Original Article

Investigation of Biological, Scolicidal and Antimicrobial Activities of the Solvent Extracts Components of *Cystoseria barbata* Seen off Sinop Province

Ulku Karaman[1,*], Tayyibe Beyza Yucel[2], Tugba Cebeci[3], Yeliz Kasko Arici[4], Murat Yucel[5], Busra Korkmaz[6]

[1]Department of Medical Parasitology, Faculty of Medicine, Ordu University, Ordu, TURKEY.
[2]Department of Medical Services and Techniques, Vocational School of Health Services, Giresun University, Giresun, TURKEY.
[3]Department of Medical Services and Techniques, Vocational School of Espiye, Giresun University, Giresun, TURKEY.
[4]Department of Biostatistics and Medical Informatics, Faculty of Medicine, Ordu University, Ordu, TURKEY.
[5]Department of Basic Sciences, Faculty of Fisheries, Sinop University, Sinop, TURKEY.
[6]Department of Pharmacognosy, Faculty of Pharmacy, Karadeniz Technical University, Trabzon, TURKEY.

ABSTRACT

Aim/Background: Seaweeds are unicellular or multicellular vegetative organisms and have a wide distribution. Their antitumoral, antiviral, antifungal, insecticidal, cytotoxic, and phytotoxic and antiproliferative activities were determined. The aim of the study was to investigate the biological, antimicrobial and scolicidal activities of *Cystoseria barbata* (*C. barbata*). **Materials and Methods:** *Cystoseria barbarata* was collected in appropriate quantities. Some of them were extracted by water vapor distillation, and some of them were extracted by Soxhlet extraction with hexane, dichloromethane, chloroform and methanol. Antimicrobial and scolicidal effects were investigated in the extracts. For the scolicidal effect, the difference between the hours at each dose for each solution and the difference between the doses at each hour were compared by ANOVA. The values of lethal concentration doses (LD_{50} and LD_{90}) were calculated using probit analysis. **Results:** The antimicrobial activities of the solutions of the solvent extracts of *Cystoseria barbarata* in DMSO against *Acinebacter baumannii ATCC BAA-747, Bacillus cereus ATCC 10876, Bacillus megaterium DSM32, Bacillus subtilis ATCC 6633, Candida albicans ATCC 10231, Citrobacter freundii ATCC 43864, Enterococcus faecalis ATCC 29212, Escherichia coli ATCC 25922, Escherichia coli ATCC 36218, Enterobacter aerogenes ATCC 13048, Klebsiella pneumoniae ATCC 13883,* Methicillin Resistant *Staphylococcus aureus ATCC 67101, Proteus mirabilis ATCC 4307, Pseudomonas aeruginosa ATCC 27853, Pseudomonas aeruginosa ATCC 9027, Salmonella typhimurium ATCC 14028, Staphylococcus aureus ATCC 29213, Staphylococcus aureus ATCC 25923* and *Staphylococcus epidermidis ATCC 12228* micro-organisms were determined. In the scolicidal effect, no change was observed in the administration of hexane extracts with respect to the duration. However, a statistically significant change was observed in the number of parasites in terms of time in others. **Conclusion:** It was determined that the solvent extracts of *C. barbarata* may have bactericidal and scolicidal effects. It is suggested that experimental studies required to investigate the effects of this algae and use it in living cells should be conducted.

Key words: *Cystoseria barbarata,* Scolicidal, Antimicrobial activity, Lethal dose, Probit analysis.

Submission Date: 12-10-2021;
Revision Date: 29-12-2021;
Accepted Date: 04-03-2022.

DOI: 10.5530/ijper.56.2s.102

Correspondence:
Assoc. Prof. Dr. Ülkü Karaman
Department of Medical Parasitology,Faculty of Medicine, Ordu University Cumhuriyet Yerleskesi Cumhuriyet Mahallesi-52200, Ordu, TURKEY
E-mail: ulkukaraman44@hotmail.com

INTRODUCTION

In the world, there are approximately 9000 species of seaweed as microalgae or macroalgae vegatative organisms. These are classified according to their pigmentation as brown (Phaeophyta), red (Rhodophyta) and green (Chlorophyta).[1]

www.ijper.org

There are bioactive components like secondary metabolites, diterpene, bromine-bound sesquiterpenes, polyphenols, flavonoids, minerals, polysaccharides, polyunsaturated fatty acids, vitamins etc. in the structure of seaweed. These components are used in the production of soothing, muscle relaxant and edema remover, human and animal food, agricultural fertilizer and polysaccharides such as agar, alginate and in the production of biofuels (such as ethanol, butanol and biogas).[2-4]

Essential oils produced by plants or herbal sources are a mixture of aromatic substances that can easily evaporate in room conditions. Essential oils, which are abundantly found in parts such as root, stem, leaf, fruit, shell and flower, are liquid at room temperature and can easily crystallize. Studies on the anticancer,[5] antileukemic,[6] antioxidant,[7,8] antimicrobial,[9] antifeedant,[10] antifouling,[11] antimalarial,[12] and cytotoxic,[13,14] activities of essential oil components of algae are available in the literature.

Brown algae are rich in polyphenols that have anti-oxidant properties, and they are an algae class that has a wide range of uses in the rubber industry, paints, ice cream, and plastic freezers due to their alginate content.[15,16] Most of these algae are found in seas. However, less than 1% can also live in freshwater environments.[17]

Hydatid cyst is endemic in countries in the temperate climate zone and is a zoonotic infection that settles in the liver and other organs in humans and causes serious complications unless treated. The first choice in treatment is surgery. In the surgical treatment of the disease, many protoscolocidal agents have been used for years to kill live protoscoleces in the cyst. Agents such as 20% or 30% hypertonic saline, 95% ethanol, and 0.5% cetrimide are used as scolicidal agents. In this process, the cyst content is aspirated, and scolicidal agents are given into it, kept for 10-15 min and aspirated again in percutaneous procedures in cases of hydatid cysts with suitable localization.[18] Studies have reported that percutaneous interventions are safe and effective.[19,20] The scolicidal effect of brown algae has not been investigated according to the source literature.

The scolocidal effect of brown algae, which has antioxidant properties, has not been investigated in the source information reached. In this direction, considering that there may be regional differences in this study, it was aimed to determine the biological activities of extracts of seaweed in organic solvents (chloroform, dichloromethane, hexane and methanol).

MATERIALS AND METHODS

Collection of Samples and Obtaining Their Extracts

barbata was collected from Akliman and center shipyard locations in Sinop province in November. In order to obtain essential oil from the algae sample, approximately 200 g of algae were collected from a 2-3 m off the shore. The collected algae were placed in polyethylene bottles and brought to the laboratory under the cold chain. Species were determined by analyzing the samples brought to the laboratory. The samples were kept at room temperature and dried. Essential oil was obtained with the Clevenger apparatus. Sixty gr of algae was weighed and put into a 2L balloon, distilled water was added on it, and extraction was performed for 4 hr with a Clevenger apparatus connected to a cooling bath (-12°C). A soxhlet device was used to obtain the extract. 30 g of algae was weighed and placed in the Soxhlet condenser accordingly. 250 mL of chloroform, methanol, dichloromethane and hexane were added into the balloon and Soxhlet extraction was performed for 24 hr. The solutions obtained at the end of the period were passed through the evaporator until dryness was obtained and the residue was weighed and stored at +4°C until antimicrobial and scolocidal activity analyses. All solvents used were 98%-95% pure and were Sigma Aldrich brand. Extractions were prepared with chloroform, methanol, dichloromethane and hexane solvents.

Analysis of Components

The retention indices (RI) of the components were determined by Kovats method using n-alkanes (C6-C30) as standard. Volatile components were identified by comparing their retention indices and mass spectra to existing analytical standards (limonene, linalool, α-terpineol, geraniol, tridecane, tetradecane, pentadecane, nonadecane, eicosan, heneicosan, docosan, trichosane, tetracosan and pentacosan) and mass spectra found in libraries. FFNSC1.2, W9N11 and NIST results were verified by comparing them with RI values in the literature.

Micro-organisms Used

A total of 18 bacterial species including gram positive *Bacillus (B). subtilis ATCC 6633, Bacillus cereus ATCC 10876, Bacillus megaterium DSM32, Staphylococcus (S.) aureus ATCC 29213, Staphylococcus aureus ATCC 25923, Enterococcus faecalis ATCC 29212, Methicillin Resistant Staphylococcus aureus (MRSA) ATCC 67101, Staphylococcus epidermidis ATCC 12228* and gram negative *Escherichia (E.) coli ATCC 25922, Escherichia coli ATCC 36218, Pseudomonas (P.) aeruginosa ATCC 27853, Pseudomonas*

aeruginosa ATCC 9027, Klebsiella (K.) pneumoniae ATCC 13883, Acinetobacter (A.) baumannii ATCC BAA-747, Enterobacter aerogenes ATCC 13048, Citrobacter freundii ATCC 43864, Salmonella typhimurium ATCC 14028 and *Proteus mirabilis ATCC 43071* for antimicrobial activity in the study. *Candida albicans (ATCC 10231)* strain was used for antifungal activity. Antimicrobial activity was also performed by the disk diffusion method as described by Demirel *et al*.[21]

Viability determination of protoscoleceses obtained from cow liver hydatid cyst was realized using 0.1% eosin solution.

Experimental Procedure

The solution was prepared as a final concentration of 15000µg / ml dissolved in sterile saline for the scolicidal effect in the study. After the solution was thoroughly homogenized, the membrane was sterilized by filtering. 100 µl of sterile saline was added to each well of the sterile 96-well microplate. Later, 100 µl of *C. barbata* extracts were added to the first wells and noted next to it. 100 µl was drawn from the first well and added to the second well, thus diluting the compound at a ratio of 1/2. This process was repeated in 8 wells and the resulting mixture was expelled. After serial dilution, 100 µl of protoscoleces with a concentration of 4500 units / ml was added to the wells. Wells 9 and 10 were left empty. As a control, only 100µl protoscoleces of a known dilution were added to these wells. Viability rates were determined by counting the viability rates of protoscoleces at 3., 4., 6., 8., 10., 12., 15., 17., 19., 22., 28. And 32 hr. Each count was made twice and averaged. In the study, 30% NaCl solution, which is widely used routinely, was used as a scolicidal agent. Its dilution and concentration of added parasites were performed by a similar method.

Microorganisms to be tested for antimicrobial effect were added to Müller Hinton Broth, yeasts were added to Sabouraud Dextrose Broth, they were left to incubation at 37°C for 18 hr, and turbidity measurements were performed with a densitometer according to Mc Farland No: 0.5 at the end of incubation. In a sterile cabinet, 100 µl of liquid media adjusted to Mc Farland No: 0.5 was added to all Mueller Hinton Agar (LAB039) and Sabouraud Dextrose Agar (LAB009) media and was spread evenly over the entire petri dish with sterile swab. 15 and 30 µL of extracts prepared in different solvents were absorbed into 6 mm sterile empty discs. The discs prepared in this way were placed in petri dishes prepared by spreading. The prepared petri dishes were left for 24 hr at 37°C incubation. After 24 hr, the zone diameters formed around the discs were measured and the values were recorded in the tables in mm. Standard antibiotic discs of Tobramycin (Bioanalyse, 10µg / disc) and Nystatin (Bioanaly, 30µg / disc) were used as a control in the study.

Statistical Analysis

Descriptive statistics of the data set were expressed as mean±standard deviation (SD). The differences between means were compared by one-way ANOVA followed by Tukey's post-hoc test. The values of lethal doses (LD_{50} and LD_{90}) were determined using probit analysis for the certain times. A $p \leq 0.05$ value was considered statistically significant. Data analyses were performed using the IBM SPSS (version 26, IBM Inc., Chicago, IL, USA) and Minitab (version 19, Minitab Inc., State College, PA, USA) statistical software.

RESULTS

The amount of oil obtained as a result of water vapor distillation of *C. barbata* was weighed and found to be 27.1 mg. From the essential oils given to GC-MS device, 17 compounds were identified and 99.81% of the essential oil was identified. The main constituents were identified as aldehyde at 51.91% and terpene and terpenoid compounds at 32.37% (Table 1). Bitki ekstrelerinin de ana materyallerinin protein, ham yağ, karbonhidrat, kül, Na aljinat, şeker, alginic acid, amgnezyum, kadmiyum, fosfor ve arsenic içerdiğine dair araştırmalar bulunmaktadır.[22,23]

The scolicidal effects of *C. barbata*

The amoebicidal effect of the hexane extract of *C. barbata* prepared in different concentrations on protoscoleces at different hours was shown in Figure 1 and Table 2.

When Figure 1 and Table 2 were examined, it was seen that the viability rate decreased in all time periods except the control group as the dose increased.

As seen in Table 2, protoscoleces vitality rate in the control group did not differ significantly according to the holding time ($p > 0.05$). Similarly, protoscoleces viability did not show any change with respect to the holding times at 117 µg / ml, 234 µg / ml and 3750 µg / ml doses ($p > 0.05$). At other doses, a statistically significant change was observed in the number of parasites in terms of time. When the administration doses were compared in each holding period, it was determined that the vitality rates showed a significant change with respect to the doses in all holding periods. 3h and 19h parasite viabilities up to 7500 µg / ml dose did not differ significantly with the control ($p > 0.05$). At a dose of 15000 µg / ml, protoscoleces viability was significantly reduced compared to control. The viability rates started

Table 1: The essential oil analysis of *C. barbata*.

No	Retention Indices	Compound Name	*C. barbata* % Area	Experimental RI	Literature RI
1	8.593	Hexanal	27.117	801	803
2	10.162	3Z-Hexenol	-	-	858
3	11.710	Heptanal	4.653	904	903
4	14.724	1-octene-3-ol	1.542	975	978
5	15.052	6-methyl-5 heptene-2-on	-	-	981
6	15.305	Furan-2-pentyl	-	-	993
7	15.621	(*2E,4E*)-Heptadienal	8.707	1008	1012
8	17.262	Melonal	2.216	1047	1045
9	17.646	Benzene acetaldehyde	-	-	1052
10	19.672	3,5-octadiene-2-on	1.958	1093	1096
11	20.055	Nonanal	4.141	1102	1101
12	25.311	β-cyclocitral	4.698	1222	1220
13	26.975	2E-decen-1-ol	3.824	1264	1268
14	33.655	α-Ionon	1.132	1430	1426
15	34.408	Geranyl acetone	3.501	1452	1453
16	35.766	1-Pentadecene	-	-	1489
17	35.898	Trans-β-Ionon	15.910	1488	1487
18	36.040	Pentadecane	3.959	1500	1500
19	36.551	Tridecanal	2.339	1505	1509
20	40.008	Hexadecane	-	-	1600
21	41.215	Benzophenon	-	-	1626
22	43.177	Heptadecane	4.227	1701	1700
23	43.750	Pentadecanal	2.741	1709	1710
24	43.963	Pentadecanone	-	-	1710
25	45.383	Tetradecanoic acid	-	-	1763
26	48.017	Hexahydrofarnesyl acetone	7.135	1848	1848
27	51.778	n-Hexadecanoic acid	-	-	1966
28	56.296	Phytol	-	-	2110
		Total	%99.81		

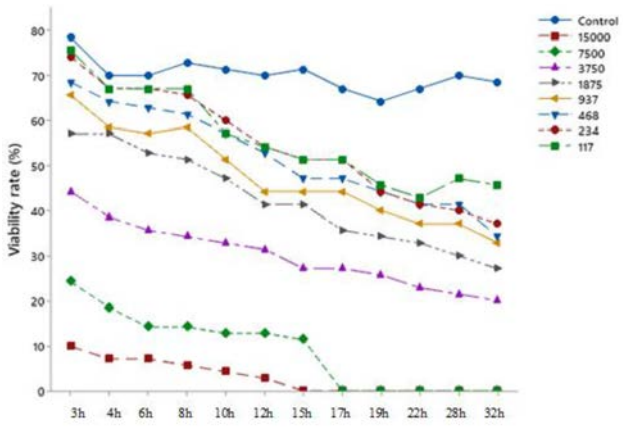


Figure 1: The scolicidal effect of the hexane extract of *C. barbata* prepared in different concentrations on protoscoleces at different hours.

to decrease significantly in 4 hr, 6 hr, 10 hr and 12 hr holding times at 3750 µg / ml dose, in 8h, 15 hr and 17 hr holding times at 1875 µg / ml dose and in 22 hr, 28 hr and 32 hr holding times at 234 µg / ml dose.

The difference between the exposure times and the doses of the plant extract in terms of parasite viability was found to be statistically significant in determining the scolicidal effect. Although this situation varied according to the extract doses, it can be interpreted that the viability rate of the parasite decreased as the dose increased. Again, a decrease in the viability of the parasite was observed depending on the exposure time. The 15000 µg / ml concentration of the hexane extract of *C. barbata* showed a very strong scolicidal effect and no live parasites were observed at the 15th hr (Table 2,

Table 2: The effect of hexane extract of *C. barbata* at different concentrations on protoscoleces according to hours.

h	15000 µg/ml	7500 µg/ml	3750 µg/ml	1875 µg/ml	937 µg/ml	468 µg/ml	234 µg/ml	117 µg/ml	Control	*p*
3	10.00^{Ac}	24.29^{Abc}	44.29^{abc}	57.14^{Aabc}	65.71^{Aab}	68.57^{Aab}	74.29^{a}	75.71^{a}	78.57^{a}	**0.003****
4	7.14^{ABd}	18.57^{Acd}	38.57^{bc}	57.14^{Aab}	58.57^{ABab}	64.29^{ABa}	67.14^{a}	67.14^{a}	70.00^{a}	**0.000*****
6	7.14^{ABd}	14.29^{Acd}	35.71^{bc}	52.86^{ABab}	57.14^{ABab}	62.86^{ABa}	67.14^{a}	67.14^{a}	70.00^{a}	**0.000*****
8	5.71^{ABCd}	14.29^{Ad}	34.29^{c}	51.43^{ABCbc}	58.57^{ABab}	61.43^{ABab}	65.71^{ab}	67.14^{ab}	72.86^{a}	**0.000*****
10	4.29^{ABCc}	12.86^{ABc}	32.86^{bc}	47.14^{ABCDab}	51.43^{ABab}	57.14^{ABab}	60.00^{ab}	57.14^{ab}	71.43^{a}	**0.000*****
12	2.86^{BCd}	$12.86A^{Bcd}$	31.43^{bcd}	$41.43^{ABCDabc}$	44.29^{ABabc}	52.86^{ABab}	54.29^{ab}	54.29^{ab}	70.00^{a}	**0.000*****
15	0.00^{Cc}	11.43^{ABc}	27.14^{bc}	41.43^{ABCDb}	44.29^{ABab}	47.14^{ABab}	51.43^{ab}	51.43^{ab}	71.43^{a}	**0.000*****
17	0.00^{Cd}	0.00^{Bd}	27.14^{c}	35.71^{ABCDbc}	44.29^{ABabc}	47.14^{ABabc}	51.43^{ab}	51.43^{ab}	67.14^{a}	**0.000*****
19	0.00^{Cb}	0.00^{Bb}	25.71^{ab}	34.29^{BCDab}	40.00^{ABab}	44.29^{ABab}	44.29^{ab}	45.71^{ab}	64.29^{a}	**0.019***
22	0.00^{Cc}	0.00^{Bc}	22.86^{b}	32.86^{BCDb}	37.14^{ABb}	41.43^{ABb}	41.43^{b}	42.86^{ab}	67.14^{a}	**0.000*****
28	0.00^{Cc}	0.00^{Bc}	21.43^{bc}	30.00^{CDb}	37.14^{ABb}	41.43^{ABb}	40.00^{b}	47.14^{ab}	70.00^{a}	**0.000*****
32	0.00^{Cd}	0.00^{Bd}	20.00^{cd}	27.14^{Dbc}	32.86^{Bbc}	34.29^{Bbc}	37.14^{bc}	45.71^{ab}	68.57^{a}	**0.000*****
p	**0.000*****	**0.000*****	**0.058**	**0.001****	**0.025***	**0.024***	**0.097**	0.058	0.996	

*: <0.05; **: <0.01; ***: <0.001

Horizontally, line means that do not share a lowercase letter are significantly different ($p<0.05$). Vertically, means that do not share a capital letter are significantly different ($p<0.05$).

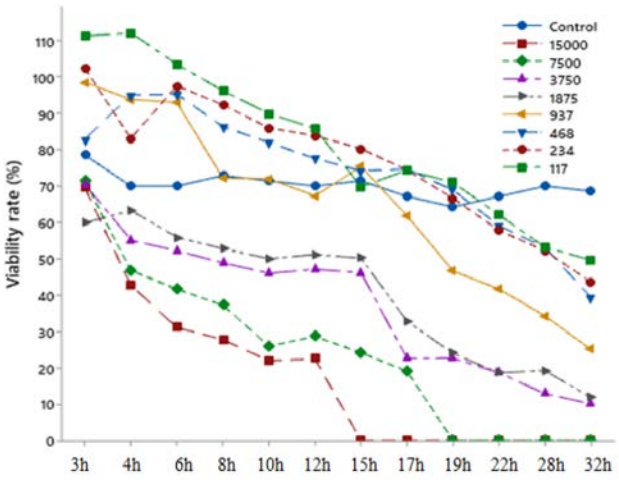


Figure 2: The scolicidal effect of the methanol extract of *C. barbata* prepared at different concentrations on protoscoleces at different times.

Figure 1). Observed at the 15th hr of the plant, LD_{50} value was 781.360 µg / ml and LD_{90} value was 7313.211 µg / ml (Table 3).

The scolicidal effect of the methanol extract of *C. barbata* on protoscoleces at different hours was given in Figure 2 and Table 4. When Figure 2 and Table 4 were examined, it was seen that the viability rate decreased as the dose increased in all periods except the control group.

According to Anova test, protoscoleces viability rate in the control group did not show a significant change with respect to the holding time as seen in Table 4

Table 3: LD_{50} ve LD_{90} values of hexane extracts of *C. barbata* against time.

Lethal Concentration Doses		
Time	**LD_{50} (95% confidence limit)**	**LD_{90} (95% confidence limit)**
3 h	4008.577 [a] (2881.349–5405.451)	12903.200 [a] (10347.817–17443.959)
4 h	2839.389 [a] (2120.855–3602.772)	11620.474 [a] (9907.554–14150.784)
6 h	2509.278 [a] (1812.474–3230.894)	10947.100 [a] (9333.642–13326.546)
8 h	2418.188 [a] (1759.802–3102.271)	10353.374 [a] (8834.447–12588.935)
10 h	1641.108 [a] (937.386–2310.039)	9723.987 [a] (8208.506–12018.002)
12 h	1010.396 [a] (245.682–1677.341)	9067.818 [a] (7583.271–11372.964)
15 h	781.360 [a] (131.709–1333.758)	7313.211 [a] (6076.983–9282.068)
17 h	641.830 [a] (183.739–1033.782)	5125.995 [a] (4265.033–6501.030)
19 h	251.881 [a] (-870.017–954.461)	5059.235 [a] (3734.855–8212.273)
22 h	97.377 [a] (-495.392–535.902)	4777.301 [a] (3921.249–6195.253)
28 h	181.195 [a] (-547.377–688.127)	4530.600 [a] (3560.648–6374.980)
32h	-117.233 [a] (-1070.470–462.392)	4307.673 [a] (3302.878–6375.428)

–, not calculated, a: µg/ml

Table 4: The effect of methanol extract of *C. barbata* at different concentrations on protoscoleces according to hours.

h	15000 µg/ml	7500 µg/ml	3750 µg/ml	1875 µg/ml	937 µg/ml	468 µg/ml	234 µg/ml	117 µg/ml	Control	*p*
3	69.64^{A}	71.43^{A}	70.36^{A}	60.00	98.57	82.86	102.50	111.43^{A}	78.57	**0.777**
4	42.75^{AB}	46.83^{AB}	55.08^{AB}	63.25	93.83	95.00	83.08	112.25^{A}	70.00	**0.208**
6	$31.14B^{Cb}$	41.67^{Bab}	52.19^{ABab}	55.89^{ab}	93.01^{ab}	95.29^{a}	97.56^{a}	103.54^{ABa}	70.00^{ab}	**0.008****
8	27.78^{BCe}	37.27^{Bde}	48.84^{ABd}	52.78^{cd}	72.22^{bc}	86.34^{ab}	92.36^{ab}	96.30^{ABa}	72.86^{bc}	**0.000*****
10	22.00^{BCd}	26.00^{BCd}	46.00^{ABcd}	50.00^{bcd}	72.00^{abc}	82.00^{ab}	86.00^{a}	90.00^{ABa}	71.43^{abc}	**0.000*****
12	22.50^{BCc}	28.67^{BCc}	47.08^{ABbc}	51.17^{bc}	67.33^{ab}	77.58^{ab}	83.83^{a}	85.92^{ABa}	70.00^{ab}	**0.000*****
15	0.00^{Cb}	24.20^{BCab}	46.15^{ABab}	50.16^{ab}	75.32^{a}	74.20^{a}	80.29^{a}	69.87^{ABa}	71.43^{a}	**0.010***
17	0.00^{Cd}	19.09^{BCcd}	22.55^{ABbcd}	32.82^{abcd}	61.82^{abc}	74.64^{a}	74.36^{a}	74.36^{ABa}	67.14^{ab}	**0.000*****
19	0.00^{Cb}	0.00^{Cb}	22.63^{ABab}	23.91^{ab}	46.64^{ab}	68.87^{a}	66.60^{a}	71.05^{ABa}	64.29^{a}	**0.002****
22	0.00^{Cc}	0.00^{Cc}	18.61^{Bbc}	18.52^{bc}	41.67^{abc}	59.07^{ab}	57.78^{ab}	62.13^{ABab}	67.14^{a}	**0.001****
28	0.00^{Cb}	0.00^{Cb}	12.79^{Bb}	19.19^{ab}	34.26^{ab}	53.03^{ab}	52.19^{ab}	53.20^{Bab}	70.00^{a}	**0.005****
32	0.00^{Cb}	0.00^{Cb}	10.00^{Bab}	11.79^{ab}	25.00^{ab}	39.29^{ab}	43.57^{ab}	49.64^{Bab}	68.57^{a}	**0.025***
p	**0.000*****	**0.000*****	**0.005****	**0.121**	**0.058**	**0.706**	**0.123**	**0.009****	**0.996**	

*: <0.05; **: <0.01; ***: <0.001

Horizontally, means that do not share a lowercase letter are significantly different ($p<0.05$). Vertically, means that do not share a capital letter are significantly different ($p<0.05$).

($p> 0.05$). Similarly, protoscoleces viability did not change in terms of holding times at 234 µg / ml, 468 µg / ml, 937 µg / ml and 1875 µg / ml doses ($p> 0.05$). In other administration doses, a statistically significant change was observed in the number of parasites with respect to time ($p <0.05$). According to Tukey test, when the administration doses were compared in each holding period, it was determined that the viability rates showed a significant change according to the doses except for the 3rd and 4th hr ($p <0.05$). In the evaluation of the concentrations compared to the control group, the viability of the protoscoleces after the 10th hr was significantly reduced at the dose of 15000 µg / ml compared to the control. At a dose of 7500 µg / ml, the viability of protoscoleces decreased significantly at 8th, 10th, 12nd, 17th, 19th, 22nd, 28th and 32nd hr compared to the control. In the evaluation between concentrations, a significant decrease in parasite viability was detected at 15000 µg / ml dose when compared to 1875 µg / ml dose at 8th hr, 937 µg / ml dose at 8th, 10th, 12th and 15th hr and 468 µg / ml and 117 µg / ml doses at 8th, 10th, 12nd, 15th, 17th, 19th and 22nd hr.

The 15000 µg / ml concentration of the methanol extract of *C. barbata* showed a very strong scolicidal effect and no live parasites were observed at the 15th hr (Table 4, Figure 2). Observed at the 15th hr of the plant, LD_{50} value was 1172.857 µg / ml and LD_{90} value was 8409.504 µg / ml (Table 5).

The scolicidal effect of the chloroform extract of *C. barbata* on protoscoleces at different hours was given in Figure 3 and Table 6. When Figure 3 and Table 6 were analyzed, it was seen that the viability rate decreases as the dose increased in all time periods except the control group.

As seen in Table 6, protoscoleces viability rate in the control group did not show a significant change with respect to the holding time ($p> 0.05$). Similarly, protoscoleces viability at the dose of 1875 µg / ml did not change according to the holding times ($p> 0.05$). In other administration doses, a statistically significant change was observed in the number of parasites in terms of time ($p <0.05$). When the administration doses were compared in each holding time, it was determined that the viability rates showed a significant change according to the doses except for the 3rd hr ($p <0.05$). Protoscoleces viability decreased significantly after the 4th hr at a dose of 15000 µg / ml compared to the control group. Similarly, a significant decrease in viability was found after 8th hr at 3750 µg / ml dose, after 10th hr with 1875 µg / ml dose, after 12th hr with 937 µg / ml and 498 µg / ml doses, and after 15th hr at 234 µg / ml and 117 µg / ml doses. When the dose was evaluated with respect to time, a significant difference was found between the 3rd hr and the 6th and later hours.

Table 5: LD_{50} ve LD_{90} values of methanol extracts of *C. barbata* against time.

Lethal Concentration Doses		
Time	**LD_{50} (95% confidence limit)**	**LD_{90} (95% confidence limit)**
3 h	–	–
4 h	4607.423[a] (1244.037–10466.526)	21612.814[a] (13756.751–61621.535)
6 h	3549.600[a] (1978.514–5294.637)	17154.363[a] (13195.221–25246.088)
8 h	3041.659[a] (1419.991–4709.445)	16615.695[a] (12740.730–24610.685)
10 h	1914.585[a] (182.958–3416.577)	14135.774[a] (10790.224–21096.103)
12 h	1358.794[a] (-325.538–2712.687)	14659.895[a] (11425.283–20933.176)
15 h	1172.857[a] (221.718–1995.102)	8409.504[a] (6634.790–11721.379)
17 h	218.137[a] (-1009.498–1033.197)	6407.168[a] (4870.545–9636.676)
19 h	5.147[a] (-929.484–537.830)	3467.523[a] (2569.355–5571.769)
22 h	-175.326[a] (-1195.739–364.989)	3246.315[a] (2399.704–5234.735)
28 h	-224.525[a] (-1208.554–270.241)	2727.980[a] (2003.996–4461.663)
32h	-464.111[a] (-2686.302–161.543)	2045.337[a] (1326.254–4873.992)

–, not calculated, a: µg/ml

The 15000 µg / ml concentration of the chloroform extract of *C. barbata* showed a very strong scolicidal effect and no live parasites were observed at the 8th hr (Table 4, Figure 3). Observed at the 8th hr of the plant, LD_{50} value was 27.626 µg / ml and LD_{90} value was 3217.174 µg / ml (Table 7).

The scolicidal effect of the dichloromethane extract of *C. barbata* on protoscoleces at different hours was given in Figure 4 and Table 8. When Figure 4 and Table 8 were analyzed, it was seen that the viability

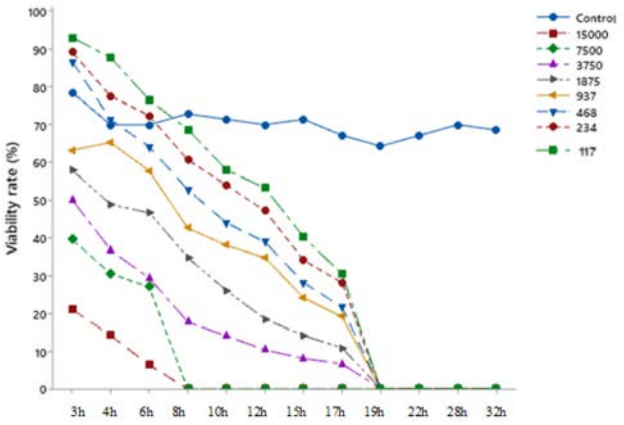


Figure 3: The scolicidal effect of the chloroform extract of *C. barbata* prepared at different concentrations on protoscoleces at different times.

Table 6: The effect of chloroform extract of *C. barbata* at different concentrations on protoscoleces according to hours.

h	15000 µg/ml	7500 µg/ml	3750 µg/ml	1875 µg/ml	937 µg/ml	468 µg/ml	234 µg/ml	117 µg/ml	Control	*p*
3	10.36^{A}	21.07^{A}	36.43^{A}	42.14	51.79^{A}	89.29^{A}	96.79^{A}	99.64^{A}	78.57	**0.054**
4	8.17^{Ae}	14.17^{ABde}	26.33^{ABcde}	36.50^{bcde}	55.33^{Aabcd}	81.50^{Aab}	87.50^{ABa}	100.00^{Aa}	70.00^{abc}	**0.000*** **
6	4.12^{ABd}	10.94^{ABcd}	21.89^{ABbcd}	41.67^{abcd}	45.96^{Aabcd}	75.08^{ABab}	72.81^{ABCabc}	95.29^{ABa}	70.00^{abc}	**0.002****
8	0.00^{Bd}	0.00^{Bd}	15.74^{ABcd}	25.00^{bcd}	38.89^{ABabcd}	$44.68^{ABCDabc}$	58.33^{ABCDab}	57.87^{ABCDab}	72.86^{a}	**0.001****
10	0.00^{Bd}	0.00^{Bd}	6.00^{ABcd}	22.00^{bcd}	42.00^{Aabc}	48.00^{ABCab}	52.00^{ABCDab}	60.00^{ABCab}	71.43^{a}	**0.000*** **
12	0.00^{Bc}	0.00^{Bc}	0.00^{Bc}	20.67^{bc}	26.33^{ABCbc}	28.67^{BCDbc}	34.75^{BCDabc}	36.92^{BCDab}	70.00^{a}	**0.000*** **
15	0.00^{Bc}	0.00^{Bc}	0.00^{Bc}	6.09^{bc}	6.09^{BCbc}	5.93^{CDbc}	15.87^{CDb}	16.03^{CDb}	71.43^{a}	**0.000*** **
17	0.00^{Bb}	0.00^{Bb}	0.00^{Bb}	0.00^{b}	4.00^{Cb}	4.00^{CDb}	8.82^{Db}	12.55^{CDb}	67.14^{a}	**0.000*** **
19	0.00^{Bb}	0.00^{Bb}	0.00^{Bb}	0.00^{b}	0.00^{Cb}	0.00^{Db}	0.00^{Db}	0.00^{Db}	64.29^{a}	**0.000*** **
22	0.00^{Bb}	0.00^{Bb}	0.00^{Bb}	0.00^{b}	0.00^{Cb}	0.00^{Db}	0.00^{Db}	0.00^{Db}	67.14^{a}	**0.000*** **
28	0.00^{Bb}	0.00^{Bb}	0.00^{Bb}	0.00^{b}	0.00^{Cb}	0.00^{Db}	0.00^{Db}	0.00^{Db}	70.00^{a}	**0.000*** **
32	0.00^{Bb}	0.00^{Bb}	0.00^{Bb}	0.00^{b}	0.00^{Cb}	0.00^{Db}	0.00^{Db}	0.00^{Db}	68.57^{a}	**0.000*** **
p	**0.001****	**0.011***	**0.005****	**0.051**	**0.000*****	**0.000*****	**0.000*****	**0.000*****	**0.996**	

*: <0.05; **: <0.01; ***: <0.001

Horizontally, means that do not share a lowercase letter are significantly different ($p<0.05$). Vertically, means that do not share a capital letter are significantly different ($p<0.05$).

rate decreased as the dose increased in all time periods except the control group.

As seen in Table 8, protoscoleces viability rate in the control group did not show a significant change with respect to the holding time ($p > 0.05$). In other administration doses, a statistically significant change was detected in the number of parasites in terms of time ($p < 0.05$). When the administration doses were compared in each holding time, it was observed that the viability rates showed a significant change according to the doses except for the 6th hr ($p < 0.05$). When compared to the control group, protoscoleces viability decreased significantly after the 4th hr at a dose of 15000 µg / ml, after 8th hr at 3750 µg / ml dose, and after 15th hr at doses of 1875 µg/ml, 937 µg/ml, 468 µg/ml, 234 µg/ml and 117 µg/ml.

The 15000 µg / ml concentration of the dichloromethane extract of *C. barbata* showed a very strong scolicidal effect and no live parasites were observed at the 8th hr (Table 8, Figure 4). Observed at the 8th hr of the plant, LD_{50} value was 337.202 µg / ml and LD_{90} value was 3840.556 µg / ml (Table 9).

Scolicidal effect of NaCl solution used at a dose of 30000 µg / ml, which is used as a scolicidal agent in routine applications, on protoscoleces at different times was given in Figure 5 and Table 10. When Figure 5 and Table 10 were examined, it was seen that the viability rate decreased as the dose increased in all time periods except the control group.

As shown in Table 10, protoscoleces viability rate in the control group did not show a significant change in terms of the holding time ($p > 0.05$). In other administration doses, a statistically significant change was detected in the number of parasites with respect to time ($p < 0.05$). When the administration doses were compared in each holding time, it was observed that the viability rates showed a significant change according to the doses except for the 6th hr ($p<0.05$). When compared to the control group, protoscoleces viability decreased significantly after the 8th hr at a dose of 15000 µg / ml.

Table 7: LD_{50} vs LD_{90} values of chloroform extracts of *C. barbata* against time.

Lethal Concentration Doses		
Time	**LD_{50} (95% confidence limit)**	**LD_{90} (95% confidence limit)**
3 h	1859.561 [a] (-29.427–3593.816)	9976.026 [a] (7077.157–17839.172)
4 h	934.311 [a] (-909.726–2285.616)	8591.347 [a] (6186.450–14633.527)
6 h	415.376 [a] (-1494.289–1592.619)	6852.126 [a] (4824.810–12421.728)
8 h	27.626 [a] (-502.873–363.522)	3217.174 [a] (2382.393–5181.073)
10 h	-19.144 [a] (-922.599–486.193)	2478.286 [a] (1924.275–3579.260)
12 h	-375.677 [a] (-1901.516–101.506)	1686.704 [a] (1137.813–3592.725)
15 h	-367.698 [a] (-6220.713–47.975)	712.908 [a] (360.148–4421.220)

Table 8: The effect of dichloromethane extract of *C. barbata* at different concentrations on protoscoleces according to hours.

h	15000 µg/ml	7500 µg/ml	3750 µg/ml	1875 µg/ml	937 µg/ml	468 µg/ml	234 µg/ml	117 µg/ml	Control	*p*
3	15.71^{Ab}	24.29^{Aab}	72.14^{Aab}	78.93^{Aab}	84.29^{Aab}	100.00^{Aab}	105.71^{Aab}	127.14^{Aa}	78.57^{ab}	**0.035***
4	12.17^{ABd}	20.25^{ABd}	47.00^{ABcd}	67.25^{ABbc}	69.25^{ABCbc}	77.33^{ABCbc}	85.50^{ABb}	126.50^{Aa}	70.00^{bc}	**0.000*****
6	2.27^{BC}	8.25^{BC}	28.70^{AB}	58.00^{ABC}	79.04^{AB}	81.31^{AB}	85.44^{AB}	94.11^{AB}	70.00	**0.269**
8	0.00^{Cc}	0.00Cc	16.67^{Bbc}	40.97^{ABCDab}	54.63^{ABCab}	56.48^{ABCa}	60.42^{ABCa}	64.35^{ABCa}	72.86^{a}	**0.000*****
10	0.00^{Cc}	0.00^{Cc}	18.00^{Bbc}	36.00^{ABCDabc}	48.00^{ABCabc}	50.00^{ABCabc}	54.00^{ABCab}	62.00^{ABCab}	71.43^{a}	**0.002****
12	0.00^{Cb}	0.00^{Cb}	8.25^{Bb}	22.50^{BCDab}	37.00^{ABCab}	41.08^{ABCab}	45.17^{ABCab}	47.17^{ABCab}	70.00^{a}	**0.003****
15	0.00^{Cd}	0.00^{Cd}	8.01^{Bd}	11.86^{CDcd}	16.03^{ABCbcd}	20.03^{BCbcd}	30.29^{ABCbc}	34.29^{ABCb}	71.43^{a}	**0.000*****
17	0.00^{Cc}	0.00^{Cc}	4.27^{Bbc}	8.55^{CDbc}	10.55^{BCbc}	12.55^{BCbc}	15.36^{BCbc}	21.36^{BCb}	67.14^{a}	**0.000*****
19	0.00^{Cb}	0.00^{Cb}	0.00^{Bb}	0.00^{Db}	0.00^{Cb}	0.00^{Cb}	0.00^{Cb}	0.00^{Cb}	64.29^{a}	**0.000*****
22	0.00^{Cb}	0.00^{Cb}	0.00^{Bb}	0.00^{Db}	0.00^{Cb}	0.00^{Cb}	0.00^{Cb}	0.00^{Cb}	67.14^{a}	**0.000*****
28	0.00^{Cb}	0.00^{Cb}	0.00^{Bb}	0.00^{Db}	0.00^{Cb}	0.00^{Cb}	0.00^{Cb}	0.00^{Cb}	70.00^{a}	**0.000*****
32	0.00^{Cb}	0.00^{Cb}	0.00^{Bb}	0.00^{Db}	0.00^{Cb}	0.00^{Cb}	0.00^{Cb}	0.00^{Cb}	68.57^{a}	**0.000*****
p	**0.000*****	**0.037***	**0.003****	**0.000*****	**0.001****	**0.001****	**0.001****	**0.000*****	**0.996**	

*: <0.05; **: <0.01; ***: <0.001

Horizontally, means that do not share a lowercase letter are significantly different ($p<0.05$). Vertically, means that do not share a capital letter are significantly different ($p<0.05$).

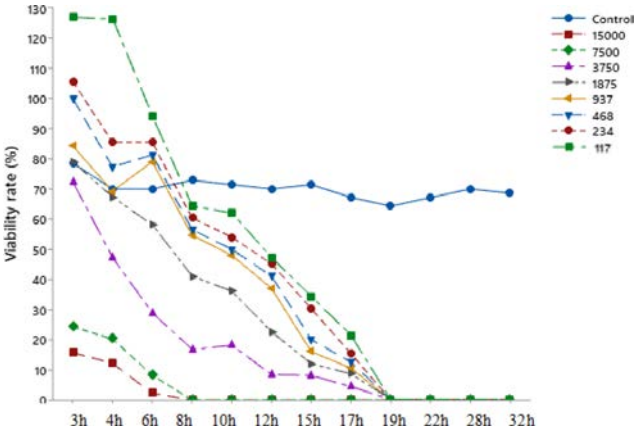


Figure 4: The scolicidal effect of the dichloromethane extract of *C. barbata* prepared at different concentrations on protoscoleces at different times.

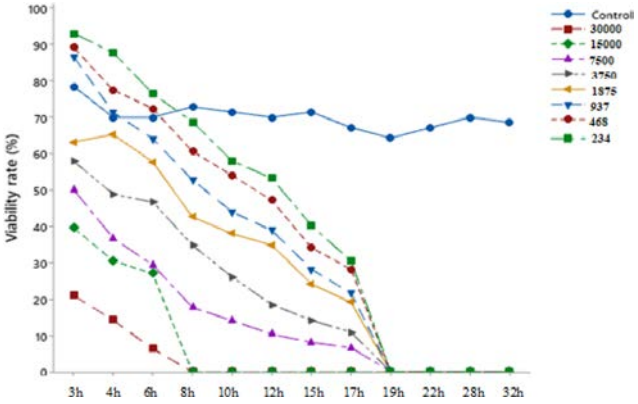


Figure 5: The scolocidal effect of NaCl prepared in different concentrations on protoscoleces at different times.

The 15000 µg / ml concentration of the NaCl extract of *C. barbata* showed a very strong scolicidal effect and no live parasites were observed at the 8th hr (Table 10, Figure 5). Observed at the 8th hr of the plant, LD_{50} value was 225.314 *µg / ml and* LD_{90} value was 3588.961 *µg / ml* (Table 11).

The antimicrobial effect of *C. barbata* according to the disk diffusion method was given in Table 12.

Table 9: LD_{50} ve LD_{90} values of dichloromethane extracts of *C. barbata* against time.

	Lethal Concentration Doses	
Time	**LD_{50} (95% confidence limit)**	**LD_{90} (95% confidence limit)**
3 h	4471.045 [a] (3065.400–6455.796)	12372.024 [a] (9481.821–18351.640)
4 h	2316.511 [a] (888.172–3762.525)	11061.901 [a] (8365.384–16818.0)
6 h	1127.383 [a] (-1052.371–2817.117)	6495.983 [a] (4172.758–17209.5)
8 h	337.202 [a] (-360.428–822.783)	3840.556 [a] (2919.631–5821.715)
10 h	26.283 [a] (-804.985–532.985)	3670.781 [a] (2783.537–5582.013)
12 h	-345.809 [a] (-1501.371–176.922)	2566.943 [a] (1852.912–4395.593)
15 h	-870.840 [a] (4681.582–-75.387)	1939.359 [a] (1198.941–5318.237)
17 h	-1097.148 [a] (-17399.315–-189.858)	1271.332 [a] (612.749–9300.063)

Table 10: The effect of different concentrations of NaCl on protoscoleces according to hours.

	30000 µg/ml	15000 µg/ml	7500 µg/ml	3750 µg/ml	1875 µg/ml	937 µg/ml	468 µg/ml	234 µg/ml	Control	*p*
1	21.07^{A}	39.64^{A}	50.00^{A}	57.86^{A}	63.21^{A}	86.79^{A}	89.29^{A}	93.21^{A}	78.57	**0.089**
2	14.25^{ABe}	30.58^{ABde}	36.67^{ABd}	48.83^{ABcd}	65.25^{Abc}	71.33^{ABab}	77.50^{ABab}	87.75^{Aa}	70.00^{ab}	**0.000*****
3	6.40^{BCb}	27.02^{Bab}	29.29^{ABCab}	46.63^{ABab}	57.74^{ABa}	64.14^{ABCa}	72.39^{ABCa}	76.52^{ABa}	70.00^{a}	**0.003****
4	0.00^{Ce}	0.00^{Ce}	17.59^{BCDde}	34.72^{ABCcd}	42.59^{ABCbcd}	$52.78^{ABCDabc}$	60.65^{ABCabc}	68.75^{ABab}	72.86^{a}	**0.000*****
5	0.00^{Cd}	0.00^{Cd}	14.00^{CDcd}	26.00^{ABCbcd}	38.00^{ABCabc}	44.00^{BCDabc}	54.00^{ABCab}	58.00^{ABab}	71.43^{a}	**0.000*****
6	0.00^{Ce}	0.00^{Ce}	10.25^{CDde}	18.42^{ABCcde}	34.83^{ABCbcd}	$38.92^{BCDEabcd}$	47.17^{ABCabc}	53.25^{ABab}	70.00^{a}	**0.000*****
7	0.00^{Ce}	0.00^{Ce}	8.01^{CDde}	14.10^{BCcde}	24.20^{BCDbcd}	28.21^{CDEbc}	34.13^{BCDb}	40.22^{BCb}	71.43^{a}	**0.000*****
8	0.00^{Cc}	0.00^{Cc}	6.55^{Dbc}	10.82^{BCbc}	19.09^{CDbc}	21.64^{DEbc}	28.18^{CDbc}	30.45^{BCb}	67.14^{a}	**0.000*****
9	0.00^{Cb}	0.00^{Cb}	0.00^{Db}	0.00^{Cb}	0.00^{Db}	0.00^{Eb}	0.00^{Db}	0.00^{Cb}	64.29^{a}	**0.000*****
10	0.00^{Cb}	0.00^{Cb}	0.00^{Db}	0.00^{Cb}	0.00^{Db}	0.00^{Eb}	0.00^{Db}	0.00^{Cb}	67.14^{a}	**0.000*****
11	0.00^{Cb}	0.00^{Cb}	0.00^{Db}	0.00^{Cb}	0.00^{Db}	0.00^{Eb}	0.00^{Db}	0.00^{Cb}	70.00^{a}	**0.000*****
12	0.00^{Cb}	0.00^{Cb}	0.00^{Db}	0.00^{Cb}	0.00^{Db}	0.00^{Eb}	0.00^{Db}	0.00^{Cb}	68.57^{a}	**0.000*****
	0.000***	0.000***	0.000***	0.001**	0.000***	0.000***	0.000***	0.000***	0.996	

*: <0.05; **: <0.01; ***: <0.001,

Horizontally, means that do not share a lowercase letter are significantly different ($p<0.05$). Vertically, means that do not share a capital letter are significantly different ($p<0.05$).

Table 11: LD_{50} ve LD_{90} values of NaCl extracts of *C. barbata* against time.

Lethal Concentration Doses		
Time	**LD_{50} (95% confidence limit)**	**LD_{90} (95% confidence limit)**
3 h	3086.654[a] (1318.303–4968.542)	15484.266[a] (11567.073–24359.6)
4 h	887.119[a] (-646.611–2076.558)	12414.023[a] (9786.271–17294.894)
6 h	64.308[a] (-1541.919–1168.582)	9668.057[a] (7523.572–13782.677)
8 h	225.314[a] (-349.472–631.635)	3588.961[a] (2824.072–5046.195)
10 h	-137.645[a] (-975.128–335.880)	3083.948[a] (2330.613–4720.362)
12 h	-321.111[a] (-1296.237–162.894)	2567.305[a] (1898.764–4118.004)
15 h	-640.361[a] (-2589.177–-0.385)	2149.418[a] (1453.708–4364.770)
17 h	-956.416[a] (-4933.866–-163.027)	1732.752[a] (1058.738–4762.764)

When the table was examined, *C. barbata* had an antimicrobial effect on *A. baumannii ATCC BAA-747, B. cereus ATCC 10876, B. subtilis ATCC 6633, Citrobacter freundii ATCC 43864, E. coli ATCC 36218, Enterobacter aerogenes ATCC 13048, K. pneumoniae ATCC 13883, P. aeruginosa ATCC 9027* and *S. epidermidis ATCC 12228* microorganisms in all solvents at a concentration of 30 µl. It was effective against MRSA ATCC 67101 at concentrations of 15 µl and 30µl. However, the concentrations of *C. barbata* were not effective in *Enterococcus faecalis ATCC 29212, E. coli ATCC 25922, Proteus mirabilis ATCC 43071* and *S. aureus ATCC 29213*.

DISCUSSION

The extracts in the presented study were obtained by drying. Studies conducted with similar methods reported that they reached effective results.[24-26] Essential oil researches used in this study performed on algae samples shows similarities with the studies including the methods of the extraction of essential

Table 12: Antimicrobial effect of C. barbata on microorganisms.

Micro-organisms	*Chloroform mg/disc*		*Dichloromethane mg/disc*		*Hexane mg/disc*		*Methanol mg/disc*		*Standart mg/disc*	
	15		15		15		15		10	30
	15µl	30µl	15µl	30µl	15µl	30µl	15µl	30µl	Tob	Nys
Acinebacter baumannii ATCC BAA-747	-	6.5	-	7	-	7	-	7	22	-
Bacillus cereus ATCC 10876	-	6.5	-	6.5	-	6.5	-	6.5	22	-
Bacillus megaterium DSM32	-	6.5	-	-	-	6.5	-	6.5	18	-
Bacillus subtilis ATCC 6633	-	6.5	-	6.5	-	6.5	-	6.5	18	-
Candida albicans ATCC 10231	-	8	-	6.5	-	6.5	-	-	-	24
Citrobacter freundii ATCC 43864	-	6.5	-	7	-	7	-	7	20	-
Enterococcus faecalis ATCC 29212	-	-	-	-	-	-	-	-	16	-
E. coli ATCC 25922	-	-	-	-	-	-	-	-	20	
E coli ATCC 36218	-	6.5		6.5	-	6.5	-	6.5	20	-
Enterobacter aerogenes ATCC 13048	-	7	-	8.5	-	6.5	-	6.5	17	-
Klebsiella pneumoniae ATCC 13883		6.5		6.5	-	7	-	7	19	-
MRSA ATCC 67101	6.5	8	-	6.5	6.5	7	7	9	20	
Proteus mirabilis ATCC 43071	-	-	-	-	-	-	-	-	19	-
Pseudomonas aeruginosa ATCC 27853	-	-	-	6.5	-	-	-	-	20	-
Pseudomonas aeruginosa ATCC 9027	-	7	-	7	-	7	-	6.5	20	
Salmonella typhimurium ATCC 14028	-	-		6.5	-	-	-	7	20	-
S. aureus ATCC 25923	-	-	-	-	-	-	-	6.5	20	-
S. aureus ATCC 29213	-	-	-	-	-	-	-	-	22	
S. epidermidis ATCC 12228	-	6.5	-	7	-	6.5	-	6.5	22	-

oils with the same extraction method and component determination by GC / MS analysis in Turkey.[12,27] The composition of essential oils obtained from seaweed contains terpenic or non-terpenic volatile compounds. Generally, these compounds include hydrocarbons and their oxygenated derivatives. Alcohol, carboxylic acid, ester, aldehyde, ketone etc. structures can also be found.[28] Terpenes, on the other hand, are formed by binding of isoprene units to each other. Monoterpenes, sesquiterpenes and diterpenes and their oxygenated forms are part of most essential oils.[29] It has been reported that they have a wide range of biological activity, especially due to the terpenic compounds in the essential oil.[30] The main areas of use are cosmetics, medicine, food industry, dentistry, oral care products, perfumery, dyeing, aromatherapy and phytotherapy.[29,31] Similarly, it is stated that aldehyde can be used in drug production by adding it to the structure of different compounds.[32] In the study, the classes and amounts of the most abundant compounds in the structure of *C. barbata* were aldehyde (43.69%), terpene and terpenoid compounds (32.37%), hydrocarbon (8.19%) and alcohol (5.37%), respectively. However, carboxylic acid compounds have not been found.

It has been reported that seaweeds are rich in minerals and some biologically active compounds such as polysaccharides, proteins, lipids and polyphenols have antibacterial, antivirus and antifungal activity.[22] It is thought that terpene and terpenoid compounds, which are abundant in the structure of *C. barbata*, can be used as antimicrobial and scolicidal agents, and there is no study about the possibility of scolicidal agent in the source literature obtained. In different studies, formol, hypertonic glucose solution, alcohol, hypertonic NaCl (3; 10; 20 %), chlorhexidine, cetrimide, $AgNO_3$, povidone iodine, alcohol, 3 % H_2O_2, albendazole solution, iodine and its compounds were reported among the first substances used for protoscolicidal purposes.[33-35] It was determined that the ones having highest risk among these substances used were formol, hypertonic saline and alcohol.[36,37] Therefore, researches thought to be different plant-derived scolicidal have been conducted. Ozcelik *et al*.[38] stated that *Allium Sativum* is fully effective on protoscoleces at 50% mg / ml concentration in 15 min, at 25% mg / ml concentration in 20 min and at 12.5% mg / ml concentration in 30 min. Again, Ozcelik *et al*.[39] found that propolis, which could also affect daughter vesicles in 10 min, can be used as a scolicidal agent. In the study presented, extracts of *C. barbata* prepared in chloroform, methanol, hexane and dichloromethane were compared control solution (NaCl) with respect to the scolocidal effect. Protoscoleces viability of the hexane extract of *C. barbata* at a dose of 15000 μg / ml decreased significantly compared to the control. The viability rates started to decrease significantly in 4 hr, 6 hr, 10 hr and 12 hr holding times at a dose of 3750 μg / ml, 8 hr, 15 hr and 17 hr holding times at a dose of 1875 μg / ml and 22 hr, 28 hr and 32 hr holding times at a dose of 234 μg / ml. Also, when the doses of methanol extract of *C. barbata* was compared in each holding period by Tukey test, it was determined that the viability rates showed a significant change with respect to the doses except for the 3rd and 4th hr (p <0.05). In the evaluation of concentrations compared to the control group, the viability of protoscoleces decreased significantly after 10 hr at a dose of 15000 μg / ml compared to the control. Similarly, protoscoleces viability of the chloroform extract of *C. barbata* at a dose of 15000 μg / ml compared to the control group decreased significantly after the 4th hr. Again, a significant decrease in viability was found after 8th hr at 3750 μg / ml dose, after 10th hr with 1875 μg / ml dose, after 12th hr with 937 μg / ml and 498 μg / ml doses, and after 15th hr at 234 μg / ml and 117 μg / ml doses. In the evaluation of the dose in terms of time, a significant difference was found between the 3rd hr and the 6th and later hr. When compared to the control group, protoscoleces viability of the dichloromethane extract of *C. barbata* decreased significantly after the 4th hr at a dose of 15000 μg / ml, after 8th hr at 3750 μg / ml dose, and after 15th hr at doses of 1875 μg/ml, 937 μg/ml, 468 μg/ml, 234 μg/ml and 117 μg/ml. In the study, LD_{50} and LD_{90} values were also examined. Of the hexane extract of *C. barbata* at the 15000 μg / ml concentration at the 15th hr, the observed LD_{50} value was 781.360 μg / ml and LD_{90} value was 7313.211 μg / ml. Of the methanol extract of *C. barbata* at the 15000 μg / ml concentration at the 15th hr, the observed LD_{50} value was 1172.857 *μg / ml and* LD_{90} value was 8409.504 *μg / ml.* Of the chloroform extract of *C. barbata* at the 15000 μg / ml concentration at the 8th hr, the observed LD_{50} value was 27.626 *μg / ml and* LD_{90} value was 3217.174 *μg / ml. Of the* dichloromethane extract of *C. barbata* at the 15000 μg / ml concentration at the 8th hr, the observed LD_{50} value was 337.202 *μg / ml* and LD_{90} value was 3840.556 μg / ml. Of the NaCl extract of *C. barbata* at the 15000 μg / ml concentration at the 8th hr, the observed LD_{50} value was 225.314 μg / ml and LD_{90} value was 3588.961 μg / ml.

As a result of the antimicrobial activity evaluation in the study, *C. barbata* had an antimicrobial effect on *A. baumannii ATCC BAA-747, B. cereus ATCC 10876, B. subtilis ATCC 6633, Citrobacter freundii ATCC 43864, E. coli ATCC 36218, Enterobacter aerogenes ATCC 13048,*

K. pneumoniae ATCC 13883, P. aeruginosa ATCC 9027 and *S. epidermidis ATCC 12228* microorganisms in all solvents at a concentration of 30 µl. It was effective against *MRSA ATCC 67101* at concentrations of 15 *µg / ml* and 30 *µg / ml*. The antioxidant properties of the solution in hot water in DMSO of *C. barbata* sample collected from Espiye district of Giresun province were investigated in the source literature.[40] However, *C. barbata* obtained off the coast of Sinop province was investigated for scolicidal and antimicrobial effects in the study presented. Similarly, Ozdemir *et al*.[41] performed both Soxhlet extraction and essential oil analysis of freeze-dried *D. membranacea* and *C. barbata*, and they realized antimicrobial activity test on the solvent extract and essential oils they obtained. Although they did not observe any activity on *E. coli ATCC 36218*, *Pseudomonas aeruginosa* and *S. epidermidis* in the antimicrobial activity test performed by the researchers, a moderate activity was observed at a concentration of 30 *µg / ml* in the study. This situation may be due to the dose and method used. Erturk and Tas,[42] conducted antioxidant and antimicrobial activity tests on the extract they prepared by keeping 7 algae samples, including *C. barbata* and *U. rigida*, which they collected from Ordu province, in ethanol. In *C. barbata* disk diffusion test, it was found that it showed the highest values between 9-16 mm on *S. aureus ATCC 25923*. In the study, it showed antibacterial effect in methanol extract. Again, Taşkın *et al*.[43] prepared methanol extracts of 6 algae samples, including *C. barbata* and *U. rigida,* collected from Canakkale and it was observed that *S. aureus ATCC 6538P* and *E. coli ATCC 29998* showed values between 11-16 mm zone diameter as a result of antibacterial activity tests. In the presented study, antimicrobial effect was observed on *E. coli ATCC 36218* and *S. aureus ATCC 25923*. The differences in the studies may have resulted from the method and dose adjustment used and the differences in the density of bacteria, fungi and parasites.

CONCLUSION

In this study, it was determined that the solvent extracts of *C. barbata* may have bactericidal and scolicidal effects. The algae extract obtained showed similar effects in different solvents. In this case, it was thought that LD90 dose could be increased for a faster effect, and the dose could be decreased for a slower effect. It is suggested that controlled experiments should be conducted for the use of *C. barbata* in living cells. In this study, it was concluded that the dose should be adjusted in order to increase the level of antimicrobial effect and necessary arrangements should be made accordingly.

Financial Support

The study was supported by Giresun University Scientific Research Projects Commission Directorate with the project numbered Fen-Bap-A-170417-111.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

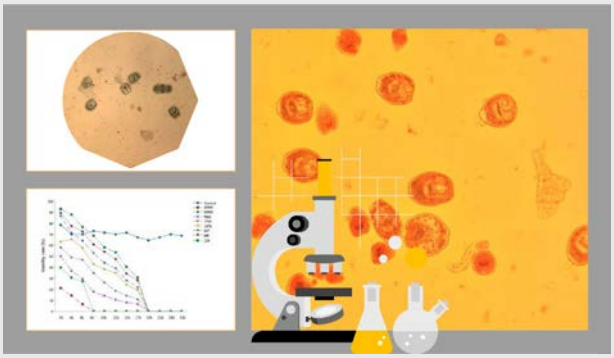
ATTC: American Type Culture Collection ***C. barbata*:** *Cystoseria barbata;* **DMSO:** dimethyl sulfoxide **LD:** Lethal concentration doses, **RI:** retention index **SD:** standard deviation.

REFERENCES

1. Miyashita K, Mikami N, Hosokawa M. Chemical and nutritional characteristics of brown seaweed lipids: A review. J Funct Foods. 2013;5(4):1507-17. doi: 10.1016/j.jff.2013.09.019.
2. Senthilkumar K, Manivasagan P, Venkatesan J, Kim SK. Brown seaweed fucoidan: Biological activity and apoptosis, growth signaling mechanism in cancer. Int J Biol Macromol. 2013;60:366-74. doi: 10.1016/j.ijbiomac.2013.06.030, PMID 23817097.
3. Sanjeewa KKA, Kim EA, Son KT, Jeon YJ. Bioactive properties and potentials cosmeceutical applications of phlorotannins isolated from brown seaweeds: A review. J Photochem Photobiol B. 2016;162:100-5. doi: 10.1016/j.jphotobiol.2016.06.027, PMID 27362368.
4. Laurienzo P. Marine polysaccharides in pharmaceutical applications: An overview. Mar Drugs. 2010;8(9):2435-65. doi: 10.3390/md8092435, PMID 20948899.
5. Gutierrez-Rodríguez AG, Juárez-Portilla C, Olivares-Bañuelos TN, Zepeda RC. Anticancer activity of seaweeds. Drug Discov Today. 2018;23(2):434-47. doi: 10.1016/j.drudis.2017.10.019, PMID 29107095.
6. Atasever-Arslan B, Yilancioglu K, Kalkan Z, Timucin AC, Gür H, Isik FB, *et al*. Screening of new antileukemic agents fromessential oils of algae extracts and computational modeling of their interactions with intracellular signaling nodes. Eur J Pharm Sci. 2016;83:120-31. doi: 10.1016/j.ejps.2015.12.001, PMID 26709080.
7. Moubayed NMS, Al Houri HJ, Al Khulaifi MMA, Al Farraj DA. Antimicrobial, antioxidant properties and chemical composition of seaweeds collected from Saudi Arabia (Red Sea and Arabian Gulf). Saudi J Biol Sci. 2017;24(1):162-9. doi: 10.1016/j.sjbs.2016.05.018, PMID 28053587.
8. Roohinejad S, Koubaa MJ, Barba FJ, Saljoughian S, Amid M, Greiner R. Application of seaweeds to develop new food products with enhanced shelf-life, quality and health-related beneficial properties. Food Res Int. 2017;99(3):1066-83. doi: 10.1016/j.foodres.2016.08.016, PMID 28865618.
9. Gümüş B, Ünlüsayın M. Determination of antimicrobial activity of two macro algae extracts. EgeJFAS. 2016;33(4):389-95. doi: 10.12714/egejfas.2016.33.4.13.
10. Asakawa Y, Yoyota M, Takemoto T, Kubo I, Nakanishi K. Insect antifeedant secoaromadendrane-type sesquiterpenes from Plagiochila species. Phytochemistry. 1980;19(10):2147-54. doi: 10.1016/S0031-9422(00)82212-8.
11. Young Cho JY, Kwon EH, Choi JS, Hong SY, Shin HW, Hong YK. Antifouling activity of seaweed extracts on the green algae *Enteromorpha prolifera* and the mussel Mytilus edulis. J Appl Phycol. 2001;13(2):117-25. doi: 10.1023/A:1011139910212.
12. Mayer AMS, Rodríguez AD, Berlinck RGS, Fusetani N. Marine pharmacology in 2007-8: Marine compounds with antibacterial, anticoagulant, antifungal, anti-inflammatory, antimalarial, antiprotozoal, antituberculosis, and antiviral activities; affecting the immune and nervous system, and other miscellaneous

mechanisms of action. Comp Biochem Physiol C Toxicol Pharmacol. 2011;153(2):191-222. doi: 10.1016/j.cbpc.2010.08.008, PMID 20826228.
13. Garcia-Dav S, Murillo-Al I, Munoz-Ocho M, Carranza-T E, Garza-Padr R, Morales-Ru E, Viveros-Va E. Bactericide, antioxidant and cytotoxic activities from marine algae of genus Laurencia collected in Baja California Sur, Mexico. Int J Pharmacol. 2018;14(3):391-6. doi: 10.3923/ijp.2018.391.396.
14. Zbakh H, Chiheb I, Motilva V, Riadi H. Antibacterial, cytotoxic and antioxidant potentials of *Cladophora prolifera* (Roth) Kutzing collected from the Mediterranean coast of Morocco. Am J Phytomed Clin Ther. 2014;2(10):1187-99.
15. Bilgin Sokmen B, Aydin S, Sagkal Y, Akyurt I. Investigation of the antioxidant activity of seaweed *Cystoseira barbata* (Stackhouse) *C. Agardh* collected from Giresun. Black Sea. J Sci. 2016;6(14):66-76.
16. Agregán R, Munekata PES, Franco D, Carballo J, Barba FJ, Lorenzo JM. Antioxidant potential of extracts obtained from macro- (*Ascophyllum nodosum, Fucus vesiculosus* and *Bifurcaria bifurcata*) and micro-algae (*Chlorella vulgaris* and *Spirulina platensis*) assisted by ultrasound. Medicines (Basel). 2018;5(2):33. doi: 10.3390/medicines5020033, PMID 29642562.
17. Zerrifi SEA, El Khalloufi F, Oudra B, Vasconcelos V. Seaweed bioactive compounds against pathogens and microalgae: potential uses on pharmacology and harmful algae bloom control. Mar Drugs. 2018;16(2):55. doi: 10.3390/md16020055, PMID 29425153.
18. Filice C, Pirola F, Brunetti E, Dughetti S, Strosselli M, Foglieni CS. A new therapeutic approach for hydatid liver cysts. Aspiration and alcohol injection under sonographic guidance. Gastroenterology. 1990;98(5 Pt 1):1366-8. doi: 10.1016/0016-5085(90)90358-8, PMID 2182372.
19. Yagci G, Ustunsoz B, Kaymakcioglu N, Bozlar U, Gorgulu S, Simsek A, *et al*. Results of surgical, laparoscopic, and percutaneous treatment for hydatid disease of the liver: 10 year's experience with 355 patients. World J Surg. 2005;29(12):1670-9. doi: 10.1007/s00268-005-0058-1, PMID 16311852.
20. Nasseri-Moghaddam S, Abrishami A, Taefi A, Malekzadeh R. Percutaneous needle aspiration, injection, and re-aspiration with or without benzimidazole coverage for uncomplicated hepatic hydatid cysts. Cochrane Database Syst Rev. 2011;19(1):CD003623. doi: 10.1002/14651858.CD003623.pub3, PMID 21249654.
21. Demirel Z, Yilmaz Koz FF, Ulku N, Ozdemir G, Sukatar A. Antimicrobial and antioxidant activities of solvent extracts and the essential oil composition of *Laurencia obtusa* and *Laurencia obtusa* var. pyramidata. Rom Biotechnol Lett. 2011;16(1):5927-36.
22. Manev Z, Iliev A, Vachkova V. Chemical characterization of brown seaweed – *Cystoseira barbata*. Bulg J Agric Sci. 2013;19(1):12-5.
23. Cirik S, Sen E. Akl. Brown algae *Cystoseira barbata* (Stackhouse) *C. Agardh* culture and changes in its chemical composition. J Fisheriessciences.com. 2010;4(4):354-61.
24. Kim YH, Kim JH, Jin HJ, Lee SY. Antimicrobial activity of ethanol extracts of *Laminaria japonica* against oral microorganisms. Anaerobe. 2013;21:34-8. doi: 10.1016/j.anaerobe.2013.03.012, PMID 23583539.
25. Peres JCF, De Carvalho LR, Goncalez E, Berian L, D'arc Felicio J. Evaluation of antifungal activity of seaweed extracts. Ciênc agrotec Lavras. 2012;36(3): 294-9.
26. Ely R, Supriya T, Naik CG. Antimicrobial activity of marine organisms collected off the coast of South East India. J Exp Mar Biol Ecol. 2004;309(1):121-7. doi: 10.1016/j.jembe.2004.03.010.
27. Ozdemir G, Horzum Z, Sukatar A, Karabay-Yavasoglu NU. Antimicrobial Activities of Volatile Components and Various Extracts of Dictyopteris membranaceae. and *Cystoseira barbata*. from the Coast of Izmir, Turkey. Pharm Biol. 2006;44(3):183-8. doi: 10.1080/13880200600685949.
28. Evren M, Tekguler B. Antimicrobial properties of essential oils. Mikrobiyol J. 2011;9(3):28-40.
29. Yaman T, Kuleasan S. Advanced extraction methods to obtain essential Oil. J Grad Sch natural and applied sci Mehmet Akif Ersoy uni. 2016 [Special Issue];1:78-83.
30. Pasayeva L, Kararenk AC, Kosar M. Traditional usage, phytochemical compositions and biological activities of Cousinia Cass. Genus J Fac Pharm Ankara. 2020;44(2):293-307.
31. Hammer KA, Carson CF, Riley TV. Antimicrobial activity of essential oils and other plant extracts. J Appl Microbiol. 1999;86(6):985-90. doi: 10.1046/j.1365-2672.1999.00780.x, PMID 10438227.
32. Ersatir M, Giray ES. Syntesis of various chalcone-coumarin hybrid compounds. Çukurova Uni J Fac Eng. 2018;35(6):111-20.
33. Feng JJ, Xiao SH, Guo HF, Ren L, Jiao PY, Yao MY, *et al*. Effects of mebendazole, albendazole, and praziquantel on alkaline phosphatase, acid phosphatase, and adenosine triphosphatase of *Echinococcus granulosus* cysts harbored in mice. Zhongguo Yao Li Xue Bao. 1992;13(6):497-501. PMID 1302436.
34. Moreno MJ, Urrea-París MA, Casado N, Rodriguez-Caabeiro F. Praziquantel and albendazole in the combined treatment of experimental hydatid disease. Parasitol Res. 2001;87(3):235-8. doi: 10.1007/s004360000334, PMID 11293572.
35. Yetim I, Erzurumlu K. Curr rent approaches in treatment of hydatid cysts of liver. J Clin Anal Med. 2013;4(1):64-71.
36. Belghiti J, Benhamou JP, Houry S, Grenier P, Huguier M, Fékété F. Caustic sclerosing cholangitis. A complication of the surgical treatment of hydatid disease of the liver. Arch Surg. 1986;121(10):1162-5. doi: 10.1001/archsurg.1986.01400100070014, PMID 3767649.
37. Katkhouda N, Fabiani P, Benizri E, Mouiel J. Laser resection of a liver hydatid cyst under videolaparoscopy. Br J Surg. 1992;79(6):560-1. doi: 10.1002/bjs.1800790628, PMID 1535261.
38. Ozçelik S, Sümer Z, Değerli S, Ozan F, Sökmen A. Can garlic (*Allium sativum*) extract be used as scolocidal agent?. Turkiye Parazitol Derg Derg 2007. 2007;31(4):318-21. PMID 18224626.
39. Özçelik S, Sümer Z, Değerli S. The scolocidal effect of propolis on protoscoleces and daugther cysts. Cumhuriyet Med J. 2015;37(1):4-8. doi: 10.7197/cmj.v37i1.5000071890.
40. Bilgin Sokmen B, Aydın S, Sagkal Y, Akyurt I. Investigation of the antioxidant activity of seaweed *Cystoseira barbata* (Stackhouse) C. Agardh collected from Giresun. The Black Sea. J Sci. 2016;6(14):66-76.
41. Ozdemir G, Horzum Z, Sukatar A, Karabay-Yavasoglu NU. Antimicrobial Activities of Volatile Components and Various Extracts of Dictyopteris membranaceae. and *Cystoseira barbata*. from the Coast of Izmir, Turkey. Pharm Biol. 2006;44(3):183-8. doi: 10.1080/13880200600685949.
42. Erturk O, Tas B. Antibacterial and antifungal effects on some marine algae. Kafkas Univ Vet Fak Derg. 2011;17;Suppl A:121-4.
43. E T, M O, E T, O K. Antibacterial activities of some marine algae from the Aegean Sea (Turkey). Afr J Biotechnol. 2007;6(24):2746-51. doi: 10.5897/AJB2007.000-2439.

PICTORIAL ABSTRACT



SUMMARY

We determined that the solvent extracts of *C. barbata* may have bactericidal and scolicidal effects. The algae extract obtained exhibited similar effects in different solvents. It was thought that LD_{90} dose could be increased for a faster effect, and the dose could be decreased for a slower effect. Our study, however, needs to be confirmed with controlled experiments for the use of *C. barbata* in living cells.

About Authors

Assoc. Prof. Dr. Ülkü Karaman, Department of Medical Parasitology, Faculty of Medicine, Ordu University, Ordu, Türkiye. She has completed he Ph.D. from Department of Medical Parasitology. Her area of research interest is medical parasitology and microbiology. He has about 22 years working in health science.

Dr. Yeliz Kaşko Arıcı, Department of Biostatistics and Medical Informatics, Faculty of Medicine, Ordu University, Ordu, Türkiye.

Dr. Tayyibe Beyza Yücel, Health Services Vocational School, Giresun University, Giresun, Türkiye.

Mr. Murat Yücel, Department of Biotechnology, Necmettin Erbakan University, Konya, Türkiye.

Dr. Tuğba Cebeci, Department of Medical Services and Techniques, Vocational School of Espiye, Giresun University, Giresun, 28600, Türkiye.

Res. Asist. Büşra Korkmaz, Department of Pharmacognosy, Faculty of Pharmacy, Karadeniz technical university, Trabzon, Türkiye.

Cite this article: Karaman U, Yucel TB, Cebeci T, Arici YK, Yucel M, Korkmaz B. Investigation of Biological, Scolicidal and Antimicrobial Activities of the Solvent Extracts Components of *Cystoseria barbata* Seen off Sinop Province. Indian J of Pharmaceutical Education and Research. 2022;56(2s):s312-s325.