© by PSP Volume 32– No. 03/2023 pages 1266-1273 Fresenius Environmental Bulletin

In vitro ANTIMICROBIAL, ANTIOXIDANT ACTIVITY, AND BIOCHEMICAL COMPOSITION OF PARASITIC PLANT *Cytinus hypocistis* FROM TURKIYE

Mustafa Eray Bozyel[1,*], Kerem Canli[2,3], Atakan Benek[4], Dilay Turu[2], Ozcan Simsek[5], Ergin Murat Altuner[4]

[1]Department of Biology, Faculty of Arts and Sciences, Canakkale Onsekiz Mart University, Canakkale, Turkiye
[2]Department of Biology, Faculty of Science, Dokuz Eylul University, Buca, Izmir, Turkiye
[3]Fauna and Flora Research and Application Center, Dokuz Eylul University, Buca, Izmir, Turkiye
[4]Department of Biology, Faculty of Arts and Sciences, Kastamonu University, Kastamonu, Turkiye
[5]Department of Forestry, Yenice Vocational School, Canakkale Onsekiz Mart University, Yenice, Canakkale, Turkiye

ABSTRACT

Cytinus hypocistis (L.) L. (Cytinaceae) is a Mediterranean parasitic plant used traditionally. This study aimed to investigate the *in vitro* antimicrobial, antioxidant activity, and biochemical composition of *C. hypocistis* ethanol extract (CHEt). The biochemical composition of CHEt was analyzed by GC-MS. The antimicrobial activity was investigated using a disk diffusion test against 39 bacteria (including 11 multidrug-resistant strains) and 2 fungi strains. Thirteen antibiotics in all strains except MDR strains and thirty-three antibiotics in MDR strains were used as positive controls. The antioxidant activity was determined by the DPPH method. The major component is 1,2,3-Benzenetriol. CHEt has antimicrobial activity against sixteen strains. CHEt displayed the highest activity against *Enterococcus faecium* (FI2) is a Gram-positive bacteria strain in 100 µL with a 23 mm inhibition zone. Also, *Proteus vulgaris* (MDR6), which is Gram-negative bacteria, is the most sensitive strain with a 24 mm inhibition zone in 100 µL of CHEt. Especially, the data from the *A. baumannii* (MDR3) strain is very striking because a higher activity has been observed than all positive controls. CHEt showed high antioxidant activity against Ascorbic acid. In light of all the results, it was determined that *C. hypocistis* has high antimicrobial and antioxidant potential.

KEYWORDS:
Cytinus hypocistis, GC-MS, disk diffusion, DPPH, Multidrug-resistant bacteria

INTRODUCTION

It is known that plants have a very large share in the continuity of human health and improving the quality of life [1]. In the historical process, plants have been used by people for very different purposes. It is known that plants were first used to make medicines, and then perfumes or dyes. The first evidence of the use of plants for medicinal purposes was found in Mesopotamia in 2600 BC. In the first evidence written in cuneiform on earthen tablets, it was stated that the contents of about 1000 plants were used in the treatment. Such plants as *Cedrus* species, *Cupressus sempervirens*, *Glycyrrhiza glabra*, and *Papaver somniferum* are found on tablets. These plants are also used today to treat many ailments, from coughs to colds [2].

Today, the leading countries of the world are focusing on applications of plant origin for the treatment of diseases, since drugs created from pure, synthetic, or semi-synthetic raw materials create undesirable side effects [3].

As a result of various studies conducted with plants, it has been determined that plants have chemicals called secondary metabolites in their structure. These substances give plants various advantages in the relationship of plants with the ecosystem, their adaptation, important events such as defense, protection, maintenance of life, and reproduction [4]. In the studies so far, an average of about 100.000 secondary metabolites found in the plant structure has been identified by isolating them from plants. This number is increasing with the addition of more than 4000 compounds every year [5].

It has long been known that various plant extracts and essential oils have antibacterial and antifungal activity for some bacteria and fungi [6]. This effect is due to antimicrobial substances in the structure of plants. Secondary compounds of biological origin that inhibit the development of microorganisms even in very low amounts are called antimicrobial substances. These substances can have a bacteriostatic or fungistatic effect that stops the reproduction of microorganisms, or they can have a bactericidal or fungicidal effect that causes the death of microorganisms [7]. Antimicrobial substances that can inhibit the growth of many microorganisms have been isolated from the leaves, stems, seeds, and roots of naturally growing plants, and the activities of

these substances have been tested on microorganisms. In the 1950s, the discovery of antibiotics resulted in the use of antibiotics as antimicrobial agents instead of plants. However, with the research conducted, the limited time when antibiotics are effective, the decrease in antibiotics released on the market, and people's awareness of the dangers that may arise as a result of excessive antibiotic use brought the preference of herbal extracts as medicine back to the forefront towards the end of the 90s [8]. The increase in bacteria that are resistant to antibiotics currently used in treatments, especially penicillin, causes the effect of these compounds in treatments to be significantly reduced. Since the mechanism of action of plants is different from that of antibiotics, plants are also able to inactivate resistant bacteria. In this case, plants, in addition to their therapeutic properties, can also be used as models in studies conducted to create new antibacterial drugs. For this reason, plant substances are being investigated to determine their properties, such as microbiological, pharmacological, and plant defense mechanisms [9].

In addition to antimicrobial agents, it is also known that there are different antioxidant substances in many plants that prevent the formation of certain compounds harmful to living things. Natural antioxidants are most often found in the structure of seeds, leaves, and stems of plants. But there are also antioxidant substances obtained from other plant tissues [10]. The most prominent among the natural antioxidants are carotenoids, vitamins, phenols, flavonoids, glutathione, and endogenous metabolites. Antioxidant substances of plant origin are singlet and triplet oxygen scavengers, free radical scavengers, peroxide degraders, enzyme inhibitors, and synergists [11]. A large number of antioxidant substances are found in the structure of vegetables and fruits [12, 13]. Various studies indicate that people who eat more fruits and vegetables are less sick, have a lower risk of heart attack and the likelihood of developing cancer, and the death rate is reduced [14].

They are most often found in green and red-leaved plants. It is also known that vitamins A, C, and E are natural antioxidants [15].

It has been indicated by studies that it will be useful to apply antioxidant-containing compounds topically to heal wounds and protect tissues from damage of oxidative reactions [16]. Some plants are used to treat newly formed wounds, while others are used to treat chronic, long-term existing wounds [17].

Cytinus is a genus of non-chlorophyllic plant species, represented by 8 species parasitizing the roots of the genus *Cistus* (Cistaceae). *Cytinus* species are rootless, sessile, and leafless; flowers are only seen when they emerge from the host plant during the generative phase [18, 19]. Only two species are distributed in Turkiye: *C. hypocistis* and *C. ruber* [20].

C. hypocistis (L.) L. (Cytinaceae) is a Mediterranean endophytic parasitic plant used traditionally for the treatment of throat tumor, dysentery, eye inflammation, and constipation and exhibits several properties such as astringent and emmenagogue [18, 21, 22].

We have determined that the antimicrobial activity of *C. hypocistis* ethanol extract (CHEt) against a wide range of microorganisms, including multidrug-resistant strains, has not been adequately studied at the end of the literature review. Therefore to avoid toxic effects and to serve as the basis for plant-based drug molecule determination studies in the later stages, ethanol was preferred as a solvent when preparing plant extract in this study.

In this study, we analyzed the antimicrobial and antioxidant activity and biochemical composition of CHEt.

MATERIALS AND METHODS

Plant samples. *C. hypocistis* samples were collected under *Cistus salviifolius* from Turgut Reis Battery, Canakkale, Turkiye (40°02'48" N, 26°21'42" E) and identified by Dr. Bozyel. The plant aerial parts were placed in sample bags and transported to our laboratory. The samples were air-dried at room conditions. The voucher specimens were deposited at the Fauna and Flora Research and Application Center, Dokuz Eylül University, Buca, Izmir, Turkiye (Personel herbarium number FFDEU-ERA1741).

Extraction method. Dried *C. hypocistis* aerial part samples were ground to obtain a fine powder, to increase the surface area for extraction. The active compounds were extracted by ethanol (Sigma Aldrich) through shaking at room temperature for two days. After filtering through Whatman No. 1 filter paper, the ethanol in the extract was evaporated at 45°C under vacuum by using a rotary evaporator (Heidolph Hei-Vap Value HL/HB-G1) [23]. The remnant was weighed and an extract stock was prepared by using a defined volume of ethanol, and 20 µL, 50 µL, and 100 µL of the extracted stock were transferred on empty sterile antibiotic disks to load 4.016, 10.04, and 20.08 mg extracts on disks respectively.

Antimicrobial activity. The disk diffusion test, which was described previously in detail by Bozyel et al. [24] was used for testing the antimicrobial activity of CHEt against 11 standard (ST), 11 multidrug-resistant (MDR), 10 clinical isolated (CI), and 7 food isolated (FI) bacteria, and 1 standard (ST) and 1 clinical isolated (CI) fungi strains. The incubation conditions for microorganisms excluding fungi strains were 37°C for 24 hours, but 27°C for 48 hours for *C. albicans* and *C. tropicalis*. Inoculum for each

microorganism was prepared in 0.9% sterile saline solution and the turbidity of all inocula was adjusted by comparing with 0.5 McFarland standard. The Petri dishes containing disks, on which the ethanol extract was loaded, incubated according to the suitable time-temperature combinations mentioned above, and the inhibition zones were observed and recorded in millimeters. Empty antibiotic disk and ethanol-loaded disk were used as negative controls. A wide range of antibiotics was used as positive controls.

Antioxidant activity. DPPH• (2,2-diphenyl-1-picryl-hydrazyl) free radical scavenging activity of CHEt and ascorbic acid as a positive control were tested with the method described by Mensor et al. [25] (2001). Decreasing of DPPH• absorbance, induced by the extract was monitored at 517 nm after incubation at 28 °C for 30 min with a spectrophotometer (BioTek Microplate Spectrophotometer) [26].

GC-MS analysis. The composition of CHEt was determined according to the protocol given in previous studies by GC-MS analysis. For the identification of chemical components, each sample was analyzed by Agilent GC 6890N-Agilent MS 5973 equipped with HP5-MS capillary column (30 mx0.25 mm; coating thickness 0.25 µm). Analytical conditions were an injector temperature of 350 °C; carrier gas helium at 1 mL/min; injection mode: split, split ratio 10:1; volume injected: 1 µL of sample in ethanol extract; oven temperature programmed from 40 °C to 350 °C at 4 °C/min; pressure: 48.2

TABLE 1
Antimicrobial activity of CHEt

Lab. Code	Microorganisms	20 µL*	50 µL*	100 µL*
ST1	*Bacillus subtilis* DSMZ 1971	-	-	15,00 ± 0,00
ST2	*Candida albicans* DSMZ 1386	-	-	-
ST3	*Enterobacter aerogenes* ATCC 13048	-	-	-
ST4	*Enterococcus faecalis* ATCC 29212	-	-	-
ST5	*Escherichia coli* ATCC 25922	-	-	-
ST6	*Listeria monocytogenes* ATCC 7644	-	-	-
ST7	*Pseudomonas aeruginosa* DSMZ 50071	-	-	17,00 ± 0,00
ST8	*Pseudomonas fluorescens* P1	-	-	-
ST9	*Salmonella enteritidis* ATCC 13076	-	15,00 ± 0,58	19,00 ± 0,00
ST10	*Salmonella typhimurium* SL 1344	-	12,00 ± 0,00	20,00 ± 0,00
ST11	*Staphylococcus aureus* ATCC 25923	15,00 ± 0,00	16,00 ± 0,00	19,00 ± 0,58
ST12	*Staphylococcus epidermidis* DSMZ 20044	-	-	-
FI1	*Enterococcus durans*	-	-	-
FI2	*Enterococcus faecium*	-	15,00 ± 0,00	23,00 ± 0,00
FI3	*Klebsiella pneumoniae*	-	-	-
FI4	*Listeria innocua*	-	-	-
FI5	*Salmonella infantis*	12,00 ± 0,00	14,00 ± 0,00	19,00 ± 0,00
FI6	*Salmonella kentucky*	-	14,00 ± 0,58	19,00 ± 0,00
FI7	*Escherichia coli*	-	-	-
CI1	*Staphylococcus aureus*	10,00 ± 0,00	12,00 ± 0,00	17,00 ± 1,15
CI3	*Staphylococcus hominis*	-	-	-
CI4	*Staphylococcus haemolyticus*	-	-	-
CI5	*Staphylococcus lugdunensis*	-	-	-
CI6	*Shigella boydii*	-	-	-
CI7	*Acinetobacter baumannii*	-	-	-
CI8	*Shigella flexneri*	-	-	-
CI9	*Staphylococcus aureus*	-	-	-
CI10	*Enterococcus faecalis*	-	-	-
CI11	*Klebsiella pneumoniae*	-	-	-
CI12	*Candida tropicalis*	-	-	-
MDR1	*Escherichia coli*	-	-	-
MDR2	*Klebsiella pneumoniae*	-	-	-
MDR3	*Acinetobacter baumannii*	-	8,00 ± 0,00	19,00 ± 0,00
MDR4	*Enterobacter aerogenes*	14,00 ± 0,00	16,00 ± 0,00	22,00 ± 0,58
MDR5	*Serratia odorifera*	11,00 ± 0,00	12,00 ± 0,00	17,00 ± 0,00
MDR6	*Proteus vulgaris*	12,00 ± 0,00	16,00 ± 0,00	24,00 ± 0,00
MDR7	*Streptococcus pneumoniae*	14,00 ± 0,00	18,00 ± 0,00	22,00 ± 0,00
MDR8	*Staphylococcus aureus* MRSA	10,00 ± 0,00	13,00 ± 0,00	18,00 ± 1,15
MDR9	*Staphylococcus aureus* MRSA+MDR	12,00 ± 0,00	14,00 ± 0,58	18,00 ± 0,00
MDR10	*Providencia rustigianii*	-	-	-
MDR11	*Achromobacter* sp.	-	-	-

"-": No activity, *: The data is given as the mean values of three replicates with standard errors

kPa; and split flow: 9.9 mL/min. The MS scan conditions were a transfer line temperature of 280 °C, an interface temperature of 280 °C, and an ion source temperature of 230 °C. Identification of the components was conducted by matching the retention times against National Institute of Standards and Technology (NIST Mass Spectrometry DATA CENTER) data library, and crosscheck was applied with previously published data [27].

Statistics. All tests were applied as triplicates. One-way analysis of variance (ANOVA), which is a parametric method was performed ($P = 0.05$). Pearson correlation coefficient was determined for any possible correlation between the intensity of antimicrobial activity and concentration. R Studio, version 3.3.2 was used for statistical analysis [28].

RESULTS AND DISCUSSION

Antimicrobial activity of CHEt. The data obtained from the study about the inhibition zone diameters are shown in Table 1. According to the results, negative controls show no activity [29]. Additionally, statistical analysis verified that the differences between the results of three replicates of each extract volume were statistically non-significant ($p>0.05$). In addition, obtaining a Pearson correlation coefficient of 0.1881 presented a very weak positive correlation between the antimicrobial activity and the volumes of extracts used. Also, thirteen antibiotics in standard, food isolated, and clinical isolated strains (Table 2) and thirty-three antibiotics in multidrug-resistant strains were used as positive controls (Table 3).

According to Table 1, CHEt shows antimicrobial activity against 16 of 41 strains. High susceptibility (≥ 15 mm) was observed in these strains [30]. The most susceptible gram-positive bacteria strain is FI2 with a 23 mm inhibition zone and the gram-negative bacteria strain is MDR6 with a 24 mm inhibition zone. In additionally the CHEt has shown highly effective results against MDR3, MDR4, and MDR7 among MDR bacteria strains. Especially, the data from MDR3 is very striking because a higher activity has been observed than all positive controls.

ST9 and ST10 have more effective results than Tobramycin, Ampicillin, and Cefuroxime. ST11 showed more effective results than Tobramycin, Ceftriaxone, and Vancomycin. FI2 has more effective results than only Tobramycin and Chloramphenicol. FI5 has more effective results than Gentamicin, Tobramycin, Ampicillin, and Cefuroxime, while FI6 showed the same effect except for Cefuroxime. MDR3 showed high activity than all antibiotics. It was determined that MDR4 and MDR5 are more effective than 14 antibiotics, MDR6 is more effective than 18 antibiotics, MDR7 is more effective than 20 antibiotics, MDR8 is more effective than 13 antibiotics, and MDR9 is more effective than 6 antibiotics.

TABLE 2
Positive controls against all strains except MDR strains

Lab. Code	1	2	3	4	5	6	7	8	9	10	11	12	13
ST1	30	26	36	44	34	37	38	41	36	44	20	52	42
ST7	15	22	18	-	-	9	-	-	-	-	-	-	-
ST9	21	15	24	23	-	28	27	16	-	16	-	28	31
ST10	24	15	35	22	-	27	27	13	-	14	-	26	21
ST11	21	14	22	31	24	21	16	25	22	29	16	30	27
FI2	28	15	28	40	30	11	31	32	24	33	26	43	34
FI5	17	14	22	22	-	28	26	14	-	17	-	26	24
FI6	12	16	24	22	-	29	30	15	-	19	-	26	27
CI1	22	18	23	-	-	-	-	-	-	-	-	-	-

"-": No activity; 1: Gentamicin; 2: Tobramycin; 3: Ciprofloxacin; 4: Cefazolin; 5: Clindamycin; 6: Chloramphenicol; 7: Ceftriaxone ; 8: Ampicillin; 9: Cephalothin; 10: Cefuroxime; 11: Vancomycin; 12: Amoxicillin/Clavulanic acid; 13: Trimethoprim/Sulfamethoxazole

TABLE 3
Positive controls against MDR strains

Lab. Code	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
MDR3	-	-	-	-	-	9	-	-	8	-	8	-	-	-	-	-	-
MDR4	16	18	32	11	-	31	32	-	-	18	-	-	30	15	33	15	-
MDR5	7	9	23	-	-	28	8	-	-	-	8	13	-	-	16	22	10
MDR6	11	11	42	-	9	22	23	9	-	20	-	9	30	10	37	32	12
MDR7	10	8	42	-	9	22	26	9	-	19	9	10	8	10	40	31	15
MDR8	-	7	-	-	45	27	8	12	-	-	26	11	24	14	-	11	12
MDR9	22	21	27	26	38	30	19	22	28	31	19	25	30	15	-	23	23

"-": No activity; 1: Gentamicin; 2: Tobramycin; 3: Ciprofloxacin; 4: Cefazolin; 5: Clindamycin; 6: Chloramphenicol; 7: Ceftriaxone ; 8: Ampicillin; 9: Cephalothin; 10: Cefuroxime; 11: Vancomycin; 12: Amoxicillin/Clavulanic acid; 13: Trimethoprim/Sulfamethoxazole; 14: Clarithromycin; 15: Aztreonam; 16: Piperacillin/Tazobactam; 17: Ampicillin/Sulbactam.

TABLE 3 (Continued)

Lab. Code	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
MDR3	-	10	-	-	-	-	8	16	8	9	-	13	10	-	-	-
MDR4	31	8	8	-	-	8	29	14	-	28	9	13	25	8	8	30
MDR5	21	9	-	8	-	8	18	14	19	29	10	10	23	8	-	-
MDR6	25	13	-	24	11	8	26	12	12	26	16	-	12	8	-	22
MDR7	27	11	-	24	13	9	29	10	10	30	13	9	13	8	-	14
MDR8	8	-	-	9	40	19	14	-	-	9	7	8	23	-	-	-
MDR9	19	36	17	21	33	18	25	9	20	56	20	8	17	-	8	22

"-": No activity; 18: Ceftazidime; 19: Rifampicin; 20: Oxacillin; 21: Piperacillin; 22: Linezolid; 23: Teicoplanin; 24: Amicasin; 25: Polymyxin B; 26: Cefoxitin; 27: İmipenem; 28: Sulbactam/Cefoperazone; 29: Colistin sulfate; 30: Furazolidone; 31: Optochin; 32: Bacitracin; 33: Cefotaxime.

TABLE 4
DPPH• free radical scavenging activity of CHEt

Concentrations, µg/mL	Content of CHEt, %	Content of ascorbic acid, %
200.000	89.88	94.67
100.000	87.00	93.39
50.000	90.77	92.08
25.000	90.77	90.09
12.500	91.21	69.94
6.250	71.61	35.79
3.125	76.10	17.70
1.075	64.82	8.74

Zucca et al. [18] reported the antimicrobial activity of aqueous, cyclohexane, and ethanolic extracts of *C. hypocistis* against *E. coli* DSM 1103, *E. cloacae* DSM 30054, *P. aeruginosa* DSM 1117, *A. baumanii* DSM 30007, *K. pneumoniae* DSM 681, *S. aureus* DSM 1104, *S. aureus* MRSA, *S. epidermidis* DSM 1798, and *E. faecalis* DSM 2570. Ethanolic extract of *C. hypocistis* showed antimicrobial activity against *A. baumanii* DSM 30007, *S. aureus* DSM 1104, *S. aureus* MRSA, *S. epidermidis* DSM 1798, and *E. faecalis* DSM 2570. When we compared two studies with each other, it appears that CHEt has high antimicrobial activity against *A. baumanii*, *S. aureus*, and *S. aureus* MRSA.

Canlı et al. [30] researched the antimicrobial activity of *Lavandula stoechas* (Lamiaceae) ethanol extract against 22 bacteria strains and 1 fungus strain. These strains were the same as tested strains in the present study. The authors found that *L. stoechas* ethanol extract showed antimicrobial activity against 21 of 23 strains. Our results presented that CHEt is more effective than all strains except *C. albicans* DSMZ 1386 (ST2), *E. aerogenes* ATCC 13048 (ST3), *E. faecalis* ATCC 29212 (ST4), *L. monocytogenes* ATCC 7644 (ST6), *P. fluorescens* P1 (ST8), *S. epidermidis* DSMZ 20044 (ST12), *E. durans* (FI1), *L. innocua* (FI4), and *E. coli* (FI7).

Antioxidant activity of CHEt. The results of the DPPH• free radical scavenging activity of CHEt and ascorbic acid are shown in Table 4. According to the results, CHEt showed the highest antioxidant activity in 12.5 µg/mL concentration. Also, EC_{50} values of CHEt and ascorbic acid were determined as 0.131 and 0.359µg/mL, respectively.

Anjum et al. [31] identified that a parasitic plant *Cuscuta* sp. ethanol extract showed the highest antioxidant activity with 50.04% inhibition. Mustarichie et al. [32] reported that a parasitic plant *Scurrula atropurpurea* ethanol extract showed the highest antioxidant activity with 21.29% inhibition. In our study, the antioxidant activity was observed with 64.82% inhibition even at the lowest concentration. It is clearly understood that CHEt has a higher antioxidant potential.

Analysis of the biochemical composition of CHEt. The GC-MS analysis of CHEt with its major components and their composition percentages are given in Table 4. According to Table 5, 1,2,3-Benzenetriol (63.23%), Ethyl gallate (9.87%), and 2-Ethyl-3-methylnaphtho[2,3-b]thiophene-4,9-dione (6.04%) are mainly found in the composition of CHEt.

Benzenediol (catechol) exhibits antibacterial, antifungal, antioxidant, antivirus, anticancer, cytotoxic, and herbicidal activity [33-35]. 1,2,3-Benzenetriol (pyrogallol) possesses antibacterial, antifungal, antioxidant, antiseptic, antidermatitic, anticandidal, antimutagenic, antipsoriatic, and pesticidal activity [36, 37]. Ethyl gallate (3,4,5-trihydroxybenzoic acid ethyl ester) shows antimicrobial, antioxidant, antiparasitic, anticancer, radical scavenger, and cytotoxic activity [38]. Palmitic acid (hexadecanoic acid) demonstrates antibacterial, antifungal, antioxidant, anticancer, anti-inflammatory, anti-androgenic, a 5-alpha-reductase inhibitor, hemolytic, hypocholesterolemic, nematicidal, pesticidal, potent mosquito larvicidal, and cytotoxic activity [39-43]. Linoleic acid (9,12-octadecadienoic acid (z,z)-) has antibacterial, antifungal, anti-inflammatory,

TABLE 5
Biochemical composition of CHEt

No	Retention Time	Components	Formula	Molecular weight, g/mol	%
1	17.586	Benzenediol	$C_6H_6O_2$	110.111	1.40
2	22.929	1,2,3-Benzenetriol	$C_6H_6O_3$	126.110	63.23
3	23.498	Androsta-2,16-diene	$C_{19}H_{28}$	256.426	4.99
4	26.646	2-Ethyl-3-methylnaphtho[2,3-b]thiophene-4,9-dione	$C_{15}H_{12}O_2S$	256.320	6.04
5	33.609	UNKNOWN	-	-	0.15
6	36.595	Ethyl gallate	$C_9H_{10}O_5$	198.173	9.87
7	36.799	Palmitic acid	$C_{16}H_{32}O_2$	256.424	2.88
8	40.746	5-Hydroxy-7,8-dimethoxy-3-(4-methoxyphenyl)-4H-chromen-4-one	$C_{18}H_{16}O_6$	328.316	1.96
9	40.965	Linoleic acid	$C_{18}H_{32}O_2$	280.445	2.09
10	41.702	Octadecanoic acid	$C_{18}H_{36}O_2$	284.477	1.01
11	57.700	UNKNOWN	-	-	0.48
12	58.835	2,3-Dihydroxypropyl stearate	$C_{21}H_{42}O_4$	358.556	5.60
13	69.118	UNKNOWN	-	-	0.30

"-": No information

antiarthritic, antihistaminic, anticoronary, hepatoprotective, and hypocholesterolemic activity [44-48]. Octadecanoic acid (stearic acid) displays antibacterial, antifungal, and anticancer activity [43, 47]. 2,3-Dihydroxypropyl stearate (octadecanoic acid, 2,3-dihydroxypropyl ester) presents antimicrobial, anticancer, antiarthritic, antiasthmatic, diuretic, hepatoprotective, and nematicidal activity [47, 49].

In the light of these data, it is considered that 1,2,3-Benzenetriol, as the main component, is responsible for antimicrobial and antioxidant activity. It is also believed that Ethyl gallate singularly or synergistically contributes to antimicrobial and antioxidant activity. On the other hand, the antimicrobial and antioxidant effects of 2-Ethyl-3-methylnaphtho[2,3-b]thiophene-4,9-dione should be investigated by advanced purification.

CONCLUSION

CHEt has antimicrobial activity against a wide range of 41 microorganism strains. High susceptibility was observed in about 39% of all strains. CHEt has high antioxidant activity against ascorbic acid. In light of all the results, it was determined that CHEt has high antimicrobial and antioxidant potential. 13 compounds were identified in CHEt, the main compounds are 1,2,3-Benzenetriol (63.23%), Ethyl gallate (9.87%), and 2-Ethyl-3-methylnaphtho[2,3-b]thiophene-4,9-dione (6.04%). Purification and further studies are recommended to determine whether the antimicrobial and antioxidant activities are caused by a single known biochemical compound, such as 1,2,3-Benzenetriol, Ethyl gallate, 2,3-Dihydroxypropyl stearate, Palmitic acid, Linoleic acid, Benzenediol, and Octadecanoic acid or by a synergistic effect. Also, some compounds found in the extract are not matching with the library. As a reason for that, this medicinal plant is proposed to contain some unknown molecules and they should be identified and their 3D structure should also be determined. The unknown compound, which consists of 0.97%, should be analyzed in detail. Also, the mode of action(s) of the extract should be determined in further studies.

ACKNOWLEDGEMENTS

The authors acknowledge the Dokuz Eylul University Scientific Research Coordination Unit for funding this research (project number 2019.KB.FEN.014).

Authors contributions. KC and EMA planned the study. KC, MEB, AB, DT, OS, EMA designed, performed the experiments, and analyzed the results. MEB conducted literature retrieval and drafted the manuscript. KC and EMA supervised the experiments, critically reviewed and edited the manuscript. All authors read and approved the final manuscript.

REFERENCES

[1] Craig, W.J. (1999) Health-promoting properties of common herbs. Am. J. Clin. Nutr. 70(3), 491-499.

[2] Newman, D.J., Cragg, G.M., Snader, K.M. (2000) The influence of natural products upon drug discovery. Nat. Prod. Rep. 17(3), 215-234.

[3] Baytop, T. (1999) Curing with plants in Turkey, in the past and today (in Turkish), Nobel Medical Books, Istanbul.

[4] Bourgaud, F., Gravot, A., Milesi, S., Gontier, E. (2001) Production of plant secondary metabolites: a historical perspective. Plant Sci. 161(5), 839-851.

[5] Verpoorte, R., van der Heijden, R.T.J.M., Ten Hoopen, H.J.G., Memelink, J. (1999) Metabolic engineering of plant secondary metabolite pathways for the production of fine chemicals. Biotechnol. Let. 21(6), 467-479.
[6] Kivanc, M., Akgul, A. (1986) Antibacterial activities of essential oils from Turkish spices and citrus. Flavour Fragr. J. 1(4-5), 175-179.
[7] Taskin, E., Caki, Z., Ozturk, M., Taskin, E., Kurt, O. (2010) Antimicrobial and antitumoral activities of marine algae. Rev. Hydrobiol. 3(1), 37-50.
[8] Sahin, G. (2007) Antibacterial effect of some *Paeonia* species collected from Turkey (in Turkish), Ankara University, Graduate School of Natural and Applied Sciences, Ankara.
[9] Kalaycioglu, A., Oner, C. (1994) Investigation of antimutagenic effects of some plant extractions with Ames-Salmonella test system (in Turkish). Turk. J. Bot. 18, 117-122.
[10] Ramarathnam, N., Osawa, T., Namiki, M., Kawakishi, S. (1988) Chemical studies on novel rice hull antioxidants. 1. Isolation, fractionation, and partial characterization. J. Agric. Food Chem. 36(4), 732-737.
[11] Larson, R.A. (1988) The antioxidants of higher plants. Phytochemistry. 27(4), 969-978.
[12] Cao, G., Sofic, E., Prior, R.L. (1997) Antioxidant and prooxidant behavior of flavonoids: structure-activity relationships. Free Radic. Biol. Med. 22(5), 749-760.
[13] Wang, H., Cao, G., Prior, R.L. (1996) Total antioxidant capacity of fruits. J. Agric. Food Chem. 44(3), 701-705.
[14] Ak, T. (2006) Determination of antioxidant and antiradical properties of curcumin (in Turkish), Ataturk University, Graduate School of Natural and Applied Sciences, Erzurum.
[15] Anwar, H., Hussain, G., Mustafa, I. (2018) Antioxidants from natural sources in antioxidants in foods and its applications. In: Antioxidants in Foods and Its Applications (Shalaby, E., and Azzam, G. M., eds.). IntechOpen, London. 3-28.
[16] Kumar, B., Vijayakumar, M., Govindarajan, R., Pushpangadan, P. (2007) Ethnopharmacological approaches to wound healing - exploring medicinal plants of India. J. Ethnopharmacol. 114(2), 103-113.
[17] Khalil, E.A., Afifi, F.U., Al-Hussaini, M. (2007) Evaluation of the wound healing effect of some Jordanian traditional medicinal plants formulated in Pluronic F127 using mice (*Mus musculus*). J. Ethnopharmacol. 109(1), 104-112.
[18] Zucca, P., Pintus, M., Manzo, G., Nieddu, M., Steri, D., Rinaldi, A.C. (2015) Antimicrobial, antioxidant and anti-tyrosinase properties of extracts of the Mediterranean parasitic plant *Cytinus hypocistis*. BMC Res. Notes. 8, 1-9.
[19] Maisetta, G., Batoni, G., Caboni, P., Esin, S., Rinaldi, A.C., Zucca, P. (2019) Tannin profile, antioxidant properties, and antimicrobial activity of extracts from two Mediterranean species of parasitic plant *Cytinus*. BMC Complement. Altern. Med. 19(1), 1-11.
[20] Guner, A., Aslan, S., Ekim, T., Vural, M., Babac, M.T. (2012) List of Turkish Flora (Vascular Plants) (in Turkish), Publication of Nezahat Gokyigit Botanical Garden and Flora Research Foundation, Istanbul, 408.
[21] Aksoy, N., Gunes Ozkan, N., Aslan, S., Kocer, N. (2017) Plants used as medicinal and edible in the folk culture of Duzce Province. In: History, Culture, and Art in Duzce (Yasayanlar, I., ed.) (in Turkish), Gaye Bookstore, Duzce, 206-221.
[22] Satil, F., Tumen, G., Selvi, S. (2017) Ethnobotanical features of parasitic plants distributed in Balıkesir/Turkey. Biol. Divers. Conserv. 10(3), 7-11.
[23] Canli, K., Bozyel, M.E., Benek, A., Yetgin, A., Akata, I. Altuner, E.M. (2021) Screening of *in vitro* antimicrobial activity of *Sedum hispanicum* ethanol extract and determination of its biochemical composition. Fresen. Environ. Bull. 30(11A), 12614-12619.
[24] Bozyel, M.E., Canli, K., Benek, A., Yetgin, A., Altuner, E.M. (2021) Biochemical composition and *in vitro* antimicrobial activity of endemic *Helichrysum arenarium* ssp. *aucheri* ethanol extract. Fresen. Environ. Bull. 30(2), 869-875.
[25] Mensor, L.L., Menezes, F.S., Leitão, G.G., Reis, A.S., Santos, T.C.D., Coube, C.S., Leitão, S.G. (2001) Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother. Res. 15(2), 127-130.
[26] Turu, D., Bozyel, M.E., Candan, K., Yakan, M.A., Benek, A, Canli K. (2020) *In vitro* antimicrobial and antioxidant activities of *Pyracantha coccinea* fruits ethanol extract. Int. J. Acad. Multidiscip. Res. (IJAMR). 4(12), 89-93.
[27] Canli, K., Yetgin, A., Akata, I., Altuner, E.M. (2017) Antimicrobial activity and chemical composition screening of *Epilobium montanum* root. Indian J. Pharm. Educ. Res. 51(3), 239-243.
[28] Canli, K., Bozyel, M.E., Benek, A., Yetgin, A., Senturan, M., Altuner, E.M. (2020) Chemical composition and *in vitro* antimicrobial activity of *Matthiola tricuspidata* ethanol extract. Fresen. Environ. Bull. 29(10), 8863-8868.
[29] Canli, K., Yetgin, A., Akata, I., Altuner, E.M. (2017) Antimicrobial activity and chemical composition screening of *Anacyclus pyrethrum* root. Indian J. Pharm. Educ. Res. 51(3), 244-248.

[30] Canli, K., Yetgin, A., Benek, A., Bozyel, M.E., Altuner, E.M. (2019) *In vitro* antimicrobial activity screening of ethanol extract of *Lavandula stoechas* and investigation of its biochemical composition. Adv. Pharmacol. Sci. 2019(2019), 1-6.
[31] Anjum, F., Bukhari, S.A., Shahid, M., Anwar, S., Afzal, M., Akhter, N. (2013) Comparative evaluation of antioxidant potential of parasitic plant collected from different hosts. J. Food Process Technol. 4(5), 1-6.
[32] Mustarichie, R., Runadi, D., Ramdhani, D. (2017) The antioxidant activity and phytochemical screening of ethanol extract, fractions of water, ethyl acetate and n-hexane from mistletoe tea (*Scurrula atropurpurea* BL. Dans). Asian J. Pharm. Clin. Res. 10(2), 343-347.
[33] Smolyaninov, I.V., Pitikova, O.V., Poddel'sky, A.I., Berberova, N.T. (2018) Electrochemical transformations and antiradical activity of asymmetrical RS-substituted pyrocatechols. Russ. Chem. Bull. 67(10), 1857-1867.
[34] Kong, Y., Chen, X., Wang, W., Chen, Z. (2011) A novel palygorskite-modified carbon paste amperometric sensor for catechol determination. Anal. Chim. Acta. 688, 203-207.
[35] Topal, S., Kocacaliskana, I., Arslan, O. (2006) Herbicidal potential of catechol as an allelochemical. Z. Naturforsch. C. 61(1-2), 69-73.
[36] Balasubramanian, S., Ganesh, D., Panchal, P., Teimouri, M., Narayana, V.S. (2014) GC-MS analysis of phytocomponents in the methanolic extract of *Emblica officinalis* Gaertn (Indian Gooseberry). J. Chem. Pharm. Res. 6(6), 843-845.
[37] Upadhyay, G., Gupta, S.P., Prakash, O., Singh, M.P. (2010) Pyrogallol-mediated toxicity and natural antioxidants: triumphs and pitfalls of preclinical findings and their translational limitations. Chem.-Biol. Interact. 183(3), 333-340.
[38] Kalaivani, T., Rajasekaran, C., Mathew, L. (2011) Free radical scavenging, cytotoxic, and hemolytic activities of an active antioxidant compound ethyl gallate from leaves of *Acacia nilotica* (L.) Wild. Ex. Delile subsp. *indica* (Benth.) Brenan. J. Food Sci. 76(6), T144-T149.
[39] Ravi, L., Krishnan, K. (2017) Cytotoxic potential of N-hexadecanoic acid extracted from *Kigelia pinnata* leaves. Asian J. Cell Biol. 12(1), 20-27.
[40] Huang, C.B., George, B., Ebersole, J.L. (2010) Antimicrobial activity of n-6, n-7 and n-9 fatty acids and their esters for oral microorganisms. Arch. Oral Biol. 55(8), 555-560.
[41] Kose, Y.B., Iscan, G., Goger, F., Akalin, G., Demirci, B., Baser, K.H.C. (2016) Chemical composition and biological activity of *Centaurea baseri*: new species from Turkey. Chem. Biodivers. 13(10), 1369-1379.
[42] Abubakar, M.N., Majinda, R.R. (2016) GC-MS analysis and preliminary antimicrobial activity of *Albizia adianthifolia* (Schumach) and *Pterocarpus angolensis* (DC). Medicines. 3(1), 3.
[43] Akpuaka, A., Ekwenchi, M.M., Dashak, D.A., Dildar, A. (2013) Biological activities of characterized isolates of n-hexane extract of *Azadirachta indica* A. Juss (Neem) leaves. Nat. Sci. 11(5), 141-147.
[44] Dilika, F., Bremner, P.D., Meyer, J.J.M. (2000) Antibacterial activity of linoleic and oleic acids isolated from *Helichrysum pedunculatum*: a plant used during circumcision rites. Fitoterapia. 71(4), 450-452.
[45] Walters, D., Raynor, L., Mitchell, A., Walker, R., Walker, K. (2004) Antifungal activities of four fatty acids against plant pathogenic fungi. Mycopathologia. 157(1), 87-90.
[46] Jang, A., Srinivasan, P., Lee, N.Y., Song, H.P., Lee, J.W., Lee, M., Jo, C. (2008) Comparison of hypolipidemic activity of synthetic gallic acid–linoleic acid ester with mixture of gallic acid and linoleic acid, gallic acid, and linoleic acid on high-fat diet induced obesity in C57BL/6 Cr Slc mice. Chem.-Biol. Interact. 174(2), 109-117.
[47] Arora, S., Kumar, G. (2018) Phytochemical screening of root, stem and leaves of *Cenchrus biflorus* Roxb. J. Pharmacogn. Phytochem. 7(1), 1445-1450.
[48] Siswadi, S., Saragih, G.S. (2021) Phytochemical analysis of bioactive compounds in ethanolic extract of *Sterculia quadrifida* R. Br. AIP Conf. Proc. 2353, 030098-1–030098-7.
[49] Tadigiri, S., Das, D., Allen, R.C., Vishnu, V.R., Veena, S.S., Karthikeyan, S. (2020) Isolation and characterization of chemical constituents from *B. amyloliquefaciens* and their nematicidal activity. J. Entomol. Zool. Stud. 8(3), 2062-2066.

Received: **30.11.2022**
Accepted: **16.01.2023**

CORRESPONDING AUTHOR

Mustafa Eray Bozyel
Department of Biology,
Faculty of Arts and Sciences
Canakkale Onsekiz Mart University
Canakkale – Turkiye

e-mail: m.erayboyel@gmail.com