

ORIGINAL ARTICLE

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Preparation of sumac extract loaded microemulsion-alg microcomposites**Fadime Nulufer Kivilcim¹, Seval Cing Yildirim², Ahmet Gultek¹**¹Inonu University, Art and Science Faculty, Department of Chemistry, Malatya, Turkey²Inonu University, Art and Science Faculty, Department of Biology, Malatya, Turkey

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Abstract

Our skin is the largest organ that covers our body against the external environment and it is in constant contact with microorganisms compared to our other organs. The bacterial infection does not develop easily on our skin due to many protective factors. However, bacterial infections occur when there is a deterioration in any of these protective mechanisms. Therapeutic plants and spices are widely used as antibacterial agents for dermatological use. In this study, it is aimed to prepare a microemulsion loaded with bioactive sumac extract and to test bioactivity of microalgae (*Chlorella sp.*) commonly used in cosmetic formulations by using an innovative approach. The microcomposites prepared by this method are expected to have a better effect on fat, bacteria and toxins on the surface of skin. Sumac fruit (*Rhus coriaria*), which is used as a spice, was extracted by using ethanol in Soxhlet apparatus. Algae-microcomposites were prepared with different amounts of sumac extract (1%, 2% and 3% (w/v)). Excess of ethanolic extract of sumac fruit was mixed in 3 ml oleic acid for 72 hours at 37°C and the insoluble fraction was removed by centrifugation. Tween 80 was added to the solution as a surfactant and PEG-400 was added as a co-surfactant at 2: 1 ratio into the solution. While the mixture was stirred at the medium intensity on the magnetic stirrer, pure water was added dropwise over solution until a homogenous clear solution was obtained. The bioactivity tests of the microcomposites were carried out using gram-positive *Staphylococcus aureus* and gram-negative *Escherichia coli* bacteria which are generally found on the skin surface. Microcomposites containing 2 % and 3 % sumac extract showed bioactive properties by inhibiting the growth of *S. aureus* and *E. coli* bacteria.

Keywords: Microalgae, sumac fruit, *Rhus*, antibacterial, dermatologic**Introduction**

Therapeutic plants and spices derived from natural sources play an important role in health care. Due to flavonoids, vitamins, essential oils and minerals that they contain, these plants are widely used in medicine and cosmetic industries. Among these therapeutic plants, sumac, *Rhus coriaria* L. (Anacardiaceae) is a wild edible medicinal plant that grows in tropical regions of the world. Sumac shows an excellent bioactive effect due to its rich antioxidant, vitamin, fatty acid and mineral content. Also, it shows antioxidant, antibacterial, antifungal, antiviral and antimicrobial properties [1].

Microalgae are unicellular, photosynthetic organisms that play a key role in aquatic ecosystems and grow in freshwater as well as in marines. Microalgae are a unique reservoir of bioactive compounds and produce secondary metabolites that are required

for cellular metabolism. These secondary metabolites include phenolic compounds, carotenoids, tannins, alkaloids, flavonoids and numerous other compounds which have cosmetically importance. Microalgae used in cosmetic products have recently gained interest in the treatment of skin problems such as anti-aging and pigment disorders [2-4].

Our skin is the largest organ that covers our body against the external environment and it is in constant contact with microorganisms compared to our other organs. Despite this intense microorganism contact, the bacterial infection does not develop easily on our skin due to many protective factors. However, bacterial infections occur when there is a deterioration in any of these protective mechanisms. The most common deteriorations are caused by gram-positive bacteria, streptococci and staphylococci [5].

Aqueous and ethanolic extracts of sumac exhibit a strong antibacterial effect against gram-positive and gram-negative bacteria such as *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Salmonella typhi*, *Proteus vulgaris*, and *Shigella flexneri* [6]. *Staphylococcus aureus* and *Escherichia coli*

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start to grow intensively when the protective factor against the microorganism changes with alteration of eating, cleaning and dressing habits [7].

In cosmetic industry and literature, algae are commonly used for dermatological and medical purposes. However, there is no commercial product and scientific data in the literature on microemulsions of sumac penetrated into algae.

Nanosystems such as microemulsions (ME) and nanoemulsions (NE) offer considerable opportunities for targeted drug delivery to and via the skin. ME and NE are stable colloidal systems composed of oil and water, stabilized by a mixture of surfactants and cosurfactants, that have received particular interest as topical skin delivery systems [8]. There is a considerable effort to manipulate their formulation and characteristics to achieve optimal bioavailability and minimal skin irritation. This includes incorporation of chemical penetration enhancers, thus reduced skin blockage and increased permeation are established.

Macroemulsions are often referred as 'coarse' or opaque emulsions due to their relatively large droplet sizes causing a turbid solution. Microemulsions are transparent, monophasic, optically isotropic and thermodynamically stable colloidal dispersions consisting of oil, water, surfactant and co-surfactant. The droplet size of the microemulsions ranges between 10-100 nm. [9-11].

The aim of this study is to synthesize bioactive microcomposite suitable for dermatological use by combining the unique properties of sumac (*Rhus spp.*) and algae (*Chlorella sp.*). The bioactive sumac extract was loaded into the microemulsion to achieve a better skin penetration.

Materials and Methods

Algae Production

As shown in figure 1, 5 % of the algae cultures were incubated in Bold's Basal medium (BBM) under aseptic conditions. 200 mL algae production medium were transferred into 500 mL flasks. *Chlorella spp.* was cultured for 15 d at 22°C under a 12-hour photoperiodic conditions at a light intensity of 5000 lux (Figure 1).

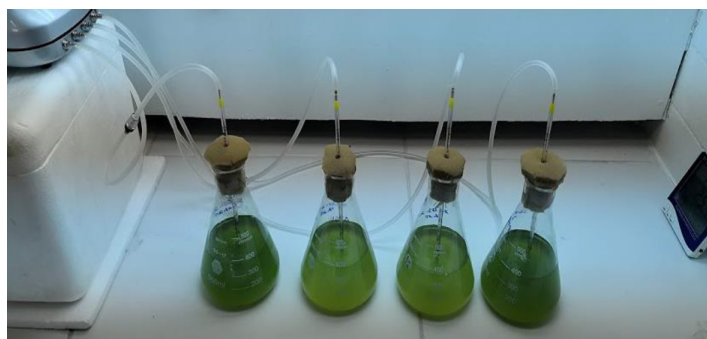


Figure 1. Photograph of the algae production unit. *Chlorella spp.* was incubated for 15 d at 22°C under 12-hour photoperiod conditions at a light intensity of 5000 lux

Sumac Extraction

The pericarp portion of the sumac was ground in the grinder. 10 g of milled sumac was placed in the soxhlet extraction apparatus. Extraction was carried out by using ethanol and excess ethanol was evaporated using a rotary evaporator.

Preparation of Microemulsion

3 mL of oleic acid was added to the ethanolic extract of the sumac fruit and stirred at 37 ° C for 72 hours and the insoluble fraction was removed by centrifugation. Tween 80 as a surfactant and PEG-400 as a co-surfactant was added into this solution at 2: 1 weight ratio. While stirring the mixture on a magnetic stirrer, distilled water was added dropwise until a homogeneous clear solution was obtained (Figure 2B). Since the particle size of the microemulsions was smaller than the wavelength of visible light, obtained mixture was transparent, not opaque, unlike conventional emulsions.

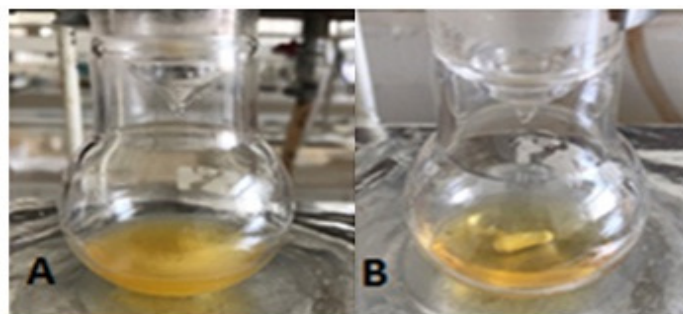


Figure 2. Preparation of microemulsion: Emulsion (A), Microemulsion (B).

Preparation of Microcomposites

At this stage of the study, dry algae biomass and microemulsions containing different amounts of bioactive sumac extract were mixed and the microcomposite was prepared (Table 1).

Table 1. Composition of microcomposite with antimicrobial agent

Treatment	Composition
Algae-Microcomposite (AMC)	Algae (w/v) ¹ , PEG (v/v), Tween-80 (v/v)
M1	AMC + S ^a (1% (w/v))
M2	AMC + S (2% (w/v))
M3	AMC + S (3% (w/v))

^a S: Sumac

¹ Concentrations were measured as dry-bases of algae

Antimicrobial Activity of Sumac extract loaded microemulsion-alg microcomposites

Typical skin bacterial contaminants used in this study were *Escherichia coli* and *Staphylococcus aureus*. *E. coli* and *S. aureus* were grown on nutrient broth at 37°C. Before measuring the antimicrobial activity of microcomposites, 0.1 mL of cultures were transferred to new broth medium and grown for 1 d. The microcomposites were fragmented into 1.2 cm diameter under aseptic conditions and the pieces were placed on nutrient agar plates, which had been previously seeded with 0.1 mL of inoculum containing approximately 10⁴-10⁵ CFU/mL of *S. aureus* and *E. coli*. The plates were incubated at 37°C for 24 h. The diameter of the inhibitory zone surrounding microcomposites pieces was measured. The experiment was done in triplicates.

Result

Microemulsions containing PEG, Tween 80 and sumac extract at different concentrations (1%, 2% and 3% weight) were prepared. Algae were added to the prepared microemulsions to

form microcomposites. Sumac extract loaded microemulsion-alg microcomposites were synthesized for antimicrobial activity tests.

Inhibition effect of Sumac extract at various concentrations is given in Table 2. The results showed a strong inhibitory effect at given concentrations except for 1 % sumac. In addition, the antibacterial effect of sumac against *S. aureus* is higher than that of *E. coli*.

Table 2. The effect of sumac extract loaded microemulsion-alg microcomposites against microorganisms

	<i>S. aureus</i>	<i>E. coli</i>
Without	-	-
M1	+	-
M2	++	+
M3	+++	++

Values were expressed as mean of 3 analysis. -, not detected; +, diameter of inhibition zone was < 1 cm; ++, diameter of inhibition zone was 1-2 cm; +++, diameter of inhibition zone > 2 cm

M1: AMC + S (1% (w/v))

M2: AMC + S (2% (w/v))

M3: AMC + S (3% (w/v))

Figure 3 shows the antibacterial results of the samples containing 2% and 3% sumac extract in the microcomposites. While the inhibition zone diameter of the microcomposite containing 2% sumac extract against *E. coli* was less than 1 cm, the inhibition zone diameter at a concentration of 3% was about 2 cm. The maximum inhibition diameter was obtained in microcomposite containing 3% sumac extract against *S. aureus*.

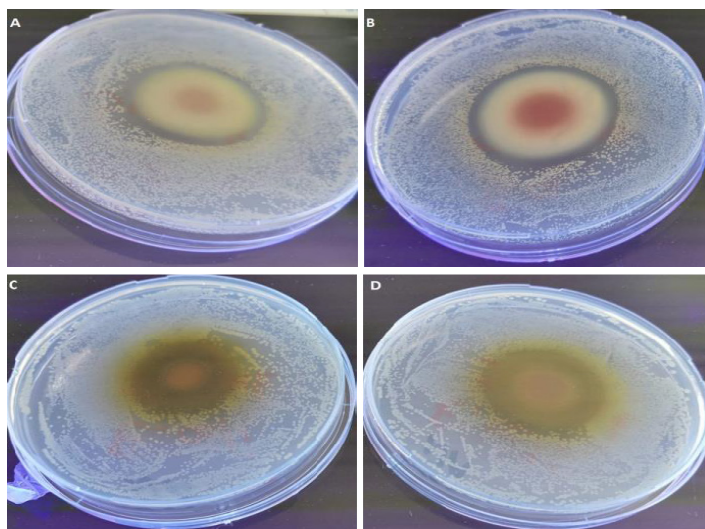


Figure 3. Photographs of antimicrobial activity of microemulsion-alg microcomposites against *S. aureus* (containing % 2 Sumac extract (A), % 3 Sumac extract (B) and *E. coli* (containing % 2 Sumac extract (C), % 3 Sumac extract (D))

Discussion

Sumac has a remarkable bioactive effect due to its rich and different antioxidant, vitamin, fatty acid and mineral content compared to all other spices. This spice has antioxidant, antibacterial, antifungal, antiviral, antifibrogen, hypoglycemic and antimicrobial

properties [12, 13]. Algae have recently attracted attention in the treatment of various skin problems [14]. Microemulsion is an emulsion preparation method and its usage in medical applications is becoming widespread since it carries the bioactive cargo to its target effectively. In this study, the extract of sumac fruit was loaded in algae microemulsion and microcomposite and its antibacterial activity was investigated. Hydrophilic species can be obtained by extraction of sumac with water and n-hexane. A more comprehensive bioactivity can be obtained by extracting both hydrophilic and hydrophobic species with the ethanolic extract. Therefore, the ethanolic extract of sumac was preferred in this study. The most important difference that separates microemulsion from macroemulsion is that the macroemulsion becomes turbidity after mixing has stopped, while the microemulsion becomes clear. Therefore, it can be understood which system is used without carrying out further analysis. In this study, it was determined that the microemulsion was prepared since the mixture was transparent as shown in Figure 2B without further analysis techniques.

Oils having antimicrobial effect in spices are usually hydroxyl group containing phenol compounds and have important antifungal, antibacterial and antioxidant properties. These phenolic compounds destroy the phospholipid layer in the cell membrane, increasing the permeability of this layer, and thus the substances inside the cell leak from the cell or the enzyme system of the bacteria is disrupted. In this case, microorganism inhibition occurs [15].

Sumac is known to exhibit antibacterial effect [16]. However, it is important to show that similar effect is observed with algae in microemulsion. Also the ratio of sumac extract in the microcomposite is highly determinant in the antibacterial effect. Experimental results show that microcomposites containing 2% and 3% sumac extract show strong antibacterial effect.

The formation of an inhibitory zone depends on the diffusion of the antimicrobial compound into culture media and growth rate of microorganisms. These parameters are influenced by the physiological state of the culture, the chemical structure and the cross-linking level of the micro-composite [17]. The antimicrobial activities of cellulose-based micro-composites containing nisin / natamycin against *S. aureus*, *L. monocytogenes* and *Penicillium spp.* Although nisin-containing cellulose microcomposite had no antimicrobial effect on *S. aureus* due to the very slow diffusion rate from micro composite to surface, neomycin containing films showed high antimicrobial effect [18].

Conclusion

It was determined that the algae-microcompositions prepared by microemulsion of sumac extracts have strong antibacterial effect against *Staphylococcus aureus* and *Escherichia coli* bacteria. Since microemulsions provide better skin penetration, algae-microcomposites prepared by this method can be used in later stages in dermatological applications.

Competing interests

The authors declare that they have no competing interest.

Financial Disclosure

There are no financial supports.

Ethical approval

No ethic approval is needed to this research.

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