



Novel formulation of oleaster bark (*Elaeagnus angustifolia*) extract-oil-emulsion gel immobilized into coconut shell hydrochar

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Abstract

This study introduced a novel formulation involving the use of oleaster bark ethanol extract-oil-emulsion gel immobilized into coconut shell hydrochar. The characterization of fish oil (FO)-emulsion gel (Eg) incorporating *Elaeagnus angustifolia* ethanol extract immobilized within coconut shell hydrochar pores and assesses its antimicrobial efficacy against *Acinetobacter baumannii*, *Staphylococcus aureus*, *Candida albicans* and *C. tropicalis* was explored. The oil extracted from the gonad of Kolyos fish (*Scomber japonicus*) was emulsified and then bioactively enriched with ethanol extract of *E. angustifolia*. Gonad emulsion containing *E. angustifolia* extract was gelled with gelatin and finally immobilized into hydrochar (H). This study examined the characteristics and surface analysis of coconut hydrochar (CH)-based *E. angustifolia* ethanol extract-Eg (H-E-Eg) using FTIR and SEM. Stability, particle size distribution, and zeta potential of H-E-Eg were determined. Notably, the stability of H-E-Eg was demonstrated with a zeta potential of -16.4 mV. Additionally, for the first time, this study investigated the time-dependent inactivation of the pathogens when exposed to H-E-Eg. A significant reduction was detected at 30 min and 1 h in *A. baumannii* (84.6% and 89.06%), *S. aureus* (74.075 and 82.12%) compared to *C. albicans* (8.3% and 9.09%) and *C. tropicalis* (46.12% and 43.2%), respectively. The maximum logarithmic decrease of *A. baumannii* was calculated as 0.9 log at the end of the 1st hour. In conclusion, *E. angustifolia* extract-emulsion gel immobilized on coconut shell hydrochar is a functional product for industries seeking sustainable and natural solutions for antimicrobial products, as well as for researchers interested in the bioactivity of plant extracts and innovative formulation techniques.

Keywords *Elaeagnus angustifolia* · *Cocos nucifera* · Hydrochar · *Scomber japonicus* · Emulsion gel · Antimicrobial

1 Introduction

In recent years, the search for natural antimicrobial agents has garnered significant attention due to the increasing challenges posed by antibiotic resistance and the negative effects of synthetic compounds on human health and the environment. One such agent is *Elaeagnus angustifolia* (*E.*

angustifolia), commonly known as oleaster and its fruit is rich in essential minerals, including sulfur, potassium, and magnesium [1]. This aromatic plant belongs to the Elaeagnaceae family and is found in many Northern Asian and European countries, including Turkey [2]. *E. angustifolia* is renowned for its diverse pharmacological activities, particularly its antimicrobial properties. It is frequently used as an analgesic and anti-inflammatory agent in the treatment of rheumatoid arthritis [3, 4], as a reparative in wound treatments [5], and as an auxiliary agent during female menopause [6]. Moreover, it is recognized for its potential anti-diabetic effects, particularly when its leaves are boiled and consumed to lower blood sugar levels [7]. Numerous studies have also highlighted the antimicrobial, antinociceptive, antitumor, and antioxidant effects of *E. angustifolia* [8, 9].

Emulsions are the most common systems used to achieve a homogeneous distribution of hydrophobic substances. These systems are defined as thermodynamically unstable mixtures that consist of an oil phase dispersed in an aqueous

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phase, or vice versa, and are stabilized by various surfactants or emulsifiers [10]. In recent years, there has been extensive research on emulsion systems and emulsifying drug delivery agents [11]. Their application across multiple fields is advantageous for enhancing the bioavailability of various substances, addressing issues in areas such as medicine, food, and waste management. Emulsion gels are produced through a two-stage process. The first stage involves preparing the emulsions, while the second stage entails creating a gel matrix by adding a suitable material to the medium [12, 13]. The resulting emulsion gels exhibit different physicochemical and dimensional properties depending on the gel matrix and the unsaturation levels of the oils used [14]. Three types of emulsion gels can be identified: bulk emulsion gels, fluid emulsion gels, and emulsion gel particles. Bulk emulsion gels are composed of proteins (such as myofibrillar protein, whey, gelatin, and sodium caseinate), polysaccharides (including carrageenan, agar, alginate, gellan gum, and inulin), as well as other compounds like saponin and glycyrrhizic acid [14–16]. To increase the bioavailability of fish oils, the ease of use of ω -3 PUFAs, and the effectiveness of drug-like substances, emulsion forms of these oils are widely studied [17]. Some methods are chosen for gelling fish oil emulsions. These are heating, the addition of enzymatic substances, the addition of acid, and the formation of cross-linked polymers with some proteins [18]. To enhance the bioavailability of fish oils, as well as the effectiveness of drug-like substances, emulsion forms of these oils are extensively studied [17]. In this study, after preparing the emulsions, hydrochar was used creating a gel matrix in the second stage for the first time.

Hydrochar, which is mostly converted from waste and plant waste, is widely used as a soil conditioner due to some of its physical properties. It is thought that its porous structure, which increases water and mineral permeability in the soil, serves as a microenvironment for the development of microorganisms [19, 20]. Furthermore, studies show that hydrochar can be used as a strong adsorption agent for water pollutants due to its ability to adsorb synthetic and organic structures [21, 22]. Therefore, hydrochar enhances the sustainability and efficacy of the formulated gel by providing a unique immobilization matrix with improved porosity and surface area for enhanced delivery of bioactive compounds. This study involved the production of hydrochar in a subcritical water medium utilizing coconut (*Cocos nucifera*) shells. Coconut belongs to the Arecaceae family and is a plant that grows in tropical countries such as Malaysia, Thailand and Indonesia. It grows up to a height of 30 m. It has drupes with a diameter of 100 mm to 250 mm [23]. It can be produced in abundance in tropical regions and is a plant with a very high economic added value. The core shell of the plant is rich in low ash content, volatile compounds, and high carbon content [24].

Several important microorganisms that have been found to cause fatal damage to the immune system weakened by COVID 19 infection, *Acinetobacter baumannii* (*A. baumannii*), *S. aureus* and *Candida albicans* (*C. albicans*) and *C. tropicalis* were investigated in this study for inhibition. *A. baumannii* Gram-negative and *S. aureus* Gram-positive bacterium that are highly resistant to many antibiotics, dangerous and can easily adhere to medical devices [25, 26]. It is a very common pathogen that increases the severity of diseases in intensive care units. Antibiotics have been used more and more, and there are even studies showing that the use of multiple antibiotics is more advantageous. *A. baumannii* can become more common in places where *Candida* yeast are present, by adhering to candida filaments, and this makes the diseases more fatal. Because *C. albicans* and *C. tropicalis* are the cause of many dangerous diseases such as meningitis [27, 28].

In this study, fish oil extracted from *Scomber japonicus* was emulsified and enriched with *E. angustifolia* extract. After that process, the obtained sample was placed in hydrochar pores and then gelled. The objective of this study was to comprehensively characterize the formulated gel using SEM, and FTIR to evaluate its antimicrobial activity against two clinically significant pathogens, namely *A. baumannii*, *S. aureus* and *C. albicans* and *C. tropicalis*.

2 Material and methods

2.1 Materials

The equipment used in this study included a domestically made fixed bed high-pressure reactor made of stainless steel, a Lyophilizer from Labconco, an Ultrasonic homogenizer (ULTRA-TURAX IKA-WERGE), a Millipore Milli-Q Advantage A10 pure water device from Darmstadt, Germany, Syringe filters with a pore size of 0.45 μ m from Agilent, a Sieve shaker of S-Coratory type, FT-IR equipment (Jasco 6700), SEM (ZEISS SUPRA 55), Autoclave (Core OT 40L), and an incubator (Core EN 055).

The materials utilized in the study included Mueller Hinton Agar (MHA), Sabouraud Dextrose Broth (SDB), and ethanol, which were purchased from Sigma. *Scomber japonicus* (*S. japonicus*) fish samples were obtained frozen from a local market in Mersin, Turkey, and were identified by Prof. Dr. Deniz Ayas. These samples were stored in a deep freezer until further analysis.

2.2 Lipid extraction using soxhlet apparatus

The extraction process was carried out according to the modified standard method [29]. *S. japonicus* gonads were cut out in an aseptic environment, placed in a sterile Stomacher Bag

(180×310 mm) and placed directly in the freezer (−20). Then, the frozen gonads were crushed with a mortar pestle and dried in an oven at 50° for 1 day. Segmented gonad samples (33 g) were weighed, which were then placed in the central chamber of the soxhlet apparatus. A 250-mL sterile and dry round-bottomed flask was filled with 200 mL of ethanol and the system was allowed to boil for 4 h. After distillation, the solvent was removed via a rotary evaporator and the lipid sample was stored at −20 °C for later use.

2.3 Plant material and ethanol extraction

E. angustifolia fruits were purchased from a food market in Mersin, Türkiye, 2023. They were brought to the laboratory and peeled, then washed and left to dry under cool conditions for about 10 days. The shells were reduced in size by some pounding in a mortar and 11 g were wrapped in a filter paper and then placed in the soxhlet apparatus. In the soxhlet apparatus, 150 mL ethanol was added to the glass flask and allowed to boil at 70 °C for 5 h. The resulting stock extract was kept in closed vials at 4 °C for analyses.

2.3.1 Chemical composition of *Elaeagnus angustifolia* ethanol extract by GC–MS

The volatile compounds of *E. angustifolia* were analyzed using mass spectrometry (MS) detection within a scanning range of $M + = 50$ –550 m/z . The extract underwent filtration through a 0.45- μ L syringe filter and approximately 1 μ L of the filtered extract was injected into the GC–MS injection port maintained at 250 °C. Elution of the extract was carried out using helium gas at a fixed pressure of 17.90 psi and a total flow rate of 1.75 mL min^{-1} . The split mode was set at a ratio of 10:1 with a flow rate of 15 mL min^{-1} . A HP-5MS capillary column (30 m×250 μ m, 0.25- μ m film thickness) was employed for the separation of the extract. The temperature program consisted of a total runtime of 94.5 min, starting at 50 °C for 2 min, followed by an increase of 4 °C min^{-1} to 100 °C for 5 min, then to 200 °C for 10 min, further to 220 °C for 10 min and finally to 300 °C for 5 min at a rate of 4 °C min^{-1} . Compounds were identified by comparing with the Wiley Registry 9th/NIST 2011 database (W9N11.L) [30].

2.4 Hydrochar synthesis from coconut (*C. nucifera*) shell

The process of producing hydrochar from coconut shells involved subjecting them to hydrothermal treatment in a subcritical water medium. A laboratory-type stainless steel reactor was utilized, and it was purged with 99% purity nitrogen gas to establish an inert environment inside. In the method, coconut shells were placed in this reactor,

which had an internal volume of approximately 220 cm^3 and was resistant to high-pressure and temperatures. Initially, a pressurized environment of 30 bar was maintained, providing subcritical conditions in which water was kept in a liquid state. Subsequently, the temperature was raised to 513 K and maintained for 1 h. Following this, the reactor was cooled to room temperature, and the resultant hydrochar was filtered using filter paper. The obtained coconut hydrochar underwent three washes with deionized water, followed by drying at 368 K for 3 h. Finally, it was sieved using 100- μ m sieves [31].

2.5 Preparation of oil- *E. angustifolia* ethanol extract-emulsion gel immobilized into CH pores

From the oils extracted from the gonads, 1 mL of oil was taken with the help of a pipette and placed in a sterile glass tube. Tween 80 (100 μ L) and 100 μ L of water were added slowly and mixed with a homogenizer for 1 min. Then, another 500 μ L of water was added and homogenized for 1 min, increasing viscosity. After this stage, 1 mL of *E. angustifolia* extract was taken and added directly to the emulsion, vortexed and homogenized again for 15 s. From this stage on, in all processes, the mouth of the bottle was covered with paraffin to prevent the evaporation of ethanol. 500 μ L of previously prepared gelatin liquid (0.15 g/mL) was added and homogenized for 15 s. The resulting *E. angustifolia* extract-Eg (E-Eg) was then added to the ultrasonic bath by adding hydrochar. The E-Eg was sonicated with hydrochar for 2 h at 25 °C. Finally, the hydrochar-*E. angustifolia* extract-emulsion gel (H-E-Eg) was homogenized for 1 min and used for experiments and analysis. E-Eg without hydrochar was used as the control group.

2.6 Characterization analysis of H-E-Eg and E-Eg

2.6.1 Physical features of H-E-Eg and E-Eg

The stability of hydrochar-based *E. angustifolia* extract emulsion gels was visually assessed for clarity over a 5-h period under storage conditions at 25 °C utilizing a 45° slope. Particle size and surface charge of H-E-Eg and E-Eg were measured using a Malvern Zetasizer equipped with MPT-2 Titrator. In this study, 100 μ L of H-E-Eg or E-Eg were diluted (1:500) with distilled water and then analyzed for size distribution and zeta potential (mV). The refractive indexes of water and oil phases were set to 1.33 and 1.38, respectively. The data were recorded as hydrodynamic diameter (nm) for particle size and zeta potential for surface charge [32].

2.6.2 FTIR analysis of H-E-Eg and E-Eg

The FT-IR spectra of H-E-Eg and E-Eg were analyzed in the FT-IR/FIR/NIR Spectrometer Frontier, ATR model, with a spectral measurement range of 4000–500 cm^{-1} .

2.6.3 SEM analysis of H-E-Eg and E-Eg

Prior to imaging of SEM, H-E-Eg and E-Eg underwent platinum coating by spraying with a Quorum 150 RES under 60 mbar pressure in liquid CO_2 [33].

2.7 Antimicrobial screening of H-E-Eg and E-Eg

Antimicrobial activity tests were conducted using agar well diffusion and logarithmic inhibition methods. The microorganisms utilized in the tests were *A. baumannii*, *S. aureus*, *Candida albicans* (ATCC 90028) and *C. tropicalis* yeasts. Prior to the experiment, bacteria and yeast were cultured in 5 mL of TSB and SDB medium, respectively. Microbial cultures were incubated for one day, and 0.5 McFarland standards were prepared for each organism.

2.7.1 Agar well diffusion method of H-E-Eg and E-Eg

Following the agar well diffusion method, MHA plates were prepared with 6-mm wells drilled into them. Samples were taken from 1-day cultures using swab sticks and evenly spread on the surface of the medium. Subsequently, 40 μL of H-E-Eg, E-Eg, *E. angustifolia* extract, and coconut hydrochar were placed into the wells. The plates were then incubated at 37 °C for 24 h. Following incubation, the clear zones of inhibition were measured using a caliper (mm). Three replicates were performed for each sample [34].

2.7.2 In inactivation test of the pathogens by H-EE-Eg

The inhibition performance of H-E-Eg and *E. angustifolia* extract-Eg were studied in the sterile 96-plate. Firstly, 190 μL of the sample was added to the well on the plate and mixed gently. Then, 10 μL of microorganism culture was added. After the process, mixing was done and left in the incubator for 1 h. Then, 190 μL of sterile-distilled water was added to the empty wells on the plate removed from the incubator. 10 μL of the incubated sample was taken and added to the well containing water (1/20). The same process was performed once again. 10 μL of the last sample was taken and smear cultivation was performed on previously prepared MHA petri dishes. Colony numbers were calculated based on logarithmic reduction and

percentage reduction values (Eq. (1)). The experiments were repeated three times [35].

$$\text{Log reduction} = \text{Log}_B^A$$

$$\% \text{ Reduction} = \frac{(A - B) \times 100}{A} \quad (1)$$

where A and B indicate the microorganisms counted before treatment and after treatment, respectively.

2.8 Statistical analyses

Statistical analyses of all tests were performed by One-way ANOVA-post hoc Tukey HSD Test ($p < 0.05$).

3 Results and discussion

3.1 Volatile compounds of *E. angustifolia* ethanol extract

According to GCMS analysis of *E. angustifolia* extract used in the study: 9-Octadecenoic acid, (E)- (52.20%), Myo-Inositol, 4-C-methyl- (12.47%), n-Hexadecanoic acid (10.03%), Cholesterol (4.44%), cis-9-Hexadecenoic acid (3.10%) were the main compounds. The others (> 2%) were 9-Octadecenoic acid (Z)-, methyl ester (2.21%), Octadecanoic acid (2.12%), 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (2.11%) (Table 1).

Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester), Octadecanoic acid, 2,3-dihydroxypropyl ester, and R-(-)-1,2-propanediol determined in the GCMS analysis of *E. angustifolia* methanolic extract were common substances also determined in Syed's study [36]. Octadecanoic acid, 2,3-dihydroxypropyl ester has previously been identified as the dominant molecule in the *E. angustifolia* plant [37]. Incilay (2014) found four main components which were named 1-hexanol, 1-octen-3 ol, hexanal, and benzaldehyde in *E. angustifolia* fruit peel and they were derivatives of hexadecanoic acid which were the components reported in this paper, too [38]. Cholesterol, which we detected in our study (4.44%), is an important bioactive component belonging to the phytosterols group. It protects against various chronic diseases, especially cardiovascular diseases, infection, cancer and diabetes. In the literature, β -sitosterol, a cholesterol-like molecule, has been detected in *E. angustifolia* [39].

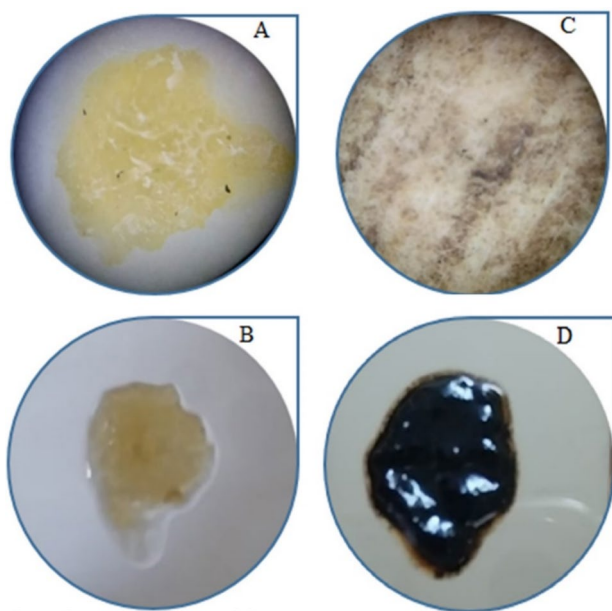
3.2 Some physical features of H-E-Eg and E-Eg

The stability, particle size distribution, zeta potential and conductivity of H-E-Eg and E-Eg are shown in Fig. 1. The pH of the E-Eg is 6.5 without hydrochar and 7.8 with hydrochar (H-E-Eg). After the fish oil was emulsified with the help of an emulsifier, *E. angustifolia* was re-homogenized

Table 1 GCMS result of *E. angustifolia* ethanol extract

Rt*	Compound	Area, %	Peak Q., %
4.345	R-(-)-1,2-propanediol	0.49	72
9.473	1,3,5-Triazine-2,4,6-triamine	0.28	64
1.319	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	2.11	95
14.174	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	1.77	90
20.863	Ethanol, 2-(2-butoxyethoxy)-, acetate	1.47	91
31.956	Myo-Inositol, 4-C-methyl-	12.47	53
32.289	Tetradecanoic acid	1.68	97
36.236	cis-9-Hexadecenoic acid	3.10	99
36.734	n-Hexadecanoic acid	10.03	99
41.393	9-Octadecenoic acid, (E)-	52.20	98
41.643	9-Octadecenoic acid (Z)-, methyl ester	2.21	62
41.898	Octadecanoic acid	2.12	91
46.711	9-Octadecenoic acid (Z)-	0.56	62
50.237	Doconexent	0.34	91
50.391	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	2.39	90
53.905	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	0.64	91
54.487	Octadecanoic acid, 2,3-dihydroxypropyl ester	1.25	90
63.342	Cholesterol	4.44	99

Rt*, Retention time; Q, Quality

**Fig. 1** Bioactive Emulsion gel (E-Eg) with 200×(A); E-Eg on 45°-slope after 5 h (B); Coconut Hydrochar-based Bioactive Emulsion gel (H-E-Eg) spread on paper with 200×(C); H-E-Eg on 45°-slope after 5 h (D)

with ethanol extract, enriched in bioactive compounds, and then bound to gelatin. Since the control didn't contain hydrochar, its other properties were the same and only its color was creamy (E-Eg) (A). While phase separation was observed in the Bioactive emulsion gel (E-Eg) at 25 °C in

the 5th hour in a 45° inclined environment (B), no phase separation in H-E-Eg was spread on paper (C) and in a 45° inclined environment (D). The image in Fig. 1D was controlled in the laboratory for 3 days and the structure did not deteriorate. The obtained *E. angustifolia* E-Eg was immobilized into hydrochar pores. Hydrochar-based *E. angustifolia* extract emulsion gel is dense, semi-liquid, gel-like and black in color (D) (Fig. 1).

In our study, bioactive emulsion gel (E-Eg) was stabilized with hydrochar. In Fig. 1, there was a clear phase separation in the gel without hydrochar (B). There was no phase separation in the gel with hydrochar (D). This showed that the hydrochar acted as a matrix. In Fig. 1, it is clear that the gel with hydrochar was spreadable on paper (C). As it is known, hydrochar has a very high water retention capacity [40, 41]. This explains that the gel will also absorb moisture over time. The zeta potential of H-E-Eg and E-Eg were -16.4 ± 0.6 mV and -17.5 ± 0.6 mV, respectively. In general, the zeta values of the particles being between -30 mV and $+30$ mV indicate that homogenate is stable [42]. Since the zeta potential of our sample (H-EE-Eg) was -16.4 mV, the sample should be considered stable. However, this may be affected by pH changes. Because studies have stated that the zeta value may change depending on the change in pH [43]. The change that may occur over time is one of the parameters that causes the negative load to increase [44]. In a study, a modified bio/hydrochar was employed for amoxicillin removal, and it was observed that the negativity (approximately -50 mV) increased as the pH conditions shifted towards pH 10 [45]. In an emulsion study involving

biochar as a stabilizer in oil–water emulsions (Pickering emulsions), the average zeta potential of alkali-treated biochar was determined to be -43.5 mV [46]. This increases the possibility of processing hydrochars and using them as potential stabilizers. As another physical feature, the level of conductivity of H-E-Eg and E-Eg were 0.0164 ± 0.002 and 0.0126 ± 0.0003 , respectively. These indicate the permeability of emulsions to electric current [47].

The mean peak particle sizes of H-E-Eg (A) and E-Eg (B) were 7.5585 and 6.223, respectively. Our study showed that H-E-Eg has a large and heterogeneous particle distribution area due to the plant-based compounds and hydrochar it contains (Fig. 2).

3.3 FTIR analysis of coconut hydrochar and H-E-Eg

The FT-IR spectra of free emulsion gel (control), coconut hydrochar single, and *E. angustifolia* E-Eg incorporated with hydrochar emulsion gel are depicted in Fig. 3.

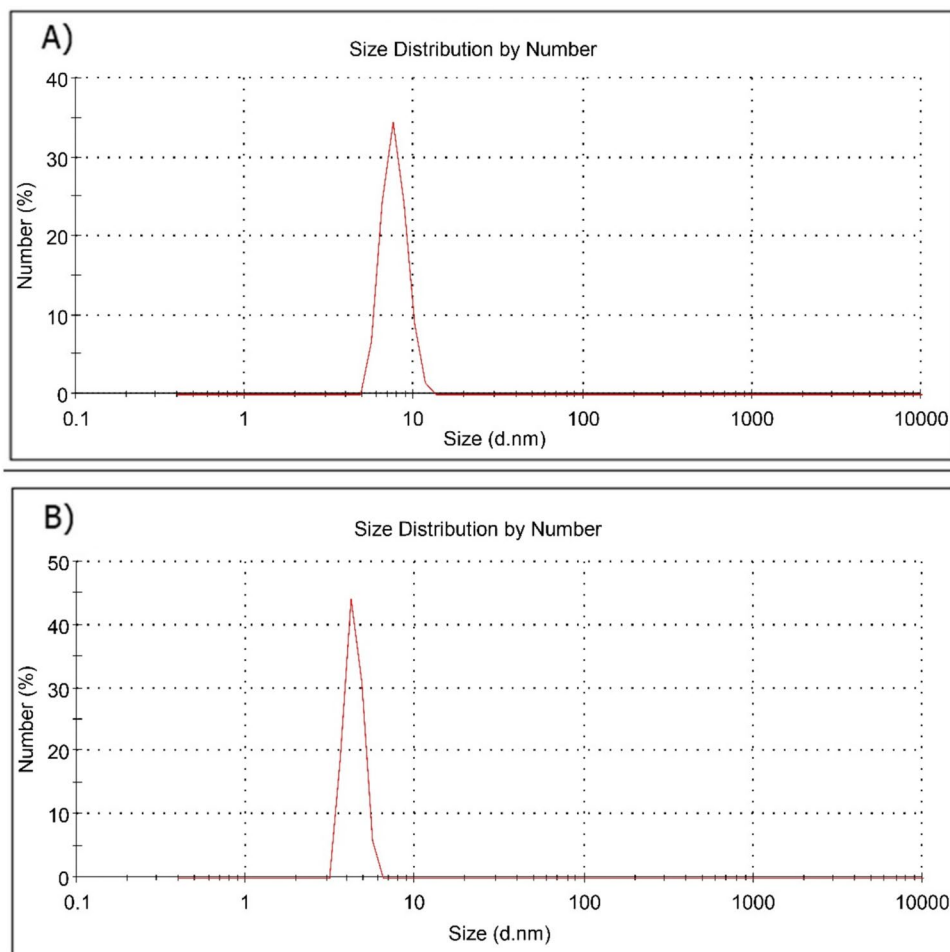
In FTIR, similar peaks were detected in control (Free-Eg) (Fig. 3A) and coconut hydrochar (CH) (Fig. 3B) samples. The aliphatic primary amines at 3331.76 cm^{-1} were at the

same point in both samples. The vibrations in Eg and CH, respectively, lipid acyl chain at 2932.1 and 2929.7 cm^{-1} , C-H bending aromatic compound at 1698.98 and 1693.3 cm^{-1} , CN at 1513.15 and 1512.65 cm^{-1} , acid or ester at $121,160$ and 1210.64 cm^{-1} indicate similar substances. Apart from Eg, more vibration was seen in hydrochar because of hydrochar's containing various molecules such as phenol [34]. In the H-E-Eg example, the density is concentrated at points 3474.55 and 2922.02 cm^{-1} (phenols and lipid acyl chains), and 2856.94 cm^{-1} (carboxylic acid). Peaks at 1457.00 and 1349.18 cm^{-1} (CH_3 bending), 1296.33 cm^{-1} (CH_3 bending), and 1247.98 cm^{-1} (C–O stretching vibrations) show that the substances coming from the oleaster extract intensely pass into the emulsion gel. The bands of 1111.11 cm^{-1} and 1030.53 cm^{-1} in CH, 1096.01 cm^{-1} in H-E-Eg, and 1111.69 cm^{-1} and 1026.6 in Eg were attributed to the C–C stretch, C–O stretch, and C–H bands [33].

3.4 SEM analysis of H-E-Eg and E-Eg

The image of the bioactive emulsion gel attached to the layered and poly structure of the hydrochar was analyzed by

Fig. 2 Size distribution chart of H-E-Eg (A) and E-Eg (B)



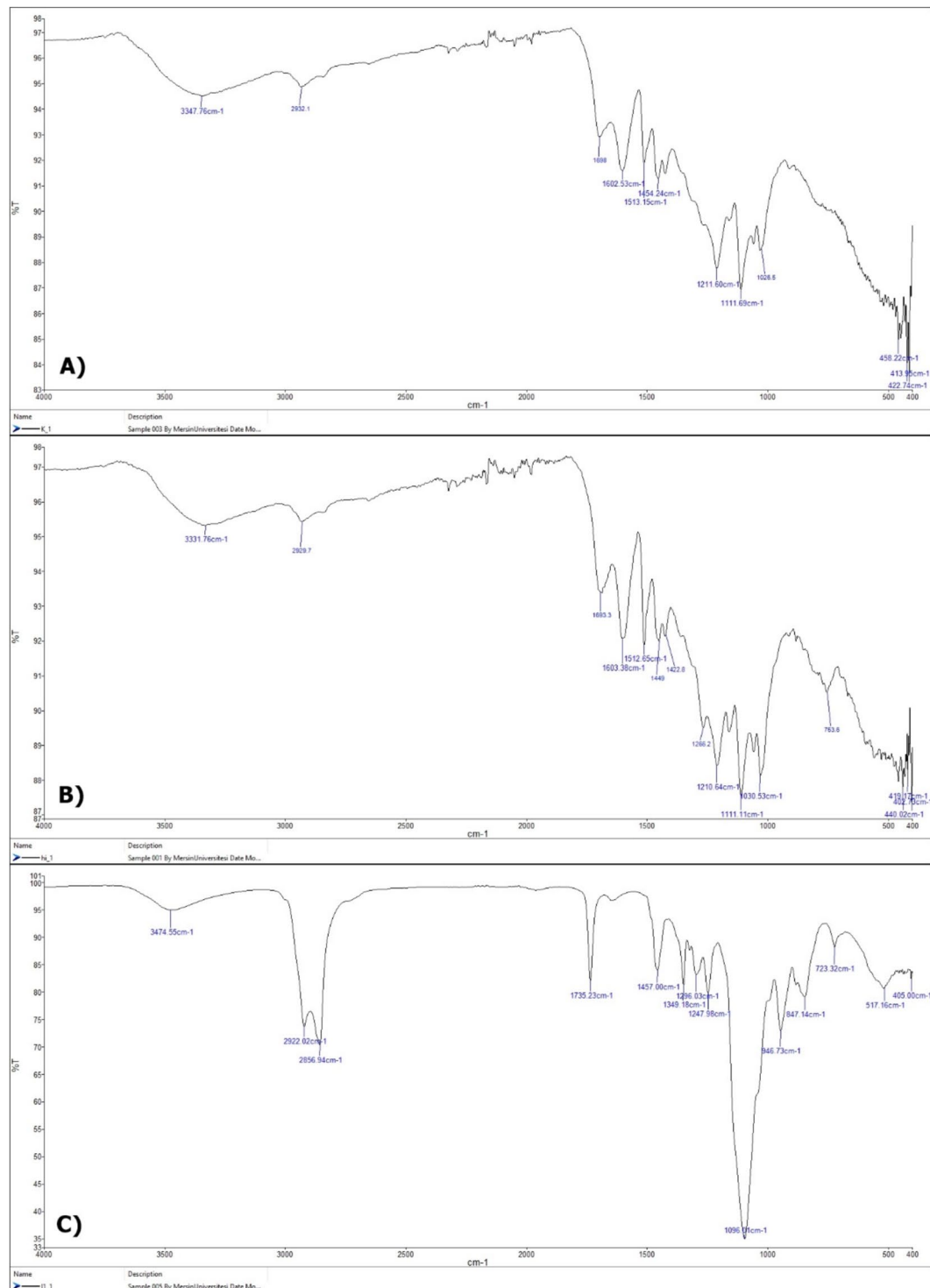


Fig. 3 FT-IR spectra of Eg (A), coconut hydrochar (B), H-E-Eg (C)

SEM. Figure 4 shows the image of the coconut shell hydrochar at (A) 2500 x, (B) 10000x and (C) 20000x, respectively. This layered structure of coconut hydrochar allowed the emulsion gel to be easily immobilized. The bioactive emulsion gel (E-Eg) looked rough due to the movement of

the plant compounds and oil it contains (D). E-Eg immobilized to the hydrochar layers appeared as dense liquid drops at 10000 x (E) and 20000 x (F) magnifications. In a previous study of the same hydrochar, the BET analysis result was calculated as 22.20 SBET (m²/g) [48]. These large pores are

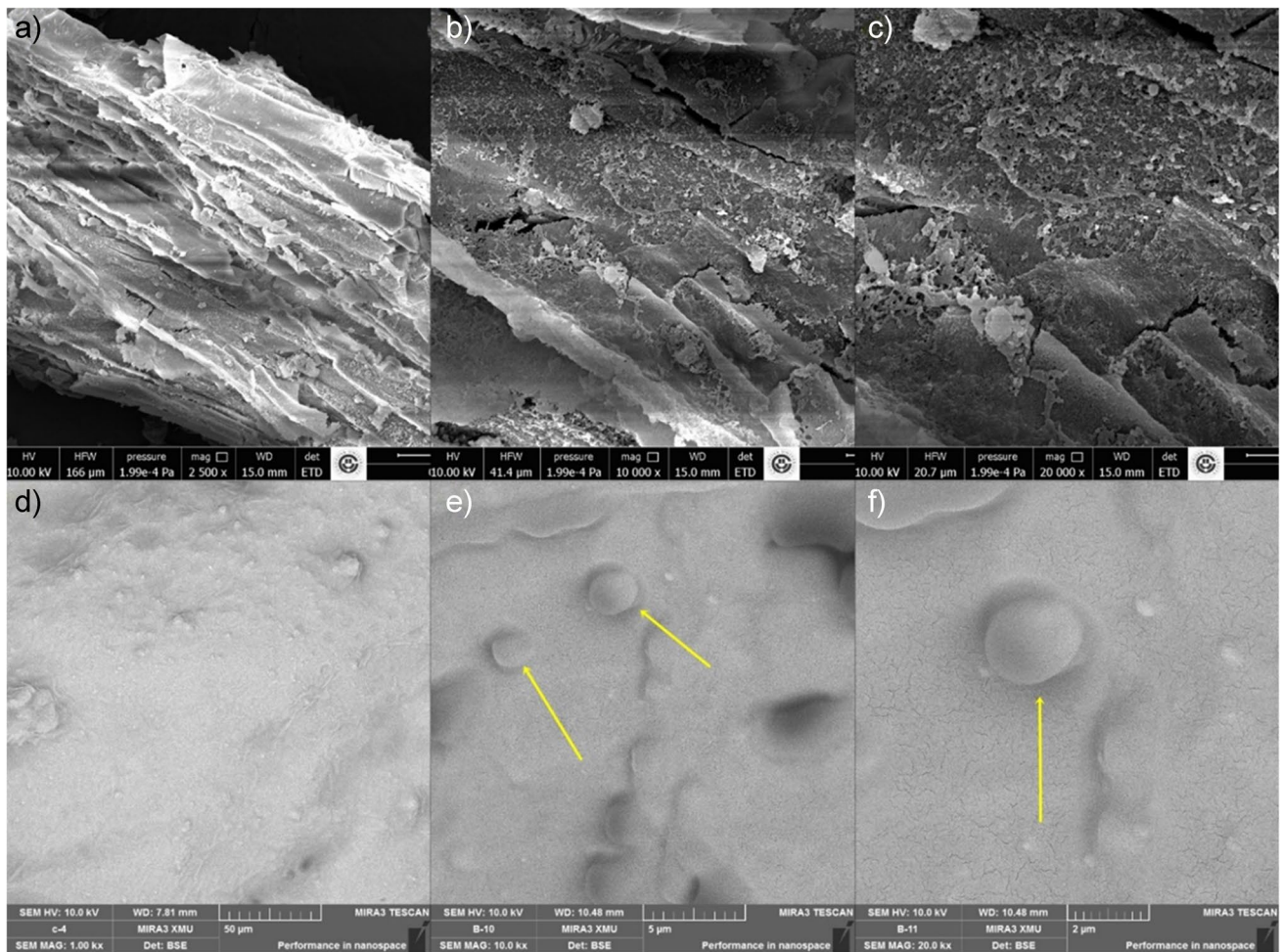


Fig. 4 Coconut hydrochar (A) 2500 x; (B) 10000 x; (C) 20000 x; *E. angustifolia* extract (D) E-Eg, 1000 x; (E) H-E-Eg, 10000 x; (F) H-E-Eg, 20000 x

filled with the emulsion gel completely covering the openings. Fish oil is the cause of the formation of droplet structures or pores in emulsion gels [18]. The layered structure of hydrochar has unique physicochemical properties for the immobilization of many bioactive materials. The high liquid absorption potential of hydrochars allows the immobilization of structures enriched in plant molecules into hydrochar layers in the bioactive polymers [31].

3.5 The inhibitions of the pathogens by H-E-Eg and E-Eg

In the agar well diffusion test, inhibition zones of H-E-Eg, *E. angustifolia* E-Eg, *E. angustifolia* ethanol extract, lipid and Hydrochar against *A. baumannii* were 2.2, 1.2, 9.6, 1.1 and 1.0 mm. *S. aureus* was inhibited with 3.2, 1.3, 8.7, 0.1, 0.0 mm by H-E-Eg, *E. angustifolia* E-Eg, *E. angustifolia* extract, lipid and hydrochar. Similarly, *C. albicans* was inhibited with 2.3, 1.1, 6.3 and 1.0 mm by H-E-Eg, *E. angustifolia*

E-Eg, *E. angustifolia* extract, lipid and hydrochar. The highest inhibition was seen by *E. angustifolia* extract alone on both microorganisms. *C. tropicalis* was inhibited with 1.2, 1.1, 6.5, 0.1, 0.0 mm by H-E-Eg, *E. angustifolia* E-Eg, *E. angustifolia* extract, lipid and hydrochar (Table 2).

Table 2 IZ (mm) of H-E-Eg, E-Eg, *E. angustifolia* ethanol extract (EE), Lipid, hydrochar, and antibiotic against *A. baumannii*, *S. aureus*, *C. albicans*, *C. tropicalis*

	<i>A. baumannii</i>	<i>S. aureus</i>	<i>C. albicans</i>	<i>C. tropicalis</i>
H-E-Eg	2.2 ^a ± 0.5	3.2 ^a ± 1.1	2.3 ^a ± 1.5	1.2 ^a ± 1.2
E-Eg	1.2 ^a ± 0	1.3 ^a ± 0.1	1.1 ^a ± 0.1	1.1 ^a ± 0.0
EE	9.6 ^b ± 1	8.7 ^b ± 1.1	6.3 ^b ± 2.1	6.5 ^b ± 1.1
Lipid	1.1 ^a ± 0.5	0.1 ^a ± 0.0	1.0 ^a ± 0.1	0.1 ^a ± 0.0
Hydrochar	1.0 ^a ± 0.1	0.0 ^a ± 0.0	1.0 ^a ± 0.5	0.0 ^a ± 0.1
Ant	22.5 ^c ± 1.1	27.2 ^c ± 1.0	18.2 ^c ± 2.5	25.2 ^c ± 2.0

Different letters in the same column indicate statistical differences. Ant, Antibiotic (128 µg/mL)

The survival rate of *A. baumannii* and *C. albicans* exposed to H-E-Eg and E-Eg is shown in Table 2. The log reduction of *A. baumannii* exposed to H-E-Eg within 30 min and 1 h was 0.8 and 0.9 log, respectively. The log reduction of *S. aureus* exposed to H-E-Eg within 30 min and 1 h was 0.7 and 0.8 log, respectively. The cells in E-Eg were reduced to 0.1 and 0.06 log within 30 min and 1 h, respectively. In contrast, the log reduction of *C. albicans* exposed to H-E-Eg was 0.4 and 0.2 log, respectively. While the yeast exposed to E-Eg was reduced to 0.03 and 0.04 log within 30 min and 1 h, respectively ($p < 0.05$). At the end of the 1 h, the highest percentage inhibitions for *A. baumannii* and *C. albicans* were 89.06% and 46.2%, respectively. *C. tropicalis* was exposed to E-Eg was reduced to 0.01 and 0.04 log within 30 min and 1 h, respectively; while *C. tropicalis* was inhibited with 0.3 and 0.2 log by H-E-Eg ($p < 0.05$) (Table 3).

In literature, the contents of parts from the *E. angustifolia* plant such as roots, stems and leaves have been previously researched using different extraction methods and their antimicrobial activity has been examined. Among these, Okmen and Turkcan (2014) found that *E. angustifolia* methanol extract compounds were a strong agent against the strains of *Bacillus subtilis*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Enterococcus faecalis*, *Yersinia enterocolitica*, *Salmonella typhimurium* (≤ 16 mm), and in the same study, the extracts were found not to be affected on *Escherichia coli* and *Candida albicans* microorganisms [49]. In another study, peptide fractions of *E. angustifolia* inhibited *S. aureus*, *E. coli*, *Proteus vulgaris*, and *Klebsiella pneumoniae* strains (≤ 20) [50]. In our study, *A. baumannii*, *S. aureus*, *C. albicans* and *C. tropicalis* microorganisms were used, and unlike the study by Okmen and Turkcan, *C. albicans* and *C. tropicalis* were inhibited by *E. angustifolia* EE (6.3 mm and 6.5 mm). No record has been found regarding the effect of *E. angustifolia* on *A. baumannii*. We detected a strong activity of *E. angustifolia* EE against *A. baumannii* and *S. aureus* (9.6 mm and 8.7 mm) than *Candida*'s. The reason for this can be explained by the following mechanism. The differences in antimicrobial mechanisms between eukaryotic and prokaryotic cells are particularly evident in terms of membrane structures and cellular components. Prokaryotic

cells have a cell wall containing a peptidoglycan layer, which provides a target for antibacterial drugs. Antibiotics inhibit peptidoglycan synthesis, making these cells more susceptible to osmotic lysis [51]. Eukaryotic cells, on the other hand, generally have more complex membrane structures and cellular metabolism, making them more resistant to the effects of antibiotics and can restrict drug entry into the cell [52]. The antimicrobial effect on *A. baumannii* and *C. albicans* depends on the active ingredients in the extract of the plant. 9-Octadecenoic acid which we detected in our study, too and is known as the dominant molecule of *E. angustifolia* [37], may be the main reason for (E)-bioactivity. In addition, Syed et al. (2023) stated that the volatile components of *E. angustifolia* extract mostly consist of compounds such as ethyl acetate, isobutyl acetate, Phytol, isoquinoline, 9-Octadecenoic acid, 1,2,3-propanetriyl ester, Squalene, Octadecanoic acid, 2,2-Dimethyl-3-(3,7,16,20-tetramethyl) [36]. In another study, they determined that *E. angustifolia* was determined that it is rich in phenolics and flavonoids and, accordingly, has a high antioxidant capacity. Among these compounds, gallic acid, catechin, and quercetin were at high levels [53]. In the emulsion gel study, when the plant extract was added to the emulsion mixture, there was a slight decrease in antimicrobial activity due to the decrease in concentration. The reason for this is that although the antimicrobial activity of the plant alone is high, the antimicrobial activities of other substances (lipid, hydrochar) compounds are somewhat low. Microorganisms were subjected to time-dependent logarithmic reduction experiments according to the colony counting method. Here, a significant reduction was detected in *A. baumannii* (84.6% and 89.06%) compared to *C. albicans* (8.3% and 9.09%) at 30 min and 1 h. It was found that logarithmic inhibitions to *A. baumannii* and *C. albicans* were similar. It is clear that this inhibition came from *E. angustifolia* EE. It is known that biochar or hydrochar alone do not have toxicity for eukaryotic cells, on the contrary, they promote growth in germination experiments of some plants [54]. In our study, the yeast selected is a eukaryotic cell and the inhibition of hydrochar on *C. albicans* is very low. In literature, the maximum logarithmic decrease of *A. baumannii* was calculated as 0.9 log at the

Table 3 Log reduction (CFU/mL) and reduction (%) on pathogens exposed to H-E-Eg and E-Eg

	Time	<i>A. baumannii</i>		<i>S. aureus</i>		<i>C. albicans</i>		<i>C. tropicalis</i>	
		H-E-Eg	E-Eg	H-E-Eg	E-Eg	H-E-Eg	E-Eg	H-E-Eg	E-Eg
Log reduction (CFU/mL)	30 min	0.8	0.1	0.7	0.1	0.4	0.03	0.3	0.01
	1 h	0.9	0.06	0.8	0.045	0.2	0.04	0.2	0.04
Percent reduction (%)	30 min	84.6 ^a ± 3.2	23.07 ^b ± 3.5	74.075 ^a ± 3.0	23.1 ^b ± 3.1	61.5 ^a ± 3.0	8.3 ^b ± 2.2	46.12 ^a ± 1.5	3.3 ^b ± 1.7
	1 h	89.6 ^a ± 3.8	13.5 ^b ± 2.1	82.12 ^a ± 2.0	10.15 ^b ± 1	46.2 ^a ± 0.2	9.09 ^b ± 2.2	43.2 ^a ± 1.6	9.01 ^b ± 1.1

Starting population ~ 1.5×10^8 , “a”, “b”, “c” on the same line indicate the difference between the data

end of the 1st hour. A 3-log₁₀ CFU/mL decrease in *A. baumannii* with the antibiotic meropenem was seen after 168 h [55]. In the meropenem + colistin study, the drugs were more effective and bacterial death increased by 3 logs in 24 h. In our study, a 0.9 log decrease after 1 h is a significant inhibition [56].

The enrichment of hydrochars with phenolic extracts is quite new in the literature. Previously, the characterization of hydrochar obtained from P and N-enriched antibiotic residues was studied [57]. A carbonized AuNPs-hydrochar nanocomposite was developed for the degradation of cephalexin [58]. However, antimicrobial researches of these studies are not available. Our study is the first to study how to make hydrochar more active than itself in terms of its bioactive properties. Hydrochar is a very efficient substance for soil microbiota on its own. It provides important contributions to the nutrition of the soil and the balance of the microbiota. It does not have ecotoxic properties [59]. It is planned in future studies to review whether it is toxic or not above a certain concentration, depending on the area where the product will be used.

4 Conclusion

In this study, the first step was to enrich the emulsion form of the oil obtained from *Scomber japonicus* gonad with *E. angustifolia*. The oils have some antimicrobial activity, but this is not sufficient. The bioactive *E. angustifolia* ethanol extract added to it made the emulsion more bioactive. Gelled emulsions provide more stability for the bioactive molecules and extend their activity life. The use of coconut shell hydrochar as an immobilization matrix provided strong penetration of the emulsion gel into the coal layers. This is due to the strong absorption properties of the hydrochar particles. In order to use H-E-Eg in industrial applications, parameters such as time-dependent shelf life or pH control should be reviewed. Several studies at various temperatures, pH levels, and different matrix concentrations should be conducted in the future.

This not only improves the environmental profile of the formulation but also provides a platform to explore new avenues in waste utilization and resource efficiency. Emulsion forms with increased effectiveness in cosmetics, food, and agriculture can be an alternative in combating microorganisms and breaking antibiotic resistance. By adding a gelled bioactive structure to the pores of hydrochar, hydrochar has found a wider application area, especially in applications such as animal feed, soil improvement, and cosmetics. Although hydrochar itself has little or no antimicrobial activity, it has become a bioactive structure even when enriched with primary and secondary metabolites.

Through the comprehensive characterization and evaluation of the antimicrobial activity of oleaster bark ethanol extract-oil-emulsion gel immobilized on coconut shell hydrochar against *Acinetobacter baumannii*, *S. aureus*, *Candida albicans*, and *C. tropicalis*, several important findings emerged. First, the incorporation of oleaster bark extract into the gel matrix demonstrates significant antimicrobial activity against both bacterial and fungal pathogens. This suggests the presence of bioactive compounds in the extract that warrant further investigation for their potential therapeutic applications.

Overall, the findings presented in this article have important implications for various sectors, including pharmaceuticals, cosmetics, and healthcare. Further research is warranted to elucidate the mechanisms underlying the observed antimicrobial activity and explore additional applications of this innovative formulation. By utilizing natural resources and sustainable materials, we can continue to move toward safer, more effective antimicrobial solutions in an increasingly environmentally conscious world. It is planned to re-evaluate and conduct necessary research according to the area in which the product we developed will be used, such as cosmetics, medicine, or agriculture.

Author contribution Elif Ayşe Erdogan Eliuz: Writing and correcting the original draft, review & editing. Discussed the results and commented on the manuscript, critical feedback, experimentation, and organization and helped shape the research. Erdal Yabalak: Writing and correcting the original draft, review & editing. Discussed the results and commented on the manuscript, critical feedback, experimentation, and organization and helped shape the research. Design of the article, corrections and, supervision. Deniz Ayas: Commented on the manuscript.

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Data availability All results data and analyses of the study are included in this published article.

Declarations

Ethical approval Not applicable.

Competing interests The authors declare no competing interests.

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