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Original Article

First screening of volatile constituents and antibacterial, antibiofilm, and anti-quorum sensing activities of *Cinclidotus* species

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ABSTRACT

Background and Aims: Bryophytes are the center of interest for natural sources due to their medicinal and traditional usage in various ailments with their interesting phytochemicals. However, there are very few studies on this subject. In the present study, we aimed to investigate mosses belonging to the genus *Cinclidotus* (Cinclidotaceae), such as *C. pachyloma, C. bistratosus, C. riparius, C. pachylomoides, C. fontinaloides* and *C. aquaticus* regarding their volatile components and biological activities.

Methods: The mosses were collected from various locations in Türkiye and then extracted with ether. The volatile components were identified and semi-quantified using GC-TQMS (Gas Chromatography and a Triple Quadrupole Mass Spectrometer). The MICs were evaluated using the broth microdilution method. A microplate-based biofilm model was used against *P. aeruginosa* PAO1 using the crystal violet assay to determine the antibiofilm activity. The anti-quorum sensing activity was carried out using the disc diffusion method.

Results: The initial screening of the selected mosses to confirm the significant potential of their volatile phytochemicals was investigated for the first time in this study. The main components were determined as linoelaidic acid, glycerol 2-hexadecanoate, and campesterol in diverse types of *Cinclidotus* species by GC/TQMS. In addition, the potency of the antibacterial, antibiofilm, and anti-quorum sensing activities of these species exhibited moderate to highly effective results.

Conclusion: All results showed that mosses are rich sources of natural compounds and good samples for biological activities. Mosses are promising candidates that could be useful in preventing or treating various pathological conditions. However, further *in vitro* and *in vivo* studies should focus on a single component or the mechanisms.

Keywords: Bryophyte, Metabolites, Moss, Phytochemicals, Activity, Volatile

INTRODUCTION

New drug molecules are discovered based on natural products (Dushenkov & Raskin, 2008). Investigations into medicinal plants have steadily increased, providing reasonable and essential information on plants and candidate molecules in drug development (Cragg & Newman, 2013). Records of Bryophytes date back to ancient times, with the first being in the first century; more Bryophyte taxa are considered medicinal plants (Drobnik & Stebel, 2014; 2015; 2018). Bryophytes are the second largest group behind only the flowering plants that grow almost all over the world and stand out with their extraordinary chemical properties and biological activities (Sabovljević, Bijelović, & Grubišić, 2001; Klavina et al., 2015; Aruna & Krishnappa, 2018), used as a remedy for many ailments in many forms in traditional medicine in China, Europe, North America, and India (Saxena & Harinder, 2004; Glime, 2007; Karim, Suleiman, Rahmat, & Abu-Bakar, 2014; Klavina et al., 2015; Nilsu et al., 2018; Ludwiczuk & Asakawa, 2019). However, the phytochemistry of Bryophytes has been neglected for a long time due to their small size and difficulties in the identification and collection of the sample in pure forms (Saxena & Harinder, 2004; Adebiyi, Oyedeji, Chikwendu, & Fatoke, 2012), but in recent years, interest in bryophytes has been increasing due to

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their rich source of novel active compounds (Asakawa, 2007; Xie & Lou, 2009; Asakawa, Ludwiczuk, & Nagashima, 2013a; 2013b). All these features of bryophytes mentioned here indicate that they are potentially important biomaterials (Klavina et al., 2015).

The term bryophyte is a common name used to express liverworts (Marchantiophyta), hornworts (Anthocerotophyta), and mosses (Bryophyta), which are taxonomically closely related groups. Today, they represent about 20.000–25.000 taxa globally and show an almost global distribution (Crum, 2001; Patiño & Vanderpoorten, 2018). According to a study by Kürschner and Erdağ in 2023, the bryophytes of Turkey are represented by 1244 taxa (1025 mosses, 215 liverworts, and 4 hornworts), of which 1% are endemic. This number tends to increase with intensive floristic studies conducted by Turkish scientists (Erdağ & Kürschner, 2017; Erata, Batan, Alataş, & Özen, 2020; Ursavaş, Keçeli, Uyar, & Ören, 2020; Kırmacı, Özenoğlu, Armağan, Aslan, & Çatak, 2022; Özenoğlu & Kırmacı, 2022). Mosses, the second largest group of the plant kingdom (more than 14.000 taxa), are not woody and are very simple land plants with typically small sizes (maximum of 60 cm) (Asakawa et al., 2013b). They contain various phytochemicals, besides important medicinal uses in different fields (Saxena & Harinder, 2004; Klavina et al., 2015). The North American tribes used mosses as medicinal plants to treat wounds, burns, tuberculosis, pneumonia, neurasthenia, and other diseases. In China, several mosses are used medicinally for burns, bruises, external injuries, snake bites, pulmonary tuberculosis, neurasthenia, fractures, convulsions, scalds, uropathy, and pneumonia. (Saxena & Harinder, 2004; Klavina et al., 2015). The mosses have also been used for medicinal purposes in Malaysia; in Egypt, some are used to prepare medicinal tea primarily for treating colds (Glime, 2017). In Germany, *Ceratodon purpureus* (Hedw.) Brid. and *Bryum argenteum* Hedw. are the remedies for fungal infections of horses besides anti-leukemic and anticancer effects (Glime, 2013; Aslanbaba, Yilmaz, Yayıntaş, Özyurt, & Öztürk, 2017). *Polytrichum commune* Hedw., a traditional Chinese moss, has been used especially for lymphocytic leukemia besides high fever, injuries related to pneumonia, and prolapsed uterine (Cheng et al., 2013).

Antibiotic resistance is a major global health concern as new strains of resistant bacteria continue to emerge, causing significant morbidity and mortality. New compounds are urgently needed to combat bacterial infections caused by resistant bacteria. The inability to discover new groups of antibiotics and the inevitable emergence of resistance to existing antibiotics has prompted scientists to explore alternative treatment options (Islam et al., 2017; WHO, 2017). Researchers have been searching for new molecules that target different mechanisms of pathogenicity because antibiotics are ineffective against resistant bacteria. Biofilm formation, one of these mechanisms, can increase the pathogenicity of the bacteria and protect them from external factors. The other mechanism is quorum sensing (QS). QS plays a role in synthesizing virulence factors, which contribute to the formation of biofilms and pathogenicity. Recently, scientists have shifted their focus towards researching antibiofilm and anti-quorum sensing molecules as alternatives to antibacterials for treating bacterial infectious diseases. Based on current research, anti-quorum sensing, and antibiofilm compounds, especially those found in natural resources, would be effective in controlling the resistance problem (Uroz, Dessaux, & Oger, 2009; Nithya, Begum, & Pandian, 2010).

The genus *Cinclidotus* from mosses, which constitutes the main material of this study, is represented by 9 taxa, 3 of which are endemic to our country (Erdağ & Kürschner, 2011; Ursavaş & Çetin, 2014). According to Erdağ and Kürschner (2011), the southern part of Türkiye is an excellent site condition for a main speciation center for this hygrophytic complex. Recently, there have been limited studies on the biologically active chemical components of Bryophytes. According to the literature review, there was no detailed study on *Cinclidotus* (Cinclidotaceae) species from Bryophytes. The only research on the genus investigating the allelopathic effects of *C. pachylomoides* Bizot on pepper and corn plants (Turkyılmaz Unal, İşlek, Ezer, & Düzelten, 2017). Therefore, the study aimed to analyze the volatile components using GC/TQMS and to evaluate the antibacterial, antibiofilm, and anti-quorum sensing activities of *Cinclidotus* species.

MATERIALS AND METHODS

Plant materials

The species were collected from different localities in Türkiye. The authentic samples were deposited in the Herbarium Aydın Adnan Menderes University (AYDN). The collection sites and herbarium number of the species are displayed in Table 1.

Extraction of the samples

The mosses (*Cinclidotus* spp.) were extracted with ether (500 mL) via maceration in a 2 L Erlenmeyer and shaken randomly in a cool and dark place for approximately two months. Then, the extracts were filtered through filter paper and in a small column with a small amount of Celite. The samples were weighed in a precision balance (Table 2).

Volatile compounds

The volatile profile was determined according to Issa-Issa, Hernández, Lipan, López-Lluch, and Carbonell-Barrachineta, (2021) with slight modifications. The identification and semi-quantification of the volatile compounds were carried out using a Shimadzu GC2030 gas chromatograph and a TQ8040 NX triple quadrupole mass spectrometer as the detector, GC-MS (Shimadzu Scientific Instruments, Inc., Columbia, MD, USA)

Table 1. The list of *Cinclidotus* species and their localities

No	Species name	Collector no: (M. Kırmacı)	Herbarium no:	Locality*
C1	*C. pachyloma* E.S. Salmon	MKIR 8579	AYDN 4009	1*
C2	*C. bistratosus* Kurschner & Lübenau-Nestle	MKIR 8578	AYDN 4008	2*
C3	*C. riparius* (Host ex Brid.) Arn.	MKIR 8573	AYDN 4003	1*
C4	*C. fontinaloides* (Hedw.) P. Beauv.	MKIR 8575	AYDN 4005	1*
C5	*C. aquaticus* (Hedw.) Bruch and Schimp.	MKIR 8574	AYDN 4004	1*
C6	*C. pachylomoides* Bizot	MKIR 8572	AYDN 4002	1*

(*) Locality 1: Antalya/Manavgat/ Köprülü Canyon, Köprü River, on Limestone Rock, 200 m, 18/06/2019 N 37° 11' 13,75" E 31° 10' 50,95" (*) Locality 2: Isparta/Sütcüler/ Kesme, Between Kesme-Sütçüler, in the stream, on Limestone Rock, 960 m, 19/06/2019 N 37° 27' 50,73" E 31° 14' 56,39"

Table 2. The extracted number of *Cinclidotus* species

No	Species name	The Plant List*	Plant amount g	Ether extract mg	Yield %
C1	*C. pachyloma* E.S. Salmon	Accepted	130.8	832.5	0.64
C2	*C. bistratosus* Kurschner & Lübenau-Nestle	Accepted	141.97	1292.9	0.91
C3	*C. riparius* (Host ex Brid.) Arn.	Accepted	57.61	227.5	0.39
C4	*C. fontinaloides* (Hedw.) P. Beauv.	Accepted	69.07	490	0.71
C5	*C. aquaticus* (Hedw.) Bruch and Schimp.	Accepted	145.64	4260.4	2.92
C6	*C. pachylomoides* Bizot	Accepted	74.22	667.6	0.90

(*) http://www.theplantlist.org/

with an AOC- 6000Plus. A SAPIENS X5MS column, 30.0 m length × 0.25 mm inner diameter × 0.25 µm film thickness (Teknokroma, Barcelona, Spain) was used. For the analysis of the samples, approximately 1.0-2.5 mg of essential oil was dissolved in 1 mL of hexane, and 1 µL was injected. Helium was used as the carrier gas at a pressure of 53.5 kPa, a total flow of 12.0 mL/min, and the flow control mode was adjusted at a linear velocity of 36.3 cm/s, and a split ratio of 1:5 was used. The temperature was 50°C, then 5°C/min ramp to 300°C. The injection temperature was 280°C, the ion source temperature was 200 °C, and the interface temperature was 250°C. Mass spectra were obtained from electron ionization (EI) at 70 eV, with an even time of 0.2 s and a spectral range of 45–400 m/z. Most volatiles were identified according to the retention indices calculated using the C7 to C16 n-alkane mixture (Sigma-Aldrich, Steinheim, Germany), the retention indices of the standards, and comparing the mass spectra obtained with those of the standards from the NIST 14 and Wiley 229 spectrum libraries. All analyses were performed in triplicate, and the results were expressed as a percentage of the relative area (Table 3).

Antibacterial activity

In the antibacterial activity tests, *Staphylococcus aureus* ATCC 25923, methicillin-resistant *S. aureus* ATCC 43300 (MRSA), *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Klebsiella pneumoniae* ATCC 13883 were used as test bacteria. The extracts were dissolved in dimethyl sulfoxide (10% DMSO). The minimum inhibitory concentration (MIC) values were determined using the broth microdilution method (CLSI, 2018). Dilutions ranging from 10 mg/mL to 0.078 mg/mL were prepared in Mueller Hinton Broth (MHB) (Difco, Detroit, MI, USA). The final test concentration of the bacteria was adjusted to 5×10^5 CFU/mL with the overnight subcultures. The microplates were incubated at 35°C for 18-24 hours. After the incubation period, the MIC values (mg/mL) were noted as the last well that completely inhibited the visual bacterial growth. MHB and 10% DMSO were used as the negative controls. Ciprofloxacin (Sigma, USA), ampicillin (Sigma, USA), and ofloxacin (Sigma, USA) were used as reference drugs.

Antibiofilm activity

Before performing the antibiofilm activity test, the MIC values of the extracts against *Pseudomonas aeruginosa* PAO1 were detected. No antibacterial activity was observed. The antibiofilm activity was determined by an *in vitro* microplate-based biofilm model against *P. aeruginosa* PAO1 using the crystal violet assay (Bali, Türkmen, Erdönmez, Sağlam, 2019; Eryılmaz, Kart, Gürpınar, 2019; Jardak, Mnif, Ayed, Rezgui, Aifa, 2021).

Table 3. Concentrations (%) of volatile compounds in the *Cinclidotus* species

Code	Compounds	RT	*C. riparius*	*C. fontinaloides*	*C. aquaticus*	*C. pachylomoides*	*C. pachyloma*	*C. bistratosus*
1	Heptanal	5.883	0.01	-	-	-	-	0.02
2	4-Methyl-3-Hexanol	5.96	0.01	-	-	-	0.17	-
3	3-Hexanol	6.873	0.20	-	0.21	0.20	0.15	0.33
4	4-Methyl-2-Pentanol	7.112	0.21	-	-	0.22	0.02	0.37
5	2-Heptenal	7.257	0.01	-	-	-	-	-
6	6-Undecanone	7.379	-	-	-	-	-	-
7	Benzaldehyde	7.424	-	-	-	-	-	-
8	2,4-Dimethyl-2-pentene	7.492	-	-	-	-	-	0.05
9	Hexanoic acid	7.561	-	-	-	-	-	-
10	4-Ethylcyclohexene	8.325	0.02	-	-	-	-	-
11	Octanal	8.496	-	-	-	-	-	-
12	2-Ethoxy-butane	8.967	-	-	-	-	-	-
13	*o*-Cymene	9.15	0.02	0.05	0.02	0.12	-	-
14	Limonene	9.297	0.10	0.28	0.09	0.70	0.09	0.03
15	Eucalyptol	9.381	0.01	0.01	-	0.06	-	-
16	γ-Terpinene	10.118	0.01	0.02	-	0.04	-	-
17	Heptanoic acid	10.299	-	-	-	-	-	-
18	Linalool	11.267	0.08	0.07	-	-	0.02	-
19	Nonanal	11.403	0.02	-	-	0.02	0.01	0.04
20	Camphor	11.507	0.02	-	-	-	-	0.03
21	2,4-Dimethyl-3-pentanol	12.107	-	-	-	-	-	-
22	3-Methyl-3-pentanol	12.255	-	-	-	-	-	-
23	2-Hydroxy-2-methyl-butyric acid	12.637	-	-	-	-	-	-
24	2-Bornanone	12.722	0.42	0.76	-	2.45	0.15	-
25	Octanoic acid	13.134	0.01	-	-	-	-	-
26	3-Methyl-3-heptanol	13.176	-	-	-	-	-	-
27	Borneol	13.442	0.07	0.03	-	-	-	-
28	Levomenthol	13585	0.01	-	-	-	-	-
29	Terpinen-4-ol	13688	0.02	0.01	-	-	-	-
30	1-(2-Methylphenyl)-ethanone	13.797	-	-	-	-	-	-

Table 3. Continued

31	*α*-Terpineol	14091	0.01	-	-	-	-	-
32	Estragole	14.149	0.05	0.02	-	-	-	-
33	Decanal	14.36	-	-	-	-	-	-
34	3-Ethyl-4-methyl-1H-pyrrole-2,5-dione	15.009	-	-	-	-	-	-
35	Linalyl acetate	15.593	0.33	0.09	-	-	0.03	-
36	Nonanoic acid	15.938	0.06	-	0.04	0.06	0.03	0.04
37	Anethole	16.654	0.11	0.02	-	-	-	-
38	2,4-Decadienal	16.866	0.03	0.03	0.40	0.15	0.05	-
39	Thymol	16.948	0.06	-	-	-	-	-
40	Tridecan-1-ol	17.309	-	-	0.02	-	-	-
41	2-Ethyl-hexanoic acid	17.427	-	-	-	-	-	-
42	Fenchol	17.519	0.04	-	-	-	-	-
43	3,5-Dimethyl-3-hexanol	17.653	-	-	-	-	-	-
44	*α*-Terpinyl acetate	18324	0.09	-	-	-	-	-
45	Eugenol	18.454	0.02	-	0.02	-	-	-
46	Hexyl nonanoate	18.622	-	-	-	-	-	-
47	2-Ethyl-3-hydroxyhexyl 2-methylpropanoate	18.988	0.07	-	-	0.06	-	-
48	Tetradecane	19.763	0.01	-	-	-	-	-
49	Caryophyllene	20.389	0.08	-	-	-	-	-
50	*o*-Benzoquinone	21.244	-	0.03	-	-	-	-
51	*β*-Ionone	21.792	-	-	0.03	-	-	-
52	Dihydroactinidiolide	23.041	0.04	0.04	0.00	0.00	0.06	0.03
53	Dodecanoic acid	23.665	0.27	0.05	0.04	0.41	0.81	0.09
54	2,2,4-Trimethyl-1,3-pentanediol di-isobutyrate	24.445	0.06	-	-	-	-	-
55	Tetradecanal	25.025	-	0.01	-	-	-	-
56	Methyl didecanoate	25.263	0.11	0.12	0.14	-	0.12	-
57	1-Tetradecanol	26.502	-	0.02	-	-	-	-
58	1-Nonadecene	26.9	0.23	0.12	-	6.12	-	-
59	Hexadecanal	27.368	-	-	-	-	-	-
60	1-Heptacosanol	28.112	-	-	-	-	-	-
61	Nonacosane	28.186	-	-	-	-	-	-
62	Pentadecanoic acid	28.276	0.37	0.35	0.17	-	-	-

Table 3. Continued

63	2-Butyl-2-octenal	28.477	-	0.32	0.25	-	-	-
64	1-Hexadecanol	28.761	-	-	0.22	-	-	0.13
65	Ethyl eicosanoate	29.054	-	-	-	-	-	-
66	1-Octadecene	29.125	-	-	-	-	-	-
67	Isopropyl myristate	29.728	-	-	0.03	-	-	-
68	Tetradecanoic acid	29.809	-	-	-	0.58	0.98	-
69	3,7,11-Trimethyl-1-dodecanol	29.897	-	-	-	-	-	-
70	9-Eicosen-1-ol	30.026	-	1.47	1.24	1.38	0.33	-
71	6,10,14-Trimethyl-2-pentadecanone	30.106	3.44	-	0.14	1.19	-	-
72	3,7,11,15-Tetramethyl-1-Hexadecanol	30.162	1.51	0.28	0.49	0.74	-	-
73	3-Methyl-heptadecane	30.599	-	-	-	-	-	-
74	Octadecanal	30.917	0.25	-	-	-	-	-
75	9-Tricosene	31.106	-	-	-	-	-	-
76	*β*-Farnesene	31.579	1.18	1.12	0.10	0.49	-	0.04
77	9,12,15-Octadecatrienoic acid	31.973	-	-	0.17	-	-	-
78	Palmitoleic acid	32.075	-	1.74	0.55	-	5.86	-
79	Hexadecanoic acid	32.571	4.03	10.22	12.34	4.29	9.83	7.18
80	Ethyl hexadecanoate	33.171	0.30	0.46	5.50	-	0.29	0.62
81	Arachidonic acid	34.217	5.70	3.77	2.06	3.05	2.02	2.39
82	Tetracosanoic acid	34.441	-	-	-	-	-	-
83	Octacosanol	34.929	-	-	-	-	-	-
84	Isopropyl palmitate	35.022	-	-	-	-	-	-
85	Phytol	35.349	1.81	4.63	1.59	3.54	2.96	2.52
86	Linoelaidic acid	35.857	2.86	12.89	19.27	4.31	12.96	7.84
87	9,12-Tetradecanyl acetate	35.966	3.64	11.50	15.66	1.31	10.24	12.31
88	9-Octadecenoic acid	36.037	3.42	4.11	-	1.63	2.94	3.51
89	Octadecanoic acid	36.345	0.92	1.17	-	1.07	-	0.94
90	Ethyl 9,12,15-octadecatrienoate	36.421	0.30	0.68	-	-	-	0.70
91	Ethyl Oleate	36.552	0.24	0.46	0.48	-	-	-
92	17-methyl-Octadecanoate	36.924	-	-	-	-	-	-
93	Methyl arachidonate	38.835	-	-	-	-	0.88	-

Table 3. Continued

94	8,11,14-Eicosatrienoic acid	39.005	-	-	-	-	-	-
95	*β*-Carotene	39.837	-	0.14	-	-	-	-
96	Glycerol 2-hexadecanoate	42.23	8.45	6.27	10.36	12.82	15.71	19.08
97	Isopropyl linoleate	45.032	-	1.32	-	-	-	-
98	1-Glyceryl stearate	45.391	4.79	1.74	-	6.86	4.02	10.32
99	Squalene	46.806	0.42	0.30	0.37	-	0.63	0.69
100	2-Hexyl-1-decanol	48.023	0.19	-	0.23	0.32	-	0.79
101	Batilol	48.439	-	-	-	-	-	-
102	*α*-Tocopherol	49.985	5.64	3.63	3.08	7.68	2.59	3.02
103	Cholesterol	50.980	0.68	0.70	-	-	1.03	-
104	16-Dehydropregnenolone	52.533	2.69	-	0.53	1.44	1.39	0.53
105	Campesterol	52.68	26.79	16.26	11.19	19.22	11.96	12.45
106	Stigmasterol	53.121	-	2.90	1.83	3.69	0.87	3.49
107	*β*-Sitosterol	54.257	17.41	9.78	11.14	13.79	10.79	10.44
	Total		**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**

[†]n.d: not detected

Biofilm formation

Pseudomonas aeruginosa PAO1 was incubated in Brain Heart Infusion (BHI) Broth for 24 h at 37°C. A final inoculum was prepared in BHI Broth with 2% sucrose, containing approximately 106 CFU/mL of P. aeruginosa.100 µL of the bacterial suspensions were added to 96-well microtiter plates for each test condition. The plates were incubated at 37°C for 72 h to form mature biofilms.

Treating the biofilm cells with the extracts

After the mature biofilms formed, the wells were washed with sterile phosphate-buffered saline (PBS, pH 7.2) to remove non-adhered cells. Then, the extracts (10 mg/mL) were transferred into the mature biofilm wells. The plates were incubated at 37°C for 24 h. After the incubation, the contents of the wells were aspirated and washed with PBS. The plates were dried at room temperature for 1 h. Then, 100 µL of 0.5% crystal violet solution was added to each well to stain the biofilm cells. After 30 min, the wells were washed with PBS, followed by adding a solution of acetone-alcohol (30:70 v/v) into the wells to dissolve the dye bound within the biofilm matrix. BHI Broth with 2% sucrose and 10% DMSO were used as negative controls. The optical density of the dissolved crystal violet dye was measured using a microplate reader (Thermo Scientific Multiskan GO Microplate Spectrophotometer, Vantaa, Finland) at 620 nm (OD 620 nm). The percentage biofilm inhibition values were calculated according to the following formula:

% Biofilm inhibition = [(OD (growth control)/ OD (sample)) /OD (growth control)] x 100

Anti-quorum sensing activity

The anti-quorum sensing activity was determined using the disc diffusion method with *Chromobacterium violaceum* ATCC 12472 as the reporter bacteria (Gajdács & Spengler, 2020; Batohi et al. 2021). The bacterial suspension was adjusted to $1.5x10^8$ CFU/mL with the overnight culture and inoculated on Luria Bertani Agar. Then, sterile blank discs (6 mm diameter; Bioanalyse®, Ankara, Türkiye) impregnated with 20 L twenty microliters of the extracts (10 mg/mL) were placed on the medium. After incubation at 30°C for 24 h, the plates were observed for a zone of violacein inhibition. The formation of an inhibition zone around the disc was noted as the potential anti-quorum sensing activity.

RESULTS AND DISCUSSION

Bryophytes are used in traditional medicine for many health disorders (Singh, Singh, Nath, Sahu, & Singh Rawat, 2011). the main purpose of this study was to determine the volatile components using GC/MS (Table 3) and analyze the antibacterial,

antibiofilm, and anti-quorum sensing activities of *Cinclidotus* species.

In previous studies, several p-terphenyl derivatives from the ethyl acetate extract of *Homalia trichomanoides* (Hedw.) Brid. displayed antifungal activity (MIC: 2.0, 2.0, and 0.6 µg/mL) against *Candida albicans* (Wang, Yu, & Lou, 2005). In another study, the methanol extracts of the mosses exhibited moderate antimicrobial activity against Gram-negative and Gram-positive bacteria (Dulger, Yayıntaş, & Gonuz, 2005). The ethanol extract of *Bryum argenteum* against all bacteria and fungi exhibited an antibacterial effect by the microdilution method (Sabovljevic, Sokovic, Sabovljevic, & Grubisic, 2006). The mosses were highly active against Gram-positive and Gram-negative bacterial and fungal strains (Singh, Rawat, & Govindarajan, 2007). The methanol extracts (80%) of some mosses also showed antibacterial activity, especially *Hylocomium splendens* (Hedw.) Schimp. extract was the strongest (Kang, Kim, Liu, Jovel, Towers, 2007). However, some are used as a remedy for infections and skin diseases in humans with marked antimicrobial effects (Singh et al., 2007). The antioxidant activity of ethanol extracts from *Atrichum undulatum* (Hedw.) P. Beauv. and *Polytrichum formosum* Hedw. was stronger than that of *Pleurozium schreberi* (Willd. ex Brid.) Mitt. and *Thuidium tamariscinum* (Hedw.) Schimp. (Chobot, Kubicová, Nabbout, Jahodář, Hadacek, 2008). The water extract of *Ptychostomum moravicum* (Podp.) Ros&Mazimpaka were found to have moderate antioxidant activity (Pejin, Bogdanovic-Pristov, Pejin, & Sabovljevic, 2013). The water extracts of the Peat moss called *Sphagnum* sp. showed anti-inflammatory and antioxidant effects, suggesting that they can suppress inflammation and prevent oxidative stress and cellular damage (Choi et al., 2014). The ethanolic extract from the Chilean native

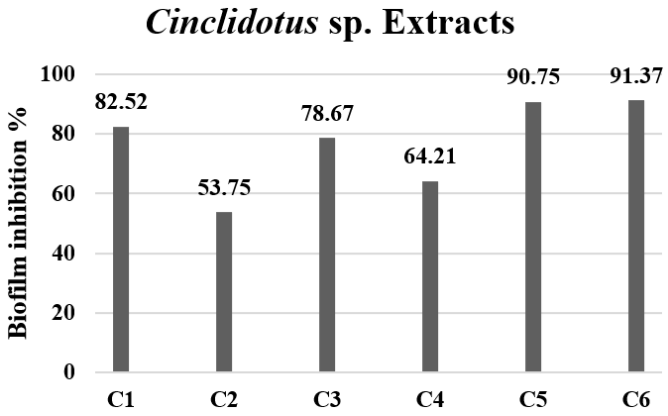

Figure 1. Antibiofilm activity of *Cinclidotus* spp. Extracts C1: *C. pachloma*, C2: *C. bistratosus*, C3: *C. riparius*, C4: *C. fontinaloides*, C5 C5: *C. aquaticus*, C6: *C. pachylomoides*

moss *Sphagnum magellanicum* Brid. created inhibitory effects against Gram-negative and Gram-positive bacteria. In addition, the presence of vanillic, chlorogenic, syringe, caffeic, gallic,

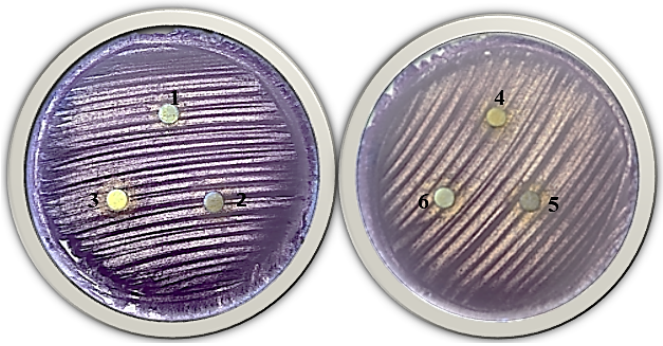

Figure 2. QS inhibitory activity of the *Cinclidotus* spp. Extracts C1: *C. pachloma*, C2: *C. bistratosus*, C3: *C. riparius*, C4: *C. fontinaloides*, C5: *C. aquaticus*, C6: *C. pachylomoides*

3-4 hydroxybenzoic, p-coumaric, and salicylic acids was determined by RP-HPLC (Montenegro, Portaluppi, Salas, Diaz, 2009). The butanol fractions of some mosses have significant effects against Gram-positive bacteria, especially against *Staphylococcus aureus* (Singh et al., 2011). The ethanol and chloroform extracts of the common mosses from Northern Europe exhibited antimicrobial activity against several bacterial strains, but the most potent one was *Polytrichum commune* Hedw. (Klavina et al., 2015). Ethanol and water extracts of *Cinclidotus fontinaloides* and *Palustriella commutata* (Hedw.) Ochyra exhibited a significant antibacterial effect, especially in the *C. fontinaloides* and *P. commutata* ethanol extracts. In addition, the potent antioxidant effects of these mosses were also observed (Yayıntaş, Alpaslan, Karagul, Yilmaz, & Sahiner, 2017). The extract from *Ptychostomum capillare* (Hedw.) Holyoak & N. Pedersen showed a 3-5% biofilm inhibition against *S. epidermidis*; and reduced the effect of H2O2 (Onbasli & Yuvali, 2021). Mosses from the Anatolian flora were also evaluated for their potential antimicrobial, antioxidant, anthocyanin, and allelopathic effects (Yayıntaş et al., 2017; Turkyılmaz Ünal et al., 2017). All results showed that mosses are rich sources of natural compounds and good samples for biological activities. Therefore, this preliminary information shows that it is noteworthy to investigate the *Cinclidotus* species. Consequently, among the tested extracts, C1 and C2 exhibited an antibacterial effect against S. aureus ATCC 25923 and S. aureus ATCC 43300 (MRSA) with a MIC value of 10 mg/mL (Table 4). Nevertheless, none of the extracts showed antibacterial activity against the test bacteria except for these. The percentage biofilm inhibition values of C1, C2, C3, C4, C5, and C6 were determined as 82.52%-53.75%-78.67%-64.21%-90.75% and 91.37%, respectively (Figure 1). The appearance of a transparent inhibition zone around the disc indicated the potential occurrence of QS inhibitory activity (Figure 2). However, the other tested Cinclidotus sp. extracts exhibited anti-quorum sensing activity.

Table 4. Minimum inhibitory concentration (MIC) values (mg/mL) of *Cinclidotus* species extracts against the tested bacteria

Cinclidotus sp. Extracts	Gram-positive Bacteria			Gram-negative Bacteria		
	***Staphylococcus aureus* ATCC 25923**	***Staphylococcus aureus* ATCC 43300(MRSA)**	***Enterococcus faecalis* ATCC 29212**	***Escherichia coli* ATCC 25922**	***Klebsiella pneumoniae* ATCC 13883**	***Pseudomonas aeruginosa* ATCC 27853**
C1	10	10	-	-	-	-
C2	10	10	-	-	-	-
C3	-	-	-	-	-	-
C4	-	-	-	-	-	-
C5	-	-	-	-	-	-
C6	-	-	-	-	-	-
Ampicillin	0.0016	0.05	NT	NT	NT	NT
Ciprofloxacin	NT	NT	0.0625	NT	0.0625	NT
Ofloxacin	NT	NT	NT	0.001	NT	0.008
DMSO (10%)	-	-	-	-	-	-

"-": represents no activity. C1: *Cinclidotus pachloma,* C2: *C. bistratosus,* C3: *C. riparius,* C4: *C. fontinaloides, C5* C5: *C. aquaticus,* C6: *C. pachylomoides* NT: not tested

CONCLUSION

In this study, the volatile compounds of the tested *Cinclidotus* species collected from different localities of Türkiye were analyzed by GC/MS and examined in terms of their biological activity. The result of the phytochemical analysis in this study is that there are bioactive components in ether extracts. The investigated extracts showed reasonable activity at the concentrations evaluated. Based on the data presented, it was concluded that mosses are promising candidates that could be useful in the prevention or treatment of various pathological conditions. However, further *in vitro* and *in vivo* studies should focus on a single component or the mechanisms.

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