



Eco-Friendly Medicinal Soap with High Antimicrobial Efficacy: A Natural Formulation from Virgin Olive Oil and *Eucalyptus globulus* Essential Oil

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Abstract:

This study aimed to formulate and characterize an eco-friendly medicinal soap by combining the saponifiable and emollient properties of virgin olive oil with the antimicrobial and antioxidant potential of *Eucalyptus globulus* essential oil. The essential oil was extracted by hydrodistillation and characterized in accordance with ISO standards, while virgin olive oil was evaluated to ensure its suitability for saponification. Soap formulations were prepared using the hot saponification method and enriched with varying concentrations of essential oil (5%, 8%, and 12%) and 5% extra virgin olive oil. The resulting products were assessed for their organoleptic and physicochemical properties, as well as their antimicrobial and antioxidant activities. Antimicrobial efficacy was determined against selected ATCC bacterial and fungal strains using the agar diffusion method, while antioxidant activity was evaluated using the DPPH assay. The extracted essential oil exhibited a yield of 0.8% and met standard physicochemical criteria. The formulated soaps demonstrated moderate yet significant antioxidant activity, reaching 47.53% at 12% essential oil concentration, and strong antimicrobial effects, particularly against *Klebsiella pneumoniae* (50 mm) and *Escherichia coli* (44.5 mm). The inclusion of extra virgin olive oil further improved the overall performance, indicating a synergistic interaction between olive oil polyphenols and eucalyptus bioactive compounds. These findings suggest a sustainable and effective approach for developing plant-based therapeutic soaps with promising applications in natural dermocosmetics and personal hygiene.

1. Introduction

Soap has held a fundamental role in hygiene and public health throughout history, serving as one of the earliest and most accessible methods for

reducing microbial contamination and promoting cleanliness [1]. Its source comes to ancient civilizations such as the Mesopotamians and Egyptians, who developed rudimentary soap-like substances by combining animal fats with alkaline

ash, laying the foundation for early cleansing agents [2]. Over centuries, soap formulation advanced significantly, especially in the Mediterranean region, where the use of refined olive oil led to the creation of gentler, more effective soaps that not only cleansed but also nourished the skin [2]. In contemporary times, the role of soap extends far beyond basic cleaning; it has become an integral part of personal care and well-being, with specialized formulations developed to address diverse skin types, sensitivities, and dermatological conditions [3,4]. This evolution reflects ongoing research into the biochemical properties of ingredients and the consumer demand for products that combine efficacy with skin health benefits. However, commercial soaps predominantly incorporate synthetic additives like triclosan and sodium lauryl sulfate, substances known for their potent antimicrobial and surfactant properties but frequently lacking natural moisturizing agents such as glycerin [5,6]. These compounds can disturb the delicate balance of the skin microbiota, often resulting in skin barrier impairment characterized by dryness, irritation, and increased susceptibility to inflammation [7,8]. The disruption of microbial homeostasis, or skin dysbiosis, associated with such ingredients has fueled a consumer-driven shift towards organic and plant-based formulations that aim to preserve skin health through gentler bioactive components [9]. Furthermore, the widespread and repeated use of antibacterial agents like triclosan has raised growing concerns over the development of microbial resistance, potentially compromising both individual and public health by fostering resistant strains [10,11]. This evolving landscape underscores the imperative for innovative, sustainable soap formulations that harmonize efficacy with microbiome preservation and long-term skin health. Virgin olive oil, rich in vitamins A and E, provides an excellent moisturizing and protective base [12]. Essential oil (EO) from *Eucalyptus globulus* is conceived to be potential sources of bioactive substances with a high level of biological activity [13,14]. It was employed as antiseptic agents and exhibited high hand sanitizer activity against *Bacillus subtilis* and *Enterococcus faecalis* bacteria [15]. Leaf waste of *Eucalyptus grandis* have been explored in the development of virgin coconut oil based liquid soap [16]. Meanwhile, it has been established that olive oil performs well for making soap. [17,18]. On the other hand, *E. globulus* EO offers antimicrobial and antioxidant activity, recognized by international regulatory authorities [14,19].

Therefore, an early approach, by combining these natural ingredients (virgin olive oil and *E. globulus*

EO) into a medicinal soap that is both skin-friendly and functionally potent has great significance.

While both components are known individually for their beneficial properties [20], their synergistic use in soap formulation remains underexplored, especially in a context prioritizing safety, efficacy, and sustainability.

Hence, this study presents a novel, eco-designed soap enriched with *E. globulus* EO at different concentrations, formulated exclusively with virgin olive oil. The investigation aims to evaluate the antimicrobial and antioxidant efficacy of these formulations, offering a compelling alternative to conventional soaps in the context of natural dermocosmetics.

2. Material and Methods

2.1. Microbial strains

Standard microbial strains from the ATCC collection were used in this study, including *Staphylococcus aureus* (ATCC 6538), *Staphylococcus epidermidis* (ATCC 12228), *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 4352), and *Candida albicans* (ATCC 10231).

Their significance as prevalent skin pollutants or opportunistic pathogens served as the selection criteria.

2.2. Plant material

Fresh leaves of *Eucalyptus globulus* were harvested at the end of the growth cycle in February 2025 from a forest located in the Sidi Cherif commune, near Mostaganem City in northwestern Algeria. The leaves were firstly washed, then, dried in a dark and dry environment.

2.3. Essential oil extraction

The *Eucalyptus globulus* essential oil was extracted by hydrodistillation using a Clevenger-type apparatus. The oil yield was calculated as the percentage ratio of the weight of the extracted oil to the weight of the dried *Eucalyptus* leaves [19]. The obtained EO was stored in amber glass vials at 4 °C until further analysis of its antimicrobial and antioxidant properties.

2.4. Essential oil physicochemical characteristics

The physicochemical characterization of the essential oil was conducted following the standard ISO procedures. Optical rotation was carried out

according to ISO 3515:2004 [21], which evaluates the ability of the oil to rotate the plane of polarized light. The refractive index was determined according to ISO 6320 [22], providing information about the oil's purity and molecular composition. Density was assessed at 20 °C using the method outlined in ISO 279:1998 [23], which is essential for quality control and standardization

2.5. Olive oil physicochemical characteristics

The acid value of the virgin olive oil was carried out following the procedure described by El Guerrouj et al [24]. Briefly, 75 mL of a diethyl ether and 95% ethanol (v/v) mixture was used to dissolve 10 g of olive oil. After, this solution was titrated (0.1 N KOH) under continuous stirring until a stable endpoint was reached.

A% (oleic acid) = $(V - V_0) * (N * M/10 * m)$
Saponification value of virgin olive oil was measured according to the procedure detailed in ISO 3657 [25]. Briefly, in a flask fitted with a reflux condenser, 2 g of the oil sample were mixed with 25 mL of alcoholic potassium hydroxide solution and heated for 30 minutes. After cooling to room temperature, a few drops of phenolphthalein indicator were added, and the solution was titrated with 0.5 M hydrochloric acid until the pink endpoint vanished

$$SV = \frac{[(V_0 - V_1) \times N \times M]}{m}$$

2.6. Natural soap formulation and characterization

Natural soap was prepared from virgin olive oil using a hot saponification process with a 30% potassium hydroxide (KOH) solution. The KOH solution is gradually added to 100 g of olive oil while stirring continuously, a stick blender is used to accelerate the reaction until the obtention of a thick paste. This latter is heated at ± 80 °C for 3 hours, with occasional stirring, until a soap paste is formed. After saponification, the paste was divided into two portions: one left unchanged (0% superfatting), and the other enriched with 5% extra virgin olive oil (5% superfatting). *E. globulus* essential oil (5%) was incorporated into the soap under stirring, and the procedure was repeated with 8% and 12% essential oil. The soaps were kept for two weeks before being used for the determination of the antimicrobial activities, and the assessment of physicochemical parameters. Clarity and color were checked by the physicochemical parameters assessed to determine soap quality included moisture content, pH, free alkali content, and alcohol-insoluble matter. Naked

eyes against the white background, and the odor was smelled [11].

2.7. Soap physico-chemical properties

The following physico-chemical parameters were assessed to determine the soap quality: moisture content, pH, free alkali content, and alcohol insoluble matter. As for the moisture content according to ISO 672:1978 [26], a 5 g soap sample (WEIGHT wet) was placed in a porcelain dish, which was then heated in an oven for 2 hours at a temperature of 105 °C. Once the heating is complete, the soap sample is cooled and re-weighed (WEIGHT dry) to determine its final weight following the formula

$$\text{Moisture Content (\%)} = \frac{\text{WEIGHT}_{\text{wet}} - \text{WEIGHT}_{\text{dry}}}{\text{WEIGHT}_{\text{wet}}} \times 100$$

The pH was determined using a digital pH meter. First, 5 g of soap were dissolved in 100 mL of distilled water. Then, pH electrode previously rinsed and dried was dipped into the soap mixture. The pH value is recorded once it has stabilized, According to ISO 4316: 1977 [27].

For the determination of free alkali content, a soap sample 5 g was dissolved in 100 mL of 96% of ethanol. A few drops of phenolphthalein indicator were then added. The solution was heated until a deep purple color appears. Titration was carried out with a 0.1 N acid solution according to the method adopted by ISO 456:1973 [28]

Alcohol insoluble matter was achieved by mixing 5 g of soap with 50 mL of warm ethanol, and shook it hard to dissolve everything. Then, the solution is filtered through a pre-weighed filter paper, and rinsed with 20mL of warm ethanol. After that, the filter paper is dried at 105°C for an hour and weighed again to determinate residue weight according to ISO 673:1981 [29].

2.8. Essential oil and soap antioxidant activity

The antioxidant activity of the extracts was determined using the DPPH radical scavenging assay, according to the protocol described by Atoui et al [30] for essential oil where methanol was substituted by ethanol, with methanol replaced by ethanol, and to the protocol described by Prieto Vidal et al [31] for soaps. This assay is based on the ability of antioxidant compounds to reduce the DPPH radical, resulting in a decrease in absorbance measured at 517 nm. A greater reduction in absorbance indicates a stronger antioxidant capacity of the sample.

2.9. Essential oil and soap antimicrobial activity

The antimicrobial activity of *Eucalyptus globulus* essential oil and soap was preliminarily evaluated using the disc diffusion method, as described by Amokrane-Aidat [32], against six microbial strains: *Staphylococcus aureus* (ATCC 6538), *Staphylococcus epidermidis* (ATCC 12228), *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 4352) and the yeast *Candida albicans* (ATCC 10231).

Discs were impregnated with 20 μ L of essential oil, while DMSO served as the negative control. Bacterial suspensions were standardized to 10^8 CFU/mL (Optical density 0.08–0.13 at 625 nm). Using a sterile swab, the standardized inoculum was evenly spread over Mueller–Hinton agar plates. The agar surface was then aseptically covered with soap discs, antibiotic discs, and essential oil discs. The diameters of the inhibition zones around the discs were measured after 24 h of incubation.

3. Statistical Analysis

The experimental data were analyzed by analysis of variance (ANOVA) using JMP Pro Version 14.0 (SAS, USA). Means were compared with Tukey's test, and differences were considered statistically significant at $P < 0.05$.

4. Results and Discussions

4.1. Essential oil extraction yield and properties

In this study, the extraction yield of the EO of *E. globulus* was found to be 0.8%. The physicochemical parameters of this EO demonstrated the following results: an optical rotation of $+5^\circ \pm 0.03$, indicating the presence of optically active compounds that may contribute to the oil's biological properties, a refractive index of 1.467 ± 0.080 , which reflects the oil's purity, and a density 0.908 ± 0.006 at 20 $^\circ$ C, which is within the

typical range reported for EOs of similar botanical origin.

4.2. Determination of physicochemical parameters of olive oil

The quality of virgin and extra virgin olive oil was assessed through free acidity, expressed as a percentage of oleic acid, in accordance with International Olive Council (IOC) standards. This parameter reflects the hydrolytic degradation of triglycerides during fruit ripening, harvesting, handling, or storage [33]. The virgin olive oil sample showed a free acidity of 1.07%, well below the IOC limit of 2%, while the extra virgin sample had a value of 0.20%, significantly lower than the 0.8% threshold. These findings confirm that both oils meet the regulatory specifications for their respective categories, with the extra virgin sample demonstrating an exceptionally low acidity.

A saponification value of 184.04 mg KOH/g was obtained for the olive oil sample tested in this study indicating the amount of potassium hydroxide, expressed in milligrams, required to saponify 1 gram of oil and providing information on the average molecular weight of the triglycerides in the sample.

4.3. Formulation and organoleptic characterization of soap

To prepare the soap paste, virgin olive oil was saponified with an alkaline solution, and continuously cooked for three hours at 85 $^\circ$ C. The final product was a translucent, olive-green paste, smooth in texture, with the distinct odor of traditional Algerian soap, commonly referred to as Saboun Dzair.

4.4. Physico-chemical properties of the prepared soap

Table 1 summarizes the physicochemical properties of the olive oil soft soap, providing key information on its composition, appearance, and quality.

Samples	Moisture content	pH	Free alkali content test	Alcohol insoluble matter
Soap	$25.42^a \pm 1.3 \%$	$10.04^a \pm 0.03$	$0.70^b \pm 0.03 \%$	$1.84^a \pm 0.26 \%$
Soap (0 % Extra virgin olive oil + 5% <i>E. globulus</i> essential oil)	$25.88^a \pm 0.9 \%$	$10.09^a \pm 0.07$	$0.059^a \pm 0.002 \%$	$1.89^a \pm 0.13 \%$
Soap (0 % Extra virgin olive oil + 8% <i>E. globulus</i> essential oil)	$24.98^a \pm 1.7 \%$	$10.15^a \pm 0.05$	$0.057^a \pm 0.004 \%$	$1.92^a \pm 0.76 \%$
Soap (0 % Extra virgin olive oil + 12% <i>E. globulus</i> essential oil)	$25.86^a \pm 1.1 \%$	$10.20^a \pm 0.01$	$0.055^a \pm 0.001 \%$	$1.76^a \pm 0.53 \%$

Soap (5 % Extra virgin olive oil + 5% <i>E. globulus</i> essential oil)	24.42 ^a ± 1.5 %	10.21 ^a ± 0.04	0.078 ^a ± 0.009 %	1.78 ^a ± 0.16 %
Soap (5 % Extra virgin olive oil + 8% <i>E. globulus</i> essential oil)	24.46 ^a ± 1.07 %	10.17 ^a ± 0.06	0.059 ^a ± 0.003 %	1.82 ^a ± 0.71 %
Soap (5 % Extra virgin olive oil + 12% <i>E. globulus</i> essential oil)	25.05 ^a ± 1.4 %	10.13 ^a ± 0.02	0.091 ^a ± 0.004 %	1.94 ^a ± 0.36 %

Table 1: Physicochemical properties of prepared olive oil soft soap

Data are expressed as mean values ± standard deviation. A d: means in the same column with different letters are significantly different ($P < 0.05$). The prepared soap showed a moisture content value of 25.86 %, representing the highest recorded among all formulations, and remaining within the maximum limit of 60% set by the Indonesian National Standard (SNI 06-4085-1996) [34]. Its pH value ranged from 10.04 to 10.21, confirming its safety for use. The free alkali content (0.7%) exceeded the permissible limit of 0.1% defined by the same standard for unsupplemented soap, in contrast, the soaps enriched with virgin olive oil and *Eucalyptus globulus* essential oil showed significantly lower free alkali values, ranging from 0.055% to 0.091%, all falling within acceptable safety limits. The alcohol-insoluble matter content did not exceed the 2% limit in any of the tested soap formulations, markedly lower than the reference value of 17.6% [35].

4.5. Biological properties

4.5.1. Antioxidant activity

The antioxidant activity of *E. globulus* essential oil, extra virgin olive oil, soap enriched with different concentrations of essential oil and 5% extra virgin olive oil, is summarized in Tables 2 and 3. The antioxidant activity of soap increased significantly with the addition of *E. globulus* essential oil in a concentration-dependent manner. Incorporation of 5% extra virgin olive oil further enhanced this effect, highlighting a synergistic interaction. Although less potent than the synthetic antioxidant BHT, the enriched soaps demonstrated considerable natural antioxidant potential.

4.5.2. Antibacterial and antifungal activities

The results of antibacterial activity of olive oil soap, essential oil of *Eucalyptus globulus* and soap enriched with different concentration of essential oil are summarized in Table 4. The evaluation of antimicrobial activity revealed that soap enriched with *E. globulus* essential oil significantly inhibited all tested strains in a concentration-dependent manner. The addition of 5% extra virgin olive oil

slightly enhanced this effect, suggesting a possible synergistic interaction. In contrast, plain soap and olive oil showed weak activity, whereas the essential oil alone was the most effective, highlighting the key role of *E. globulus* in boosting the antimicrobial potential of the soap.

Table 2: Evaluation of antioxidant ability of *E. globulus* essential oil, extra virgin oil and BHT

Samples	DPPH (CI ₅₀ mg/mL)
<i>Eucalyptus globulus</i> essential oil	19.03 ± 0.84 ^c
Extra virgin olive oil	9.25 ± 0.19 ^b
BHT (standard synthetic antioxidant)	0.0146 ± 0.00 ^a

Data are expressed as mean values ± standard deviation. a–c: means in the same line with different letters are significantly different ($P < 0.05$).

Table 3: Evaluation of antioxidant potential of Soap and soap enriched with 5 % extra virgin olive oil containing different concentration of *E. globulus* EO.

Samples	DPPH (inhibition percentage %)	
Soap (0 % Extra virgin olive oil)	Enriched soap	% DPPH inhibition
	0% <i>E. globulus</i> essential oil	5.44 ± 1.5 ^a
	5% <i>E. globulus</i> essential oil	5.94 ± 2.45 ^b
	8% <i>E. globulus</i> essential oil	13.18 ± 1.78 ^c
	12% <i>E. globulus</i> essential oil	17.65 ± 2.7 ^d
Soap (5 % Extra virgin olive oil)	Enriched soap	% DPPH inhibition
	0% <i>E. globulus</i> essential oil	8.06 ± 1.82 ^a
	5% <i>E. globulus</i> essential oil	10.00 ± 1.54 ^b
	8% <i>E. globulus</i> essential oil	25.12 ± 4.31 ^c
	12% <i>E. globulus</i> essential oil	47.53 ± 3.39 ^d

Data are expressed as mean values ± standard deviation. a–d: means in the same line with different letters are significantly different ($P < 0.05$).

Table 4. Evaluation of antibacterial and antifungal properties of extra virgin olive oil, *Eucalyptus globulus* essential oil and prepared soap.

Samples	<i>E. coli</i>	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>
Soap	9 ± 1.41 ^F	10 ± 1.41 ^F	9.85 ± 0.21 ^F	9.1 ± 0.56 ^F	9.25 ± 1.06 ^D	8.35 ± 0.91 ^G
Virgin olive oil	11.5 ± 0.70 ^{EF}	11.65 ± 0.49 ^{EF}	10.6 ± 0.56 ^{EF}	10.15 ± 0.35 ^{EF}	11.5 ± 0.70 ^{CD}	9.6 ± 0.84 ^{FG}
<i>E. globulus</i>	44.5 ± 2.12 ^A	32.75 ± 1.06 ^A	19.85 ± 1.20 ^A	50 ± 1.41 ^A	22.95 ± 1.34 ^A	26 ± 1.41 ^A
Soap (0 % Extra virgin olive oil + 5% <i>E. globulus</i> essential oil)	14.87 ± 0.88 ^{DE}	14 ± 1.41 ^{DEF}	13.45 ± 0.77 ^{DE}	11 ± 1.41 ^{DEF}	12.85 ± 1.20 ^{BCD}	11.85 ± 1.20 ^{EFG}
Soap (0 % Extra virgin olive oil + 8% <i>E. globulus</i> essential oil)	16.9 ± 0.14 ^{CD}	16.75 ± 1.06 ^{CD}	16.5 ± 0.70 ^{BC}	13.8 ± 0.56 ^{BCD}	13.1 ± 1.13 ^{BC}	14.65 ± 0.91 ^{CDE}
Soap (0 % Extra virgin olive oil + 12% <i>E. globulus</i> essential oil)	19.85 ± 1.20 ^{BC}	19.35 ± 0.91 ^{BC}	16.85 ± 0.21 ^{BC}	15.65 ± 0.91 ^{BC}	14.3 ± 0.98 ^{BC}	17.6 ± 0.84 ^{BC}
Soap (5 % Extra virgin olive oil + 5% <i>E. globulus</i> essential oil)	15.65 ± 0.49 ^{CDE}	15.25 ± 1.76 ^{CDE}	14.8 ± 0.28 ^{CD}	12.75 ± 1.06 ^{CDE}	14.6 ± 0.56 ^{BC}	13 ± 1.41 ^{DEF}
Soap (5 % Extra virgin olive oil + 8% <i>E. globulus</i> essential oil)	19.5 ± 0.70 ^{BC}	18.85 ± 1.20 ^{BC}	17.25 ± 0.35 ^{ABC}	14.7 ± 0.28 ^{BC}	15.65 ± 0.49 ^B	16.9 ± 0.14 ^{BCD}
Soap (5 % Extra virgin olive oil + 12% <i>E. globulus</i> essential oil)	22.7 ± 0.42 ^B	22.4 ± 0.56 ^B	18.9 ± 1.27 ^{AB}	17.35 ± 0.91 ^B	16.1 ± 0.56 ^B	20.6 ± 0.84 ^B

Data are expressed as mean values ± standard deviation. A–G: means in the same column with different letters are significantly different (P < 0.05)

5. Discussion

The EO extraction yield obtained from *E. globulus* grown in Mostaganem region (Algeria) explored in this study was lower than those reported for plants grown elsewhere. For example the same plant grown in central Saudi Arabia had a yield of 2.5 % [36]. Previous studies also showed that essential oil content varies among species, ranging from 0.8 to 2.0 ml/100 g of dried material [24]. The yield of essential oils was 0.23% for Djelouli et al [37] and 2.2 % for Muhizi et al [38]. El Guerrouj et al. [24] reported that trees growing at higher altitudes tend to produce higher essential oil yields, with values ranging from 0.25% to 2.8 %. Variations in genotype, plant age and geoclimatic factors such as soil composition, height, temperature, and sunlight exposure could have significant effects on the phytochemical profile and overall essential oil yield in plants [39]. In 2025, a study on 28 Jordanian olive oil samples reported acidity values ranging from

0.12 ± 0.02 to 1.46 ± 0.10% oleic acid [40]. High acidity typically reflects poor post-harvest handling, such as over ripeness, inadequate storage, or excessive heat during extraction, which promote enzymatic and chemical degradation [41]. The saponification value was also determined to accurately calculate the required amount of potassium hydroxide for saponification. Our result was higher than that reported by Ajab et al [42], who found 178 mg KOH/g of olive oil. The standard range of saponification values varies between 184 and 196 mg KOH/g according to ISO 3657 [25]. Based on this, the soap formulation was precisely prepared with a superfatting level of 0 %. Regarding the saponification index reported by Altwaiq et al [40], the 28 olive oil samples showed values ranging from 88.90 ± 0.61 to 199.80 ± 0.30 mg KOH/g. This variation is mainly attributed to the chemical composition of olive oil, as the saponification index is inversely proportional to the length of the fatty acid chains: the shorter the fatty acids, the higher the saponification value. [43]

The texture of the formulated soap was soft and sticky to the touch, yet rinsed off easily, a distinctive property of soft soaps. This soap, widely used in North African hammams, is an essential element in traditional women's beauty rituals. The soft soap, known as *Saboun Dzair* in Algeria and *Saboun Beldi* in Morocco and Tunisia, is applied to the entire body and is especially valued for its softening and brightening properties, which contribute to skin purification [44].

Its value of moisture content confirms that the soap formulation complies with standard quality requirements, ensuring adequate consistency, physical stability, and microbiological safety, while preventing excessive moisture that could reduce shelf life or compromise texture [45,46].

The pH is influenced by one of the soap's key components, a strong base, specifically potassium hydroxide (KOH) and The types of fatty acids present in the oils used for saponification [47].

The pH value is an essential quality parameter in soap production, as it directly affects the skin's condition after use [48]. According to SNI 2588:2017, the acceptable pH range for soap is 4–10. A pH that is too low (strongly acidic) may cause skin irritation [49], while a high (strongly alkaline) pH can make the skin rough and scaly [50]. Healthy human skin has a pH of 5.40–5.90. More complete saponification reduces the soap's alkalinity, improving compatibility with the skin's natural pH [49]. Although soap alkalinity can protect against pathogens by neutralizing the skin's acid mantle, excessively high pH may be corrosive and cause irritation or dehydration [51]. Adding extra fats or oils during formulation (superfating) helps mitigate these effects.

The free alkali content test was conducted to determine the amount of residual alkali not reacting with fatty acids during saponification. This analysis involved titrating the soap sample with hydrochloric acid (HCl) to neutralize unreacted alkali. Although the measured value was higher than the standard, the formulation was deliberately superfatted at 5%, meaning an intentional excess of fatty acids was added. This surplus oil neutralizes any remaining alkali, thereby minimizing the risk of skin irritation. Thus, despite the elevated value (The free alkali content decreased from 0.7% to 0.055%), the final product remains safe and suitable for topical use. Moreover, superfating enhances the moisturizing properties of the soap, representing a functional benefit in cosmetic applications [52].

The soap's composition and purity, particularly the ratio of soap to unsaponifiable components, is often identified by its solubility in ethanol. A range of values around the reference standard indicates that the formulation's consistency and quality are

supported by an appropriate concentration of soap ingredients and few additions or contaminants. The solubility rate of the soap in warm ethanol reaches 98.16%, indicating a high level of purity. This low amount of insoluble matter suggests the absence of significant impurities, such as excess reactants or unsaponified substances [53].

Essential oils are recognized as natural antioxidants and are increasingly used as alternatives to synthetic agents [54]. The antioxidant activity of Eucalyptus globulus essential oil (19.03 ± 0.84 mg/mL) and extra virgin olive oil (9.25 ± 0.19 mg/mL) was markedly lower than the BHT standard (0.0146 mg/mL), but comparable to values previously reported for Eucalyptus globulus extracts [55,56]. Extra virgin olive oil exhibited strong antioxidant capacity, reaching 98.45% inhibition at 25 μ L/mL, consistent with its high polyphenol content [57].

The soap enriched with 5% extra virgin olive oil and 12% Eucalyptus globulus essential oil showed moderate antioxidant activity ($47.53 \pm 3.39\%$), higher than that of essential oil alone, suggesting a synergistic effect between olive oil polyphenols and essential oil constituents. However, the efficacy remained below that of pure olive oil, likely due to matrix effects of saponified fatty acids reducing antioxidant availability. These results align with previous findings on commercial soaps [46] and are comparable to formulations enriched with plant extracts, such as rosemary and lavender ($40.44 \pm 0.15\%$) [58].

The inhibition zones reached 50 mm for *K. pneumoniae*, 44.5 mm for *E. coli*, and 32.75 mm for *S. aureus*. These values are considerably higher than those reported by [59] (11, 9, and 14 mm, respectively), highlighting the influence of botanical origin, geographical location, and extraction conditions on the chemical composition and antimicrobial activity of essential oils. For *P. aeruginosa*, the inhibition zone (22.9 mm) largely exceeded that reported by Čmiková et al. [13] (4.33 mm). Similarly, *S. aureus* and *C. albicans* showed larger inhibition zones (32.75 mm and 26 mm) compared to 5.67 mm and 14 mm, respectively, in their study. These findings indicate a markedly stronger antimicrobial and antifungal activity of the tested oil.

According to [60], *E. globulus* essential oil produced inhibition zones of 11–34 mm against *E. coli* and 18–85 mm against *S. aureus* depending on the concentration (20–60 μ L). In contrast, our study achieved a 44.5 mm inhibition zone against *E. coli* with only 20 μ L, underscoring the high potency of our oil, likely attributable to its bioactive composition and its geographical origin (Mostaganem forest).

The soap enriched with 5% extra virgin olive oil and 12% essential oil exhibited notable antimicrobial activity, combining the antioxidant and moisturizing effects of olive oil with the strong antimicrobial properties of *E. globulus*. The inhibition zones obtained (22.7 mm for *E. coli* and 22.4 mm for *S. aureus*) were higher than those observed with soap containing only 12% essential oil (19.85 mm and 19.35 mm), suggesting a synergistic effect or enhanced diffusion of active compounds [61].

Olive oil alone displayed relatively weak activity (11.5 mm against *E. coli* and *P. aeruginosa*, 11.65 mm against *S. aureus*, and 9.6 mm against *C. albicans*), indicating that its role is more synergistic than directly antimicrobial [55]. By contrast, soap enriched with 5% olive oil and 8% essential oil achieved 19.5 mm (*E. coli*) and 18.85 mm (*S. aureus*), compared with much lower values for the base soap without enrichment (9 mm, 10 mm, and 8 mm for *E. coli*, *S. aureus*, and *C. albicans*). These findings are consistent with previous studies [62] which reported comparable values with plant-based soaps.

6. Conclusion

This study demonstrated that the essential oil of *E. globulus* extracted from the Mostaganem region, although obtained at a relatively low yield (0.8%), exhibited remarkable biological properties. The oil showed strong antimicrobial and antifungal effects, with inhibition zones reaching up to 50 mm for *K. pneumoniae* and 44.5 mm for *E. coli*, clearly surpassing values reported in the literature. Extra virgin olive oil, characterized by very low acidity and strong antioxidant capacity, also contributed to the bioactivity of the formulations.

The soap produced from olive oil and enriched with different concentrations of *E. globulus* essential oil presented improved physicochemical quality, acceptable safety parameters, and enhanced biological properties. In particular, the formulation containing 5% extra virgin olive oil and 12% essential oil demonstrated a balance between antioxidant activity (47.53%) and significant antimicrobial performance, suggesting a synergistic interaction between olive oil polyphenols and *E. globulus* bioactives.

Overall, these findings confirm that the combination of virgin olive oil and *E. globulus* essential oil represents a sustainable and effective approach for producing plant-based therapeutic soaps. Such formulations hold promising potential for applications in natural dermocosmetics and personal hygiene.

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- **Ethical approval:** The conducted research is not related to either human or animal use.
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