Research Paper

Bioactive Compounds and Antimicrobial Activity of Glasswort *Salicornia europaea*

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This study aims to determine the chemical and physical composition and antibacterial activity of glasswort. Soxhlet extraction of *Salicornia europaea* was performed using hexane and analyzed by Gas chromatography-mass spectrometry. Alpha-tocopherol content was analysed by using liquid chromatography with tandem mass spectrometry. The elemental analysis was investigated by inductively coupled plasma mass spectrometry. The crystallographic texture and composition of powder samples were determined by X-ray diffraction. The minimum inhibition concentrations and inhibition zones extracts of *Salicornia europaea* were performed by spectrophotometric broth microdilution and disc diffusion methods, respectively, against 4 bacteria (*Escherichia coli*, *Klebsiella pneumonia*, *Bacillus subtilis*, *Staphylococcus aureus*) and 2 fungi (*Candida albicans*, *Candida parapsilosis*). Alpha-tocopherol quantity and oil content of *Salicornia europaea* were found to be 2.1±0.3 mg/100 g and 15.15 %, respectively. The detected minerals were also found as sodium 447, magnesium 51.98, K 45.86, Ca 14.27 ppm. The highest minimum inhibition concentration was 0.9 mg/ml against *Escherichia coli* in methanol extract ($p<0.05$). The highest mean inhibition zones diameter was 3.8 mm for methanol extracts against *Bacillus subtilis* ($p<0.05$). As a result, the extract and oil of *Salicornia europaea* can be used as potential bioactive and antimicrobial agents for pharmaceutical and cosmetics applications.

Key words: Antimicrobial, bioactive, raw material, glasswort, *Salicornia europaea*

The Glasswort *Salicornia europaea (S. europaea)* is a plant species in the family of spinach (Amaranthaceae) which is a sea related plant found in wet or sandy grounds, that is common in Atlantic and Mediterranean coasts. *S. europaea* is an annual halophytic plant[1] which is consumed by humans due to its taste. There are very similar and hardly distinguishable species of *S. europaea* in nature[2-3] that seven species of the genus Salicornia were reported, *Salicornia pusilla*, *Salicornia europaea*, *Salicornia obscura*, *Salicornia ramosissima*, *Salicornia nitens*, *Salicornia fragilis*, *Salicornia dolichostachya*.

The plants mainly used for their medicinal or aromatic properties in pharmacy or perfumery are defined as medicinal and aromatic plants (MAPs) in the EU[4], and thus, plant extracts are extensively used as active ingredients in cosmetics. The cosmetic concept is certainly very different from the past though some of the ingredients are still the same[5]. Herbal products claim to have less side effects, commonly seen with products containing synthetic agents. Therefore, the increasing demand for natural products and extracts is leading to the over-exploitation of natural plant resource[6]. In this regard, studies are being made on the search for new raw materials and the discovery of new crops. Especially, there are many plant species which are found in nature and are beginning to be used in cosmetics due to their content as antioxidant, anti-inflammatory, antiseptic, emollient, antiseborrheic, anti keratolytic activity and antibacterial[7].

Up to date, to our knowledge, there has been no research on pharmaceuticals and cosmeceutical potential of *S. europaea*. Therefore, this study aims to investigate the chemical and physical composition and antimicrobial

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Accepted 08 March 2021
Revised 03 January 2021
Received 25 October 2020
Indian J Pharm Sci 2021;83(2):238-246

activity of *S. europaea* for pharmaceuticals and cosmeceutical applications.

MATERIALS AND METHODS

Sample preparation and chemical analysis:

Salicornia europaea samples were collected from Adana-Tuzla coastal areas and taken to the laboratory and washed and dried in air. For determination of moisture content, the sample was taken in a flat-bottom dish and kept in an air oven at 105° for 2, 4, 6 h and allowed to cool at room temperature in a desiccator and then weighed. The procedure is repeated until successive weighing agrees to as constant weighing. The loss in weight was regarded as a measure of moisture content.

The air-dried and coarsely powdered, approximately 20 g. *S. europaea* was placed in Soxhlet extractor with a 100 ml Hexane. The extracts were then concentrated to dryness under reduced pressure and controlled temperature by a rotary evaporator. The obtained oils were dried over anhydrous $CaCl_2$ and then analyzed by triple quad Gas chromatography-mass spectrometry (GC-MS) that was also re-analyzed with GC-MS Hewlett Packard Gas collection device (GCD) equipped with a mass selective detector (MSD) for comparisons. The oils were analyzed by GC-MS using Hewlett Packard GCD (model 6890) and Hewlett Packard mass spectrometry (MS) (model 5972) equipped with a mass selective detector (MSD). An HP-5 column (30 m×250 µm i.d.×film thickness 0.25 µm) and HP 18 593B automatic injection system was used. 30 ml of essential oils was transferred into 1 ml of diethyl ether (Merck) and injected to the GC-MS sampling port. The chromatogram was produced by holding the oven temperature to 45° for 5 min initially and then increasing the temperature to 130° at a rate of 2° per min followed by an increase at a rate of 3° per min to 170° and programmed to 220° at a rate of 10° per min then kept constant at 220° for 5 min. MSD conditions were as follows: capillary direct interface temperature 250°, ionisation energy 70 eV, mass range, 33-330 amu, Electro-magnetic voltage (Atune+200), scan rate 5 scan per s. Helium was used as the carrier gas at a flow rate of 1.5 ml/min. The components were identified by comparison of their mass spectra with Wiley GC-MS and National BIM Library. Relative percentage amounts of the separated compounds were calculated automatically from peak areas of the total ion chromatograms. The Kováts index of each compound was determined using C7-C40 Saturated Alkane Mixture, a certified reference material, which contains each C7-C40 component in a concentration of 1000 µg in ml of hexane.

1g ground *S. europaea* was digested using a 12 ml mixture of nitric acid/perchloric acid (8/2) (v/v) at the temperature of 100° and then the obtained acidic solution was diluted to 20 ml with ultrapure water and filtered through Whatman® to analyze by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Agilent-7500 ce Octopole Reaction System) for measuring elemental content of *S. europaea*.

About 9.82 g of fresh *S. europaea* was transferred into a 500 ml beaker containing 200 ml olive oil and incubated for 1 mo after which it was treated in an ultrasonic water bath for 1.5 h. to analyze alpha-tocopherol content of the *S. europaea* samples using liquid chromatography with tandem mass spectrometry (LC/MS-MS). Official Method (2.432) International Union of Pure and Applied Chemistry (IUPAC), Thermo Scientific Quantum Access Model LC/MS-MS were used for the analysis.

Powdered *S. europaea* was attached to the specimen holder (stub), and elemental composition, the surface topography of the crystal structure by scanning the sample surface with high energy electron beam Scanning with an electron microscope type display Electron Microscope (SEM), energy dispersive X-ray analysis (EDX) were performed in order to know the chemical composition of *S. europaea*.

S. europaea was dried and powdered to a size below 22 microns in grinders for analyses. The powdered sample was placed inside of the aluminum sample holder to ready suitable for pressing vertically with the least orientation. The crystallographic texture and composition of powder samples were determined at standard angular resolutions up to 20×20 by X-Ray Diffraction (XRD).

Antimicrobial analysis:

Dried *S. europaea* was pulverized to obtain *S. europaea* extracts and the other fractions. The method was carried out with different residence times of the samples weighed 15 g and 30 g in parallel, and the retention time in the extraction was determined as 24, 48 and 72 h. Accordingly, powder of *S. europaea* was weighed (0.001 sensitivity) and put into beakers in which 100 ml of ethanol, methanol, acetone and distilled water were added.

The antimicrobial activities of ethanol, methanol, acetone and aqueous extracts of *S. europaea*

were investigated by using disc diffusion and spectrophotometric broth microdilution methods. For both methods, four bacterial and two fungal strains were used to investigate antimicrobial activities of the *S. europaea* extracts. The bacterial strains were two gram negative bacteria *Escherichia coli* (ATCC 25293) and *Klebsiella pneumonia (K. pneumonia)*, and two gram positive bacteria *Bacillus subtilis (B. subtilis)* (ATCC 6633) and *Staphylococcus aureus*, and the fungal strains were *Candida albicans (C. albicans)* and *Candida. parapsilosis (C. parapsilosis)*. The inoculums of microorganisms were prepared in 4 ml Triptic Soy Broth for bacteria, 4 ml Sabouraud Dextrose Broth for yeasts incubated at 37° overnight. After 24 h, the microorganism suspensions were adjusted to 0.5 McFarland standard turbidity and were kept at +4° to use for disc diffusion and spectrophotometric broth microdilution experiments[8].

Spectrophotometric broth microdilution method for screening was performed on 96-well elisa plates, and firstly 50 µl of Mueller Hinton Broth (MHB) medium were added into all wells. The two-fold serial dilutions of 50 µl of *S. europaea* extract was made on all x-axis along of plate. Negative and positive controls (Ampicillin for bacteria, Fluconazole for yeast) were performed in columns 11 and 12. Then, 10 µl culture of microorganisms was inoculated on all wells except medium control wells. All plates were incubated at 37° for 24 h and the growth turbidity was measured at 600 nm and 415 bacteria and yeasts, respectively. As the calculation of minimum inhibition concentration (MIC) values, the optical density was read both before (T_0) and after 24 h incubation (T_{24}). For each plate, MIC was calculated using the following formulas (Eqn 1 and Eqn 2): The OD for each replicate at T_0 was subtracted from the OD for each replicate at T_{24}[9]. Percent of growth (%)=$OD_{test}/OD_{control}\times100$ Eq. 1. Percent of inhibition (%)=1-$OD_{test\ well}/OD_{corresponding\ control\ well}\times100$ Eq. 2. Inhibition graph was plotted; R^2 and then MIC (the lowest concentration of test material which results in 99.9 % inhibition of growth) were calculated on the obtained linear slope[10-11]. Screening with the method of Disc Diffusion Assay, microorganism cultures assayed by McFarland 0.5 was spread onto MH (Mueller Hinton) agar plates. Paper discs (6 mm diameter) were placed on agar to load 20 µl extracts prepared from *S. europaea*, the results were recorded in the growth inhibition zones (mm) surrounding the disc using a digital caliper. All data related to antimicrobial activity were obtained by averaging the triplicate analyzes.

RESULTS AND DISCUSSION

The hexane-extracted oil yield of *S. europaea* cultivated was found to be 15.15 %. To find vitamin E (Alpha-tocopherol), *S. europaea* were extracted in hexane and olive oil to elucidate vitamin E concentration in the Liquid chromatography-mass spectrometry (LC-MS) that the alpha-tocopherol amounts were 2.1±0.3 mg per 100 g with Hexane and 551.98 µg/ml with olive oil which showed that olive oil with *S. europaea* samples contains alpha-tocopherol much more than that the Hexane extraction (Table 1).

The Oleic acid, as a component of the essential oil of *S. europaea,* was found to be 36.55 %. The fatty acid composition (percent) of oil from glasswort (*S. europaea*) is presented in Table 2. The amount of sodium, magnesium, potassium and calcium (Na, Mg, K and Ca) minerals in the *S. europaea* were found 447, 51. 98, 45.86, 14.27 ppm by ICP-MS. Alternatively, the results are also confirmed by EDX analyses in fig. 1.

The crystallographic texture and composition were determined using XRD phase analysis, and Strontium Lanthanum Iron (III) Tin (IV) Oxide was found to be 54.5 % (Table 3). Tin (IV) oxide is used as abrasive, bulking and opacifying agent in cosmetic product[12]. The surface morphology characterized by SEM and SEM image of *S. europaea* is given in micrograph enlarged scale bars of 40 µm, 50 µm and 400 µm (fig. 2).

As a result of the disk diffusion method, the zone measurements of ethanol, methanol, acetone and

TABLE 1: LC/MS/MS RESULTS OF ALPHA TOCOPHEROL

Sample Name	Test/ (Analysis) (Parameter)	Method	Unit	Result
Olive oil	Alpha-tocopherol	Determination of tocopherols and tocotrienols in vegetable oils and fats by high performance liquid chromatography. Official Method. 2.432. IUPAC.	µg/ml	147.17
Olive oil with *Salicornia europaea*	Alpha-tocopherol	Determination of tocopherols and tocotrienols in vegetable oils and fats by high performance liquid chromatography. Official Method. 2.432. IUPAC.	µg/ml	551.98

TABLE 2: THE FATTY ACID COMPOSITION OF OIL FROM GLASSWORT (*S. europaea*) BY GC/MS

RT (min)	KI	Fatty Acids	Formula	MW	Amount (%)
36.27	2315	Palmitic acid	$C_{16}H_{32}O_2$	256.42	5.12
37.71	2387	Palmitoleic acid	$C_{17}H_{32}O_2$	268.43	3.27
38.21	2411	Oleik acid	$C_{18}H_{34}O_2$	282.46	36.55
49.38	3120	7-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.49	11.31
49.52	3128	8-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.49	10.63
49.94	3153	9-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.49	13.77
50.18	3167	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296.49	2.73
50.31	3175	10-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.49	3.48
50.41	3181	Stearic acid	$C_{19}H_{38}O_2$	298.50	4.07
58.94	3553	11-Octadecenoic acid, methyl ester	$C_{21}H_{42}O_2$	326.56	3.35
61.40	3684	11-Eicosenoic acid, methyl ester	$C_{21}H_{40}O_2$	324.54	3.42
72.77	4285	2,6,10,14,18,22-Tetracosahexaene	$C_{30}H_{50}$	410.39	2.30

RT: Retention Time; KI: Kovats Index; MW: Molecular Weight

TABLE 3: XRD RESULTS SHOWING COMPOUNDS CONTAINED IN *S. europaea*

%	Ref.Code	Compound Name	Chem. Formula
*16.6	98-017-4207	Perovskite	$Mo_1O_3Sr_1$
*19.0	98-004-4727	Calcium-alpha	Ca_1
*54.5	98-007-2169	Strontium Lanthanum Iron(III) Tin(IV) Oxide	$Fe_1La_1O_6Sn_1Sr_1$
*9.9	98-018-0530	Potassium Iron Dioxide	$Fe_1K_1O_2$

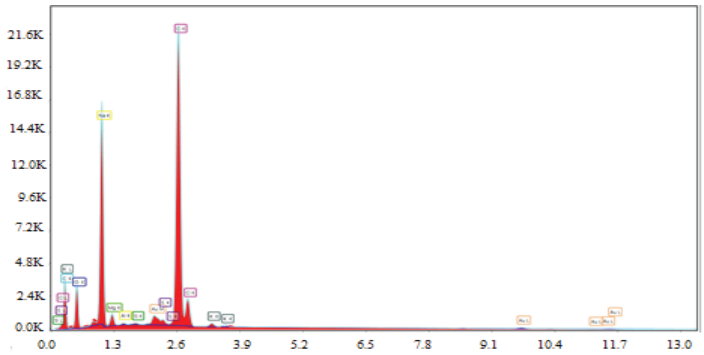

Fig.1: Graph of EDX analysis showing presence of elemental

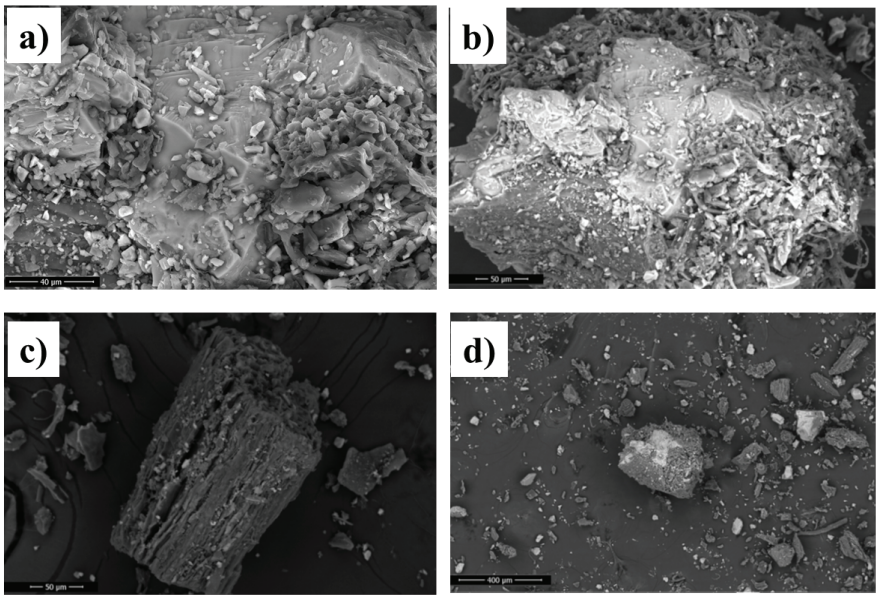

Fig. 2: SEM micrograph scale bars of 40 µm for micrographs (a), 50 µm (b), 50 µm (c), 400 µm (d).

TABLE 4: INHIBITION ZONE DIAMETERS OF *S. europaea* EXTRACTS WITH *E. coli*, *K. pneumoniae*, *B. Subtilis* AND *S. aureus* ACCORDING TO THE DISC DIFFUSION METHOD

Extracts (h)		Inhibition Zone (mm)+SEM*			
Weight (g)	Acetone	*E. coli*	*K. pneumoniae*	*B. subtilis*	*S. aureus*
	24	$0.0^{a}_{\pm 0,0}$	$0.0^{a}_{\pm 0,0}$	$0.0^{b}_{\pm 0,0}$	$0.2_{\pm 0,2}$
15	48	$0.0^{a}_{\pm 0,0}$	$0.0^{a}_{\pm 0,0}$	$0.5^{b}_{\pm 0,5}$	$0.3^{bc}_{\pm 0,3}$
	72	$0.0^{a}_{\pm 0,0}$	$0.0^{a}_{\pm 0,0}$	$0.6^{b}_{\pm 0,6}$	$0.0^{b}_{\pm 0,0}$
	24	$0.0^{a}_{\pm 0,0}$	$0.0^{a}_{\pm 0,0}$	$0.0^{b}_{\pm 0,0}$	$0.7^{a}_{\pm 0,4}$
30	48	$0.0^{a}_{\pm 0,0}$	$0.0^{a}_{\pm 0,0}$	$0.0^{b}_{\pm 0,0}$	$1.1^{a}_{\pm 0,0}$
	72	$0.0^{a}_{\pm 0,0}$	$0.0^{a}_{\pm 0,0}$	$0.0^{b}_{\pm 0,0}$	$0.4^{bc}_{\pm 0,4}$
	Ethanol				
	24	$0.0^{a}_{\pm 0,0}$	$0.0^{a}_{\pm 0,0}$	$1.9^{a}_{\pm 0,2}$	$0.0^{b}_{\pm 0,0}$
15	48	$0.0^{a}_{\pm 0,0}$	$0.0^{a}_{\pm 0,0}$	$0.4^{b}_{\pm 0,4}$	$0.0^{b}_{\pm 0,0}$
	72	$0.0^{a}_{\pm 0,0}$	$0.0^{a}_{\pm 0,0}$	$0.2^{b}_{\pm 0,2}$	$0.0^{b}_{\pm 0,0}$
	24	$0.0^{a}_{\pm 0,0}$	$0.0^{a}_{\pm 0,0}$	$1.0^{bc}_{\pm 0,5}$	$1.4^{ad}_{\pm 0,1}$
30	48	$0.0^{a}_{\pm 0,0}$	$0.0^{a}_{\pm 0,0}$	$0.0^{b}_{\pm 0,0}$	$0.3^{bd}_{\pm 0,3}$
	72	$0.0^{a}_{\pm 0,0}$	$0.0^{a}_{\pm 0,0}$	$0.0^{b}_{\pm 0,0}$	$0.0^{b}_{\pm 0,0}$
	Methanol				
	24	$1.5^{b}_{\pm 0,4}$	$0.0^{a}_{\pm 0,0}$	$0.7^{b}_{\pm 0,7}$	$0.0^{b}_{\pm 0,0}$
15	48	$1.9^{b}_{\pm 0,3}$	$1.3^{a}_{\pm 0,8}$	$1.3^{b}_{\pm 0,7}$	$0.9^{a}_{\pm 0,5}$
	72	$2.1^{b}_{\pm 0,7}$	$1.4^{a}_{\pm 0,9}$	$1.1^{bc}_{\pm 0,7}$	$1.0^{a}_{\pm 1,0}$
	24	$0.9^{ab}_{\pm 0,9}$	$1.2^{a}_{\pm 0,6}$	$2.0^{a}_{\pm 0,1}$	$0.0^{b}_{\pm 0,0}$
30	48	$1.5^{b}_{\pm 0,2}$	$1.7^{a}_{\pm 0,9}$	$3.8^{a}_{\pm 0,6}$	$1.9^{a}_{\pm 0,2}$
	72	$0.0^{a}_{\pm 0,0}$	$2.4^{a}_{\pm 1,7}$	$2.7^{ac}_{\pm 0,6}$	$1.5^{ac}_{\pm 0,2}$
	Aqueous				
	24	$0.0^{a}_{\pm 0,0}$	$0.0^{a}_{\pm 0,0}$	$0.0^{b}_{\pm 0,0}$	$0.0^{b}_{\pm 0,0}$
15	48	$0.0^{a}_{\pm 0,0}$	$0.0^{a}_{\pm 0,0}$	$0.0^{b}_{\pm 0,0}$	$0.0^{b}_{\pm 0,0}$
	72	$0.0^{a}_{\pm 0,0}$	$0.5^{a}_{\pm 0,5}$	$0.0^{b}_{\pm 0,0}$	$0.0^{b}_{\pm 0,0}$
	24	$0.0^{a}_{\pm 0,0}$	$0.0^{a}_{\pm 0,0}$	$0.0^{b}_{\pm 0,0}$	$0.0^{b}_{\pm 0,0}$
30	48	$0.0^{a}_{\pm 0,0}$	$0.0^{a}_{\pm 0,0}$	$0.0^{b}_{\pm 0,0}$	$0.0^{b}_{\pm 0,0}$
	72	$0.0^{a}_{\pm 0,0}$	$0.0^{a}_{\pm 0,0}$	$0.0^{b}_{\pm 0,0}$	$0.0^{b}_{\pm 0,0}$
	A	10.1±0.1	12.0±0.4	4.3±0.8	7.7±1.1
	F	-	-	-	

aqueous extracts of *S. europaea* against bacteria and yeasts were summarized in fig. 2. The highest mean inhibition zone diameter was found to be 3.8 mm for methanol extracts of 300 mg/ml prepared at 48 h incubation against *B. subtilis* followed by methanol extracts of 300 mg/ml prepared at 24 h incubation against *C. albicans* (2.5 mm) (Table 4 and Table 5).

MIC values of ethanol, methanol, acetone and aqueous extracts against all pathogens ranged from 8, 35 to 4671, 03 mg/ml. The highest MIC value was observed against *E. coli* in methanol extract of 300 mg/ml prepared at 72 h. The MIC values of ethanol, methanol, acetone and aqueous extracts after 24 h incubation with microorganisms are summarized in Table 6 ($p<0.05$).

The antimicrobial activity of the ethanol extract of 300 mg/ml prepared at 72 h against *B. subtilis* was found to be significantly different ($p<0.05$) from distilled water extract of 300 mg/ml prepared at 48 h. the methanol extract of 300 mg/ml prepared at prepared at 24 h against *C. albicans* and aqueous extract of 300 mg/ml prepared at 48 h against *C. parapsilosis* were found to be significantly different ($p<0.05$) from other extracts.

The extracts mostly below 100 µl showed antimicrobial activity against microorganisms. The highest MIC value was found as 8.35 µl for methanol extract of 300 mg/ml prepared at 72 h against *E. coli* followed by acetone extract of 300 mg/ml prepared at 72 h against *E. coli* (MIC=8.75 mg/ml), acetone extract of 300 mg/ml prepared at 72 h against *K. pneumoniae* (MIC=9.11 mg/ml), ethanol extract of 300 mg/ml prepared at 24 h against *S. aureus* (MIC=9.57 mg/ml), acetone extract of 300 mg/ml prepared at 48 h against *C. parapsilosis* (MIC=9.64 mg/ml) (Table 7).

The aim of this study is to investigate the chemical and physical composition and antimicrobial activity

TABLE 5: INHIBITION ZONE DIAMETERS OF *S. europaea* EXTRACTS WITH *C. albicans* VE *C. parapsilosis* ACCORDING TO THE DISC DIFFUSION METHOD.

	Extracts (h)	Inhibition Zone (mm)+SEM*	
Weight (g)	acetone	*C. albicans*	*C. parapsilosis*
15	24	$2.0^{a}_{\pm 1,0}$	$0.0^{b}_{\pm 0,0}$
	48	$0.8^{a}_{\pm 0,7}$	$0.0^{b}_{\pm 0,0}$
	72	$2.2^{a}_{\pm 1,1}$	$0.0^{b}_{\pm 0,0}$
30	24	$0.2^{a}_{\pm 0,1}$	$0.1^{a}_{\pm 0,0}$
	48	$0.0^{a}_{\pm 0,0}$	$0.1^{a}_{\pm 0,0}$
	72	$0.1^{a}_{\pm 0,1}$	$0.0^{b}_{\pm 0,0}$
	ethanol		
15	24	$1.4^{a}_{\pm 1,3}$	$0.0^{b}_{\pm 0,0}$
	48	$1.1^{a}_{\pm 0,6}$	$0.0^{b}_{\pm 0,0}$
	72	$1.4^{a}_{\pm 0,5}$	$0.0^{b}_{\pm 0,0}$
30	24	$1.0^{a}_{\pm 0,8}$	$0.0^{b}_{\pm 0,0}$
	48	$2.8^{a}_{\pm 0,5}$	$0.0^{b}_{\pm 0,0}$
	72	$1.5^{a}_{\pm 1,3}$	$0.0^{b}_{\pm 0,0}$
	methanol		
15	24	$0.0^{a}_{\pm 0,0}$	$0.0^{b}_{\pm 0,0}$
	48	$0.0^{a}_{\pm 0,0}$	$0.0^{b}_{\pm 0,0}$
	72	$0.0^{a}_{\pm 0,0}$	$0.0^{b}_{\pm 0,0}$
30	24	$2.5^{a}_{\pm 0,9}$	$0.0^{b}_{\pm 0,0}$
	48	$2.3^{a}_{\pm 2,3}$	$0.0^{b}_{\pm 0,0}$
	72	$0.7^{a}_{\pm 0,7}$	$0.0^{b}_{\pm 0,0}$
	aqueous		
15	24	$0.0^{a}_{\pm 0,0}$	$0.0^{b}_{\pm 0,0}$
	48	$0.0^{a}_{\pm 0,0}$	$0.0^{b}_{\pm 0,0}$
	72	$0.0^{a}_{\pm 0,0}$	$0.0^{b}_{\pm 0,0}$
30	24	$0.4^{a}_{\pm 0,4}$	$0.0^{b}_{\pm 0,0}$
	48	$0.0^{a}_{\pm 0,0}$	$0.0^{b}_{\pm 0,0}$
	72	$0.0^{a}_{\pm 0,0}$	$0.0^{b}_{\pm 0,0}$
	A	-	-
	F	$1.1\pm_{0.18}$	$2.4\pm_{0.1}$

of *S. europaea* for pharmaceuticals and cosmeceutical applications. Therefore, GC-MS, ICP/MS and LC/MS-MS analyzes are performed to determine its chemical composition, while 4 bacteria *E. coli, K. pneumonia, B. subtilis, S. aureus* and 2 fungi (*C. albicans, C. parapsilosis*) are used to determine antimicrobial and antifungal activation.

The hexane-extracted oil content of fresh *S. europaea* cultivated was found to be 15.15 %. Anwar *et al.*[13] investigated the analytical characterization of seed oil of *Salicornia. bigelovii* and found oil percentage to be 29.2 %. Eganathan *et al.*[14] reported 22.4 % oil in seeds of *S. brachiate* by using hexane-extracted. Anwar *et al.*[13] examined analytical characterization of *Salicornia. bigelovii* seed oil and found as 13.42 % which is lower than the present study. In the moisture analysis, the humidity rate of *S. europaea* was 8 %. Min *et al.*[15] studied chemical composition of *Salicornia. herbacea* and reported moisture content as 9.09 % that is similar to the present study. The amount of Na, Mg, K, Ca minerals in the *S. europaea* were 447, 51. 98, 45.86, 14.27 ppm respectively according to ICP/MS and EDX. Austenfeld *et al.*[16] reported Ca and Mg content in the seeds of *S. europaea* to be 30 and 138 mmol respectively. Lima *et al.*[17] studied the mineral compositions of *Salicornia ramosissima* species grown at different salinity rates for Na, K, Ca, Mg found to be 8.36-17.4 mg/g, 1.41-2.23 mg/g, 0.41-1.45 mg/g and 0.17- 0.23 mg/g respectively. Bertin *et al.*[18] investigated Na, K, Mg and Ca in *Sarcocornia ambigua* collected from two different localities and reported that mineral contents of Na, K, Mg and Ca were found as 10.19 mg/g, 2. 9 mg/g, 0.92 mg/g ve 0.54 mg/g from Palhoça beach, and 16.57 mg/g, 1.81 mg/g, 1.30 mg/g ve 0.53 mg/g from Barra da Lagoa respectively. Yabalak *et al.*[19] examined mineral and trace elements from the methanolic extract of *Arum dioscoridis* sm. by using ICP-MS, and found the mineral content for Na, Mg, K and Ca as 636.0 ppm, 1301.4 ppm, 4142.0 ppm and 14406.6 ppm respectively. Yabalak *et al.*[20] studied trace element content of *O. munzurense* that Na, K, Mg, Ca concentrations were as 13.13, 128.1, 38.3 and 161.57 ppm respectively. The regional differences in the soil and plant structure are effective in the elemental composition of a plant which then may be the reason of the detected the low concentration in the present study compared to the given other studies.

There have been several studies conducted on the antimicrobial activity of seagrasses metabolites for many years[21-23]. Some seagrasses species such as *Halodule pinifolia* and *Cymodocea rotundata* have predominant antimicrobial agents against *S. aureus*, *Vibrio cholerae*, *Shigella dysenteriae*, *Salmonella paratyphi* and *Shigella boydii*[24]. Kumar *et al.*[25] reported the antibacterial properties of different extracts of *Cymodocea serrulata*, *Halophila ovalis* and *Zostera capensis* tested against some pathogens such as *S. aureus*, *Bacillus cereus*, *B. subtilis* and the best activity were seen in ethyl acetate and methanol extract. Similarly in the present study, methanol extract was more active than the others against microorganism according to disc diffusion method.

Essaidi *et al.*[26] reported that antibacterial performance of the methanolic extract of *S. herbacea* against some pathogens by disc diffusion method. While the extract of 20 mg/ml had not any activity on *E. coli* and

TABLE 6: MIC VALUES OF *S. aureus* EXTRACTS INCUBATED SEPARATELY WITH *E. Coli*, *K. pneumoniae*, *B. subtilis and S. aureus* FOR 24 H ACCORDING TO SPECTROPHOTOMETRIC MICRODILUTION METHOD

Extracts (h)		MIC(mg/ml)+SEM*			
Weight (g)	acetone	*E. coli*	*K. pneumoniae*	*B. subtilis*	*S. aureus*
	24	$14.15^{a}_{\pm 1,94}$	$94.69^{ab}_{\pm 3,2}$	$12.98^{ab} \pm_{2,4}$	$616.37^{a}_{\pm 594,1}$
15	48	$197.88^{a}_{\pm 187,1}$	$24.43^{b}_{\pm 13,4}$	$23.98^{ab} \pm_{2,4}$	$14.10^{a}_{\pm 0,5}$
	72	$13.01^{a}_{\pm 0,96}$	$16.48^{b}_{\pm 7,5}$	$22.92^{ab} \pm_{5,4}$	$24.70^{a}_{\pm 8,8}$
	24	$13.94^{a}_{\pm 2,2}$	$13.61^{b}_{\pm 2,7}$	$14.79^{ab} \pm_{2,7}$	$79.83^{a}_{\pm 67,8}$
30	48	$177.98^{a}_{\pm 165,6}$	$36.59^{b}_{\pm 22,5}$	$16.63^{ab} \pm_{2,5}$	$38.77^{a}_{\pm 22,3}$
	72	$8.75^{a}_{\pm 0,2}$	$9.11^{b}_{\pm 0,2}$	$11.83^{ab} \pm_{0,3}$	$12.98^{a}_{\pm 1,0}$
	ethanol				
	24	$12.68^{a}_{\pm 0,6}$	$10.42^{b}_{\pm 0,1}$	$19.90^{ab} \pm_{9,8}$	$27.94^{a}_{\pm 2,8}$
15	48	$12.08^{a}_{\pm 0,2}$	$10.82^{b}_{\pm 0,5}$	$11.30^{ab} \pm_{0,9}$	$25.34^{a}_{\pm 7,8}$
	72	$11.17^{a}_{\pm 0,9}$	$11.00^{b}_{\pm 1,8}$	$10.83^{a} \pm_{0,4}$	$24.81^{a}_{\pm 7,9}$
	24	$23.90^{a}_{\pm 2,5}$	$13.83^{b}_{\pm 2,3}$	$19.90^{ab} \pm_{10,5}$	$9.57^{a}_{\pm 0,7}$
30	48	$15.96^{a}_{\pm 2,4}$	$15.15^{b}_{\pm 4,2}$	$12.76^{ab} \pm_{1,9}$	$13.15^{a}_{\pm 0,5}$
	72	$22.29^{a}_{\pm 3,7}$	$33.66^{b}_{\pm 15,6}$	$13.16^{ab} \pm_{3,3}$	$19.33^{a}_{\pm 6,0}$
	methanol				
	24	$337.71^{a}_{\pm 317,4}$	$12.96^{b}_{\pm 1,4}$	$42.62^{ab}_{\pm 12,6}$	$41.73^{a}_{\pm 18,1}$
15	48	$22.57^{a}_{\pm 0,6}$	$11.78^{b}_{\pm 0,4}$	$18.77^{ab}_{\pm 4,8}$	$20.15^{a}_{\pm 2,6}$
	72	$8.35^{a}_{\pm 0,15}$	$11.56^{b}_{\pm 0,3}$	$14.21^{ab}_{\pm 0,28}$	$47.69^{a}_{\pm 33,4}$
	24	$19.38^{a}_{\pm 1,7}$	$40.84^{b}_{\pm 9,2}$	$12.66^{ab}_{\pm 0,5}$	$35.48^{a}_{\pm 13,7}$
30	48	$67.33^{a}_{\pm 23,5}$	$151.06^{a}_{\pm 83,5}$	$16.7^{ab}_{\pm 2,6}$	$27.75^{a}_{\pm 7,41}$
	72	$13.80^{a}_{\pm 2,2}$	$36.32_{\pm 6,2}$	$22.10^{ab}_{\pm 6,8}$	$25.14^{a}_{\pm 2,4}$
	aqueous				
	24	$10.96^{a}_{\pm 0,9}$	$15.84^{b}_{\pm 1,6}$	$29.60^{ab}_{\pm 6,68}$	$47.06^{a}_{\pm 20,08}$
15	48	$10.36^{a}_{\pm 0,2}$	$43.77^{b}_{\pm 16,43}$	$49.52^{b}_{\pm 24,6}$	$20.43^{a}_{\pm 4,61}$
	72	$10.60^{a}_{\pm 0,03}$	$15.60^{b}_{\pm 1,7}$	$12.73^{ab}_{\pm 0,1}$	$75.47^{a}_{\pm 34,8}$
	24	$63.42^{a}_{\pm 26,8}$	$20.55^{b}_{\pm 6,5}$	$18.69^{ab}_{\pm 4,02}$	$24.59^{a}_{\pm 12,5}$
30	48	$11.67^{a}_{\pm,006}$	$11.31^{b}_{\pm 0,4}$	$29.21^{ab}_{\pm 4,09}$	$21.56^{a}_{\pm 10,4}$
	72	$17.11^{a}_{\pm 3,66}$	$13.32^{b}_{\pm 0,8}$	$23.19^{ab}_{\pm 1,4}$	$14.5^{a}_{\pm 1,1}$
	A	16 ±0.06	16 ±0.1	32 ±0.4	32 ±0.3
	F	-	-	-	-

*SEM: Standard error of the mean. Mean MICs, standard deviation (±) and significance level were expressed by Tukey test (ANOVA, P <0.05). In the same column, values marked with different exponential letters differ statistically at 0.05 level. A: Ampicillin (16 µg/mL), F: Fluconazole (1280 µg/mL)

K. pneumoniae after 24 h incubation, the extract of 100 mg/ml inhibited them between 6 and 10 mm zone[26]. In our study, we reported that methanolic extract of 300 mg/ml had 1.5 and 0.0 mm zone against *E. coli* and *K. pneumoniae*. Santhanakrishnan *et al.* [27] reported that the MIC values of methanolic extract of *S. brachiata* against *B. subtilis* and *K. pneumoniae* were 25 and 50 mg/ml, respectively. The MIC values of *S. europaea* as 12.96 mg/ml and 42.62 mg/ml for *B. subtilis* and *K. pneumonia* were relatively lower.

Rahmani and Heydarian[28] indicated that the ethanolic extract (70 %) of *Salicornia iranica* had no antifungal activity against *Aspergillus niger* and *Candida albicans* at the end of the at 25° for 72 h incubation. The negative effect was related to the lack of fatty acid methyl esters in ethanolic extract[28]. However, we determined the various inhibition zones of *S. europaea* ethanol extract against *C. albicans* at 24, 48 and 72 h incubations. For the concentration of 300 mg/ml, the minimum zone (1.0 mm) was at 24 h incubations while the maximum zone (2.8 mm) was at 48 h incubations which indicate that Salicornia species have different contents of ethanol extracts. In another study, the ethanol extract of *S. herbacea* seed inhibited *S. aureus* with MIC of 189.5 mg/ml and *E. coli* 420 mg/ml[29]. Whereas, we showed that maximum inhibition concentration was related to *S. aureus* with MIC of 9.57 mg/ml at 24 h incubation and *E. coli* with MIC of 11.17 mg/ml at 72 h incubation, respectively.

In summary, the present results revealed bioactive compounds of *S. europaea*, especially its richness in oil and oleic acid. Besides, *S. europaea* extracts

TABLE 7: MIC VALUES OF *S. EUROPAEA* EXTRACTS INCUBATED SEPARATELY WITH *C. albicansve, C. parapsilosis* FOR 24 H ACCORDING TO SPECTROPHOTOMETRIC MICRODILUTION METHOD

	Extracts (h)	MIC(mg/mL)+SEM*	
Weight (g)	acetone	*C. albicans*	*C. parapsilosis*
15	24	$10.32^{b}_{\pm 0,1}$	$15.64^{b}_{\pm 1,8}$
	48	$14.84^{b}_{\pm 1,9}$	$9.64^{b}_{\pm 0,04}$
	72	$30.76^{b}_{\pm 2,4}$	$15.00^{b}_{\pm 2,8}$
30	24	$21.00^{b}_{\pm 5,0}$	$18.04^{b}_{\pm 0,2}$
	48	$366.09^{b}_{\pm 86,6}$	$15.36^{b}_{\pm 0,6}$
	72	$30.05^{b}_{\pm 13,7}$	$41.53^{b}_{\pm 2,3}$
	ethanol		
15	24	$58.24^{b}_{\pm 47,8}$	$19.24^{b}_{\pm 4,1}$
	48	$15.25^{b}_{\pm 2,6}$	$17.05^{b}_{\pm 2,7}$
	72	$15.59^{b}_{\pm 5,06}$	$20.12^{b}_{\pm 2,5}$
30	24	$24.71^{b}_{\pm 3,30}$	$18.38^{b}_{\pm 5,04}$
	48	$16.40^{b}_{\pm 0,37}$	$17.14^{b}_{\pm 1,4}$
	72	$11.30^{b}_{\pm 0,33}$	$13.28^{b}_{\pm 0,06}$
	methanol		
15	24	$4671.03^{a}_{\pm 33792}$	$68.18b_{\pm 5,5}$
	48	$18.71^{b}_{\pm 0,3}$	$41.30^{b}_{\pm 18,58}$
	72	$16.80^{b}_{\pm 3,1}$	$22.16^{b}_{\pm 1,7}$
30	24	$17.32^{b}_{\pm 1,45706}$	$22.30^{b}_{\pm 7,01}$
	48	$152.25^{b}_{\pm 45,01}$	$83.95^{b}_{\pm 58,8}$
	72	$11.13^{b}_{\pm,14}$	$22.78^{b}_{\pm 1,8}$
	aqueous		
15	24	$25.82^{b}_{\pm 10,9}$	$25.85^{b}_{\pm 7,5}$
	48	$18.38^{b}_{\pm 6,2}$	$623.10^{a}_{\pm 307,2}$
	72	$1576.49^{b}_{\pm 15231}$	$74.63^{b}_{\pm 31,5}$
30	24	$20.21^{b}_{\pm 3,3}$	$21.15^{b}_{\pm 0,3}$
	48	$21.13^{b}_{\pm 1,9}$	$15.26^{b}_{\pm 4,2}$
	72	$121.76^{b}_{\pm 101,9}$	$13.89^{b}_{\pm 2,6}$
	A	-	-
	F	128±3.5	128±0.5

*SEM: Standard error of the mean. Mean MICs, standard deviation (±) and significance level were expressed by Tukey test (ANOVA, P <0.05). In the same column, values marked with different exponential letters differ statistically at 0.05 level. A: Ampicillin (16 µg/mL), F: Fluconazole (1280 µg/mL)

prepared with ethanol, methanol, acetone and aqueous exhibit a potent antimicrobial and antifungal effect against several pathogenic microorganisms. Especially, the methanol extract showed a high effect against pathogens. Therefore, extract and oil of *S. europaea* can be a new source of bioactive compounds for pharmaceuticals and cosmeceutical applications.

Acknowledgments:

This is a part of S. Karan's PhD Thesis. Thanks to the Scientific & Technological Research Council of Turkey (TUBITAK-2211/C National PhD Scholarship Program for Priority Areas) for support. Part of the study is presented in the International Cosmetic Congress "Green Cosmetics" 28-30 October 2018, Antalya/ Turkey.

Research funding:

We are grateful to the Iskenderun Technical University Scientific Research Projects Office for their financial support (Project No. 2020LTP-01).

Author contribution:

All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

Conflict of Interest:

No conflict of interest declared.

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