflicts to declare

6. Acknowledgment

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