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Biological activities of essential oil and methanolic extract of endemic *Dorytoechas hastata* Boiss et Heldr. ex Bentham (Lamiaceae) from Turkey

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Extracts and essential oils from plant sources have always been a subject of interest to scientists for their biological activities and application in food, pharmacy and cosmetic industries. Here, we have explored the essential oil compositions and antimicrobial and antioxidant activities of the essential oil as well as the methanolic extract of Chalba tea, *Dorystoechas hastata* Boiss et Heldr. ex Bentham (Lamiaceae). Essential oil was isolated from the aerial parts by hydrodistillation and analyzed by gas chromatography-mass spectrometry. The main compounds were determined as eucalyptol (1,8-cineol) (25.47%) and borneol (20.06%). Phosphomolybdenum, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, hydrogen peroxide scavenging, β-carotene bleaching activity, ferric-ion reducing power (FRAP), reducing power and cupric ions (Cu^{2+}) reducing antioxidant capacity (CUPRAC) assays were utilized to determine the antioxidant activity. *D. hastata* methanolic extract showed an effective total antioxidant, DPPH scavenging, cupric ions (Cu^{2+}) and ferric ions (Fe^{3+}) reducing activities. Essential oil and methanolic extract were evaluated for antimicrobial activity by the agar diffusion assay against 15 microorganisms. Methanol extract exerted antibacterial effect against all of the bacteria tested. *Streptococcus pneumoniae* was the most sensitive bacteria to extract while *Aeromonas hydrophila* was the most sensitive bacteria to essential oil. According to present results, *D. hastata* may be potential natural antioxidant and antimicrobial agent.

Keywords: Antimicrobial activity, Antioxidant activity, Chalba tea, Phenolic compounds

Reactive oxygen species (ROS) are inevitable byproducts of cellular respiration[1]. The ROS species attack lipids in cell membranes or DNA[2,3]. The most deleterious effect of ROS is "oxidative stress"[3]. Oxidative stress, originated from an increase in ROS production or from a decrease in the antioxidant defence, is characterized by the inability of endogenous antioxidants to counteract the oxidative damage on biological targets[3,4]. Oxidative stress is responsible for causing several diseases including cancer, autoimmune disorders, arthritis, atherosclerosis, diabetes, inflammation, cardiovascular, neurodegenerative diseases, liver disease, ulcerative colitis and aging[4-7]. Antioxidants are substances that delay, prevent or remove oxidative damage to a target molecule[8]. They can interfere with the oxidation process by various mechanisms, including reacting with free radicals, chelating free catalytic metals and acting as oxygen scavengers[2].

Plants have been used to treat various illnesses, infections and even chronic diseases over thousands of years in the traditional medicine[9,10]. Plants are the significant source of phytochemicals such as alkaloids, flavonoids, phenolic compounds and tannins which are naturally occurring in plants[11]. They have numerous biological activities such as antioxidant, antimicrobial, antifungal and anticancer activities, etc. Thus, these compounds can retard oxidative degradation of lipids and extend shelf life of food[10,12-14]. The importance of dietary antioxidant intake in disease prevention has been reported in many researches. Therefore, the antioxidant capacity of natural products, particularly are used by people has raising interest[4,5].

Furthermore, food borne microorganisms are still issues of concern as they affect health and economy. Resistance to conventional antimicrobial regimes is also one of the issues of concern in healthcare. Because of antimicrobial resistance and relatively narrow spectrum of the antimicrobials, numerous investigations are focused on discovery of new natural antimicrobial especially from plants[10,12,15-17].

Dorystoechas hastata Boiss. & Heldr. ex Bentham (Lamiaceae) is a tertiary relict monotypic plant, endemic to Antalya, Turkey[18,19]. Some of the Lamiaceae members have been called "mountain tea"

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(Dağ çayı). However, Baytop[20] reported that true name of *D. hastata* is chalba, commonly known as chalba tea (çalba çayı) around the province of Antalya. The tea prepared from their dried or fresh leaves is used in treatment of colds or flu in traditional medicine. Since ancient times, *D. hastata* has been known in China as a ‘‘Super grade” medicinal herb. It has been various biological activities such as antispasmolytic, antiarthritic, cytotoxicity, sedative, tonic, and astringent activities. Their extract is also used in cases of hemorrhage, menstrual disorders and against miscarriages[21]. Although there are limited numbers studies on phenolic compounds of *D. hastata*[21-25], detailed investigation on the antioxidant and antimicobial activities are lacking. Only, antioxidant activitiy of methanol extract of *D. hastata* were studied by DPPH, TEAC and TBARS assay[24].

Hence, we conducted this study to (i) detect the main constituents of the volatile oil of *Dorystoechas hastata* Boiss & Heldr. ex Bentham (Lamiaceae); (ii) examine the antioxidant activity of methanol extract with different assays: Phosphomolybdenum, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, β-carotene bleaching activity, ferric-ion reducing power (FRAP), hydrogen peroxide scavenging, reducing power and cupric ions (Cu^{2+}) reducing antioxidant capacity (CUPRAC) assays; and (iii) determine the antimicrobial activity against 15 microorganisms.

Materials and Methods

Plant material

Dorystoechas hastata Boiss. & Heldr. ex Bentham was collected from Antalya (Konya altı-Feslikan flatland), Southern Anatolia region of Turkey (36^o48'51"N-30^o22'58"E, 1945 m) during flowering season in July 2015. The voucher specimen (Voucher no.: Aksoy2519) has been stored at the Erciyes University, Herbarium of the Biology Department.

Extraction

The aerial parts of *D. hastata* were dried at room temperature (about 25 °C) and crushed with a grinder. Grinded plants were extracted by a Soxhlet type extractor (Buchi, Switzerland) with methanol[26]. The extract was filtered through Whatman No. 1 filter paper and evaporated to dryness with a rotary evaporator (Rotavator, Buchi, Switzerland) (T<40°C). The extract was stored at 4°C after determining the yield.

Essential oil

Five hundred grams of grinded samples were submitted to steam distillation by a Clevenger-type apparatus with 5 L water for 3 h[26]. The yield of collected essential oil was measured and then dried over anhydrous sodium sulphate. Essential oil was stored at 4°C.

GC/MS assay

For identification of essential oil composition, gas chromatography/mass spectrometry (GC/MS)/quadropole detection assay using a Shimadzu QP 5050 system (Shimadzu, Duisburg, Germany) fitted with an FFAP (polyethylene glycol+2 nitrotere-phthalate) capillary column (50 m × 0.32 mm i.d., film thickness 1.2 μm) was used. The injector and detector temperatures were set at 240 and 250°C, respectively. Column temperature was kept at 120°C for 1.0 min, then increased to 220°C at 2°C/min and kept for 20 min. Carrier gas was helium at a flow rate of 10 psi (split 1:10). The injection volume of the sample was 1.0 μL. The ionization energy was at 70 eV. Qualitative analysis was based on comparison of retention times and mass spectra (Wiley, Nist and Tutor Libraries). The composition (%) of the essential oil was computed from the GC peak areas without using any correction factors[26].

Investigation of total phenolics

Folin-Ciocalteau method was used to determine of total phenolic content in methanolic extract[27]. About 40 μL aliquot of the methanolic extract (1.0 mg/mL) was mixed with Folin-Ciocalteu reagent (200 μL) and 20% sodium carbonate (600 μL). The solution was incubated for 2 h at room temperature. Absorbance measurements were performed at 765 nm by the spectrophotometer (Shimadzu UV-Vis 1240, Japan). The standard curve was formed with gallic acid. The assay was performed in triplicate. The results are mean values and expressed in terms of milligram of gallic acid equivalents (mg GAE) /g extract.

Invesitgation of total flavonoids

Aluminum chloride colorimetric assay was performed for detect of total flavonoids[28]. The extract (1.0 mg/mL, 0.5 mL) in methanol were mixed with 1.5 mL of methanol, 0.1 mL of 10% AlCl, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. The absorbances of the solutions were measured at 415 nm. Total flavonoid content was expressed as mg of quercetin equivalents (QE)/g dry extract.

Antioxidant activity assays

Phosphomolybdenum assay

The antioxidant activity of methanolic extract was assesses by phosphomolybdenum method[29]. About 4 mL of reagent solution (28 mM sodium phosphate,

0.6 M sulphuric acid and 4 mM ammonium molybdate) was added to the extract (1 mg/mL, 0.4 mL) in methanol. The tubes were stored at 95°C for 90 min. The absorbance was measured at 695 nm. The results were expressed as mg of ascorbic acid equivalents (AAE)/g extract.

DPPH assay

DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity of methanolic extract was evaluated[30]. About 0.1-2 mg/mL concentrations of the extract were mixed with 0.1 mM DPPH solution, and the mixture was incubated at room temperature for 30 min and then absorbance was measured at 517 nm. IC_{50} (the concentration causing 50% inhibition) value was determined. The results were compared to that of BHT. The inhibition (%) was calculated by following formula:

$$\%\text{Inhibition}=\frac{\left(\text{Abs}_{\text{control}}-\text{Abs}_{\text{sample}}\right)}{\text{Abs}_{\text{control}}}\times 100$$

β-Carotene bleaching method

Lipid peroxidation inhibitory activity of methanolic extract in β carotene-linoleic acid emulsion was assessed[31]. An aliquot of β carotene in chloroform was mixed with Tween 40 and linoleic acid. The chloroform was evaporated. Distilled water (50 mL) was added to the residue to form an emulsion. About 0.2 mL of the extract (1.0 mg/mL) was added to 5 mL of the obtained emulsion. After the tubes were incubated at 50°C for 2 h, the absorbance was measured at 470 nm. The same procedure for BHT (Butylated hydroxytoluene) was carried out.

CUPRAC method

The cupric ion reducing antioxidant ability of methanolic extract was detected[32]. The extract, 7.5 mM Neocuproine, 10 mM $CuCl_2$ and 1 M NH_4Ac buffer solutions were mixed. Then, total volume was completed to 4.1 mL and incubated for 30 min. The absorbance of solution was read at 450 nm. The same procedure for Trolox was carried out.

FRAP

Ability of the methanolic extract to reduce ferric ions was examined[33]. FRAP is based on the reduction of ferric 2,4,6-tris(2-pyridyl)-1,3,5-triazine [Fe(III)-TPTZ] to the ferrous complex at low pH. FRAP reagent contains 20 mM $FeCl_3$. $6H_2O$, 300 mM acetate buffer, and 10 mM TPTZ. The extract solution was mixed with FRAP reagent and stored during 30 min at 37°C and the absorbance was read at 595 nm. Fe^{+3} reducing antioxidant power of the extract was indicated as mmol/L of $Fe^{2+.}$

H_2O_2 Scavenging activity

Different concentrations of the methanol extract (25-500 µg/mL) were mixed with 43 mM H_2O_2 solution. The solution was incubated for 10 min and the absorbance was read at 230 nm[34]. The same procedure for gallic acid, BHA and BHT was carried out. Hydrogen peroxide scavenging ability was calculated using the following formula:

$$\%\text{Scavenged } H_2O_2\ \%=\frac{\left(\text{Abs}_{\text{control}}-\text{Abs}_{\text{sample}}\right)}{\text{Abs}_{\text{control}}}\times 100$$

IC_{50} values were calculated graphically.

Reducing power

Various concentrations of the extract in methanol (0.5-10 mg/mL) were added to 2.5 mL of potassium ferricyanide [$K_3Fe(CN)_6$] and incubated at 50°C for 20 min. The solution was added to 2.5 mL of trichloroacetic acid and centrifuged at 3,000 rpm for 10 min. The upper layer solution was added to $FeCl_3$ (0.5 mL). The absorbance was measured at 700 nm[35]. BHT was used as positive control. The high absorbance value reflects the high reducing capacity.

Chelating activity on Fe^{2+}

Different concentrations of the extract in methanol (1-5 mg/mL) were mixed with 0.1 mL of $FeCl_2$ (2 mM) for 10 min. The reaction was initiated by adding 0.2 mL of ferrozine (5 mM). The absorbance was read at 562 nm[34]. The same procedure for ethylene-diaminetetraacetic acid (EDTA) was carried out.

Chelating capacity was calculated using the following way:

$$\%\text{Chelating activity}=\frac{\left(\text{Abs}_{\text{control}}-\text{Abs}_{\text{sample}}\right)}{\text{Abs}_{\text{control}}}\times 100$$

Antimicrobial activity assay

The following microorganisms were used: *Aeromonas hydrophila* ATCC 7965, *Yersinia enterocolitica* ATCC 1501, *Salmonella typhimurium* NRRLE 4463, *Listeria monocytogenes* 1/2B, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Proteus mirabilis* ATCC 25933, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus cereus* ATCC 11778, Methicillin resistant *Staphylococcus aureus* ATCC 43300 (MRSA), *Streptococcus pneumoniae* ATCC 10015, *Salmonella*

enteritidis ATCC 13076, *Candida albicans* 10231, *Aspergillus parasiticus* DSM 5771 and *Aspergillus flavus* NRRL 3357.

Agar-well diffusion method was performed to detect of antimicrobial effect of the extract[36]. Test bacteria, *C. albicans* and molds (*A. parasiticus* and *A. flavus*) were suspended in sterile nutrient broth, malt extract broth and patato dextro broth (Merck). Suspensions of each microorganism were regulated to 10^6-10^7 colony-forming units (cfu)/mL and placed in flasks containing 25 mL of sterile growth medium at 45°C and then poured into Petri plates (9 cm). The wells (5 mm in diameter) were cut from the agar. About 50 µL of the extract (30 mg/mL) was added to the wells. The methanol had no effect on the tested microorganisms. For antimicrobial effect of essential oil, the disc diffusion assay was used[37]. The disc (6 mm) applied essential oil (10 µL) was placed on the inoculated agar. Then, plates were incubated at 25-37°C for 24-48 h in the inverted position. The growth inhibition zones were measured in millimeters. Inhibition zones of standard antibiotics namely tetracycline (10 mg/mL), natamycin (30 mg/mL), ampicillin (AMP, 10 µg/disk), kanamycin (K, 30 µg/disk) and penicillin (P, 10 µg/disk) were compared.

MIC and MBC

The minimum inhibitory (MIC) and minimum bactericidal concentrations (MBC) were detected for microorganisms, which were defined as sensitive to the extract or essential oil in the agar well and disc difusion methods. MICs were assessed by the micro-dilution method using the 96-well plates according to the Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations[38]. The inocula of the microorganisms were adjusted to 0.5 McFarland standard turbidity. The extract and essential oil were adjusted at 30 mg/mL and 2000 µg/mL in 10% dimethylsulfoxide (DMSO, Merck), respectively. Then, two-fold dilutions were made (3.9-30 mg/mL and 31.25- 2000 µg/mL concentrations, respectively). 95 µL of growth medium and 5 µL of the inocula were added into each well. The serial dilutions of the extract and essential oil were prepared. The last well not contain samples as a negative control. The plates were mixed on a shaker and incubated at 25-37°C for 24 h. The lowest concentration that is not visible growth was identified as MIC. Minimum bactericidal concentration (MBC) was determined by subculturing from each negative tube and control. MBC was described as the lowest concentration that zero or only one colony was observed.

Results and Discussion

The percent yields of the methanol extract and essential oil obtained from *Dorystoechas hastata* were 28.51% (w/w) and 1.4% (v/w), respectively. Total phenolic and flavonoid contents of methanol extract of *D. hastata* were 53.05±0.7 mg GAE/g extract and 7.04±0.0 mg QE/g dry extract, respectively. GC-MS was used to detect phenolic composition of the hydrodistillated volatile oil obtained from *D. hastata* (Table 1). Thirty-six compounds constituting 100% of the total oil were determined. Eucalyptol (1,8-cineol)

Table 1 — Composition of *D. hastata* essential oil

Compounds	RT[b]	%
[a]Tricyclene	5.603	0.15
α-Thujene	5.690	0.02
α-Pinene	5.936	8.39
Camphene	6.461	4.50
β -Pinene	7.429	1.40
1-Octen-3-ol	7.539	0.46
β-Myrcene	7.881	7.82
Phellandrene- α	8.559	0.10
δ-3-Carene	8.664 .	0.79
Terpinene -α	9.021	0.32
Cymene <para-> & Cymol	9.376	0.77
Eucalyptol (1,8-Cineol)	9.733	25.47
Ocimene <(E)-, β ->	10.403	0.37
γ -Terpinene	10.957	0.35
Linalool oxide	11.575	0.07
α-Terpinolene	12.348	0.33
Dimethylstyrene < α -para->	12.615	0.05
LinalooI L	13.266	4.28
α -Thujone	14.050	0.16
Fenchyl alcohol	14.231	0.05
Camphor	15.674	6.41
Borneol	17.457	20.06
4-Terpineol	17.861	0.68
Cymen-8-ol <para->	18.381	0.05
α-Terpineol	18.823	1.67
Bornyl acetate	24.365	5.15
Carvacrol	25.952	0.48
α -Copaene	30.219	0.11
Caryophyllene	32.953	6.22
α-Humulene	35.176	0.37
δ-Cadinene	39.282	0.12
Palustrol	42.240	0.11
Caryophyllene oxide	42.851	0.15
Guaiol	44.022	2.14
β -Eudesmol	47.409	0.28
Elemol -α	47.952	0.17
Total		100

[[a] Compounds listed in order of elution from a FFAP MS column; [b] Retention time (as minutes); and [c] The percentage composition was computed from the GC peak areas]

(25.47%) and borneol (20.06%) were found to be the main compounds in the oil. It was followed by α-pinene (8.39%), β-myrcene (7.82%), camphor (6.41%), caryophyllene (6.22%), bornyl acetate (5.15%), camphene (4.50%), linalool L (4.28%) and guaiol (2.14%). The remaining twent-six compounds were at the range of 0.02 and 0.79%.

Limited reports on phytochemical compositions of on *D. hastata* have been previously published. Luteolin, carnosol, luteolin-7-glucoside, rosmanol, 6-methoxyluteolin-7-glucoside, chlorogenic acid and caffeic acid previously isolated from *D. hastata* leaves[22]. Diterpenoids and two norditerpenoids were determined from the roots of *D. hastata* growing wild in Southwestern Turkey by Ulubelen *et al*.[21].
Similar to our results, Baser & Öztürk[23] have also studied essential oil compositions from different parts of *D. hastata* using various distillation techniques and detected 1,8-cineole, borneol, α-pinene, guaiolas and camphor main compounds in the essential oils. Kan *et al*.[25] identified three main components in the essential oils isolated from different parts of cultivated *D. hastata* from Konya, Turkey. These compounds were guaiol (26.5 %) from the branch essential oil, 1,8-cineole (20.6%) from the leaf essential oil and borneol (15.0%) from the aerial parts, respectively. The major components of the flower and the leaves essential oils of *D. hastata* were also determined as myrcene, 1,8-cineole, β-pinene, α-pinene, β-caryophyllene, bornyl acetate and terpinene-4-ol[39].

In phosphomolybdenum assay, Mo (VI) is reduced to Mo (V) by the antioxidant compound. The total antioxidant activity of the extract was 445.13±0.8 mg AAE/g dry extract.

In β carotene bleaching method, the oxidation of linoleic acid forms peroxyl radicals. Then, the formed free radical oxidizes and breaks down the β carotene. It loses the double bonds and therefore, its characteristic orange colour. The antioxidants in the extract can inhibit oxidation of linoleic acid and the formation of hydroperoxides[31]. The extract inhibited the bleaching of β carotene. Inhibition rate of β carotene by the extract was 65.81% and this value lower than that of BHT (84.26%) and BHA (94.33%) at 1 mg/mL.

DPPH method to estimate the free radical scavenging effectiveness of the extracts has been widely used. DPPH assay show the hydrogen donating ability of the extract. DPPH scavenging capacity was defined as the percentage inhibition of the initial DPPH absorption by extract. Fig. 1 shows an increase in the percentage DPPH inhibition with increasing concentration. The methanol extract exerted high free radical scavenging activity. The inhibition values of the extract were 18.36, 40.08, 89.16% 91.26 and 91.96% at 6.66, 8.3, 16.6, 33.3 and 66.6 µg/mL concentrations, respectively. DPPH scavenging capacity of the extract was similar to BHT at 33.3 and 66.6 µg/mL (91.47 and 92.15%, respectively). IC_{50} values of the extract and BHT were 8.69 µg/mL and 3.35 µg/mL, respectively. A low IC_{50} value means that antioxidant capacity is high.

The CUPRAC method is based on the absorbance measurement of chromophore, Cu(I)-neocuproine chelate, formed as a result of a redox reaction of antioxidants with Cu(II) neocuproine reagent[40]. The *D. hastata* extract exerted high and concentration dependent CUPRAC activity. Absorbance values of the extract ranged between 0.015 and 3.07 at 0.6-3 mg/mL. The value of the extract (2.80) was close to that of trolox (2.85) at 1 mg/mL (Fig. 2).

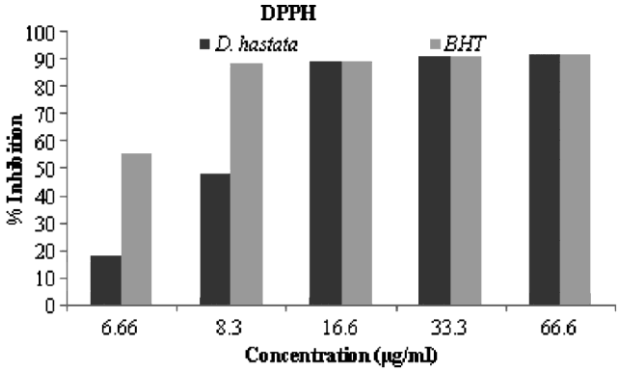

Fig. 1 — % Inhibition values of *Dorysstoechas hastata* extract and BHT as positive control by DPPH assay

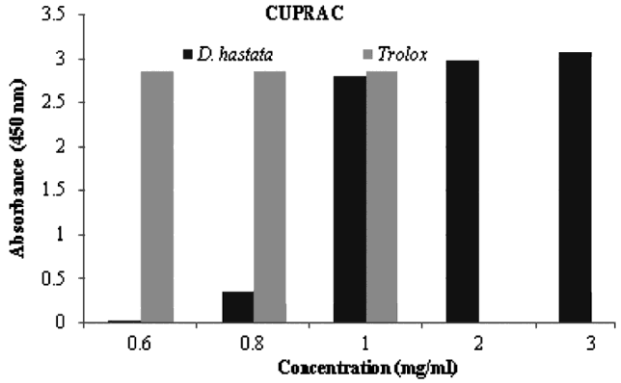

Fig. 2 — Antioxidant capacity of *D. hastata* extract by CUPRAC method

The FRAP assay measures reduction of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) in the presence of antioxidants. *D. hastata* extract exerted the high FRAP activity with 4.18 mM/L at 2 mg/mL. This value was near to that of L-ascorbic acid (4.52 mM/L) at same concentration.

D. hastata extract exerted concentration depended H_2O_2 scavenging activity (Fig. 3). H_2O_2 scavenging activity of the extract was 54.44% at 50 µg/mL. These values for BHT, BHA and gallic acid were 76.97, 64.73 and 137.61%, respectively at the same concentration. IC_{50} values were 46.39, 31.09, 23.16 and 17.62 µg/mL for *D. hastata* extract, BHT, BHA and gallic acid, respectively. The activities were in the following order: gallic acid>BHA> BHT> extract. *D. hastata* extract had a moderate H_2O_2 scavenging capacity.

Because a compound exerts antioxidant activity, its reducing potential is significant. The extract showed concentration depend reducing capacity (Fig. 4). The reducing ability of the extract was higher than that of BHT at 2.5, 5 and 10 mg/mL. The ferrous ion (Fe^{2+}) chelating activity of *D. hastata* extract was very low. The methanol extract exhibited 17.16% chelating activity on Fe^{2+}at 5.0 mg/mL, considerably lower than

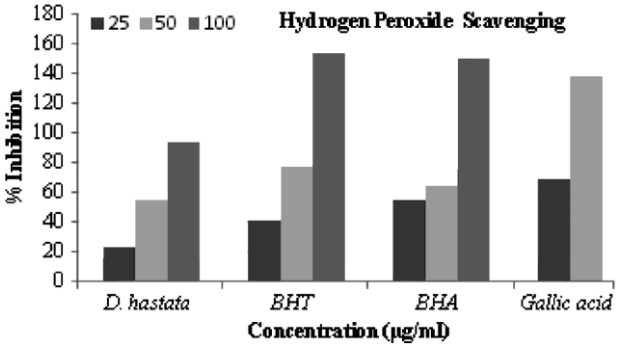

Fig. 3 — % Inihibition values of the *D. hastata* extract and standards by H_2O_2 assay

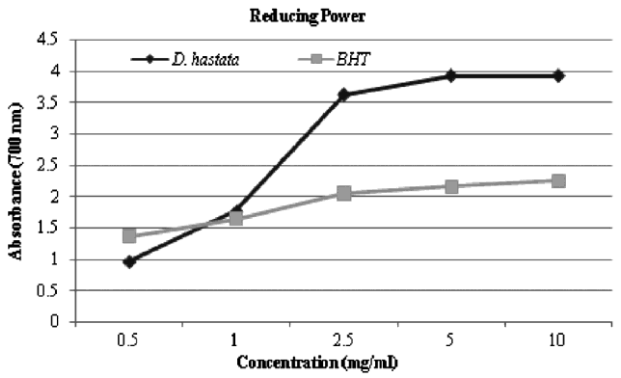

Fig. 4 —Reducing power of methanolic extract from *D. hastata*

that of EDTA (99.45%) at the same concentration. According to this result *D. hastata* extract have slightly iron binding capacity.

Chlorogenic acid, carnosic acid, coumaric acid, ferulic acid, caffeic acid, rosmarinic acid, kaempferol, quercetin, carnosol and apigenin were determined in the extracts of *D. hastata* by HPLC-DAD analysis[24]. In the same study, petroleum ether, methanol and water extracts of *D. hastata* exerted significant antioxidant, DPPH radical scavengers and effective in inhibiting lipid oxidation. Total phenolic amount of *D. hastata* methanol extract was found as 147.3 mg GAE/g[24], significantly higher compared to our finding (53.05 mg GAE/g).

In another study, Karagözler *et al*.[41] studied the antioxidant effects of the diethyl ether, ethanol, water and hot water extracts of *D. hastata* leaves using DPPH assay. Their IC_{50} values ranged from 6.17 to 20.90 µg/mL. Also their total phenolic and flavonoid amounts were found as 191.67-554.17 mg GAE/g and 24.21-69.34 mg RtE/g, respectively. In accordance with our result, authors stated that reducing activity of extracts increased with increasing concentration[41].

This is the first study to ensure information about detailed antioxidant effect of *D. hastata* extract determined by β carotene bleaching, FRAP, CUPRAC, H_2O_2 scavenging and chelating activity assays. *D. hastata* methanolic extract presented an effective total antioxidant, DPPH scavenging, cupric ions (Cu^{2+}) and ferric ions (Fe^{3+}) reducing activities.

The results of the study for antimicrobial activities of the methanol extract and essential oil of *D. hastata* are summarized in Table 2. The methanol extract exerted a broad spectrum of antibacterial activity. *Streptococcus pneumoniae* was the most sensitive bacteria (27.0 mm) to the extract while *E. coli* was the most resistant bacteria (9.0 mm). The methanol extract exhibited bigger inhibitory zones against *S. pneumoniae* than tetracycline. The essential oil of *D. hastata* showed inhibitory effect on many of bacteria tested (Table 2). Essential oil had no antibacterial activity against *Salmonella typhimurium*, *Staphylococcus aureus* (MRSA), *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Salmonella enteritidis*. *Aeromonas hydrophila* was the most sensitive bacteria (23.0 mm) to essential oil. The methanol extract and essential oil had no any antifungal activity. The methanol extract showed MIC and MBC in a range of <0.39-12.5 mg/mL. MIC and MBCs of the essential oil for bacteria were in the range of

Table 2 — Antimicrobial activity of *D. hastata* extract, essential oil and standard antibiotics (mm, inhibition zones), MIC and MBCs

Bacteria	Extract (30 mg/mL)			Essential oil			Tetracycline			Ampicillin	Kanamycin	Penicillin
	mm	MIC (mg/mL)	MBC (mg/mL)	mm	MIC (mg/mL)	MBC (mg/mL)	mm	MIC (µg/mL)	MBC (µg/mL)	mm	mm	mm
A. hydrophila	17.0	6.25	6.25	23.0	0.25	0.5	27.0	<3.9	125	32.0	19.0	37.0
Y. enterocolitica	12.0	6.25	6.25	10.0	0.5	1.0	23.0	<3.9	125	-	10.0	-
S. thyphimurium	10.0	12.5	12.5	-	-	-	15.0	62.5	125	11.0	11.0	10.0
L. monocytogenes	16.0	1.56	1.56	16.0	0.5	0.5	29.0	<3.9	<3.9	-	-	15.0
E. coli	9.0	6.25	12.5	18.0	0.5	1.0	24.0	<3.9	125	8.0	11.0	7.0
K. pneumoniae	19.0	<0.39	<0.39	15.0	0.5	0.5	48.0	15.6	15.6	23.0	7.0	33.0
S. aureus (MRSA)	10.0	12.5	12.5	-	-	-	25.0	<3.9	125	-	-	-
P. mirabilis	12.0	6.25	6.25	-	-	-	19.0	31.5	125	7.0	14.0	12.0
B. cereus	14.0	6.25	12.5	16.0	0.25	1.0	27.0	<3.9	<3.9	-	15.0	11.0
P. aeruginosa	13.0	6.25	12.5	-	-	-	23	<3.9	125	25	12	10
S. pneumoniae	27.0	<0.39	<0.39	8.0	0.5	1.0	24.0	7.8	7.8	-	-	13.0
S. enteritidis	11.0	12.5	12.5	-	-	-	25.0	<3.9	62.5	9.0	10.0	10.0
Yeast							Natamycin	MIC	MBC			
C. albicans	-	-	-	-	-	-	23.0	<3.9	62.5	-	-	-
Molds												
A. flavus	-	-	-	-	-	-	17.0	7.8	>250	-	-	-
A. parasiticus	-	-	-	-	-	-	15.0	15.6	>250	-	-	-

[-, not detected]

0.25-1.0 mg/mL. It has previously been reported that *D. hastata* essential oil showed antimicrobial activity against *E. coli*, *Acinetobacter baumanii*, *S. aureus*, *P. aeruginosa*, *C. albicans* and *Candida parapsilosis* with MIC values of 0.019-6.25 µg/mL[42].

In the present study, the antibacterial and antifungal activities of the extract and essential oil obtained from *D. hastata* against wide range of bacteria and mold were determined.

Conclusion

Above results have demonstrated that the main compounds in the essential oil *of D. hastata* are eucalyptol (1,8-cineol) and borneol. The methanolic extract of *D. hastata*, endemic to Turkey (Antalya), possesses strong antioxidant properties. The phenolic-rich extractof *D. hastata* exerted potential inhibitory effect on all bacteria tested. The essential oil of *D. hastata* showed antibacterial activity on many of bacteria tested. But, they had no antifungal activity. Overall, these observations suggest that *D. hastata* could be used as a potential natural source of antioxidant and antimicrobial compounds with broad spectrum in different industries such as food and medicine.

Conflict of Interest

Authors declare no competing interests.

References

1 Shrivastava A, Aggarwal LM, Mishra SP, Khanna HD, Shahi UP & Pradhan S, Free radicals and antioxidants in normal versus cancerous cells-An overview. *Indian J Biochem Biophys*, 56 (2019) 7.

2 Kumar D, Kumar S, Singh J, Narender, Rashmi, Vashisthaand B & Singh N, Free radical scavenging and analgesic activities of *Cucumis sativus* L. fruit extract. *Pharmacognosy*, 2 (2010) 365.

3 Mansour H, Elkady AA, Elrefaei A & Hafez HF, Radioprotective, antioxidant and antitumor efficacy of *Annona muricata* L. Leaf extract. *Indian J Biochem Biophys*, 55 (2018) 205.

4 López-Alarcónand C & Denicola A, Evaluating the antioxidant capacity of natural products: A review on chemical and cellular-based assays. *Anal Chim Acta*, 763 (2013) 1.

5 Alam MN., Bristi NJ & Rafiquzzaman M, Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity. *Saudi Pharm J*, 21 (2013) 143.

6 Mukherjee S, Pal S, Chakrabotry R, Koley H & Dhar P, Biochemical assessment of extract from *Oxalis corniculata* L.: Its role in food preservation, antimicrobial and antioxidative paradigms using *in situ* and *in vitro* models. *Indian J Exp Biol*, 56 (2018) 230.

7 Chauhan S, Sharma D &Goel HC, An *in vitro* evaluation of *Tribulus terrestris* L. Fruit extract for exploring therapeutic potential against certain gut ailments. *Indian J Exp Biol*, 56 (2018) 430.

8 Carochoand M & Ferreira ICFR, A review on antioxidants, prooxidants and related controversy: Natural and synthetic compounds, screening and analysis methodologies and future perspectives. *Food Chem Toxicol*, 51 (2013) 15.

9 Cota DL, Mishra S, Shengule SA & Patil D, Assessment of *in vitro* biological activities of *Terminalia arjuna* Roxb. Bark extract and Arjunarishta in inflammatory bowel disease and colorectal cancer. *Indian J Exp Biol*, 58 (2020) 306.

10 Gupta S, Bhagat M, Sudan R, Rajput S & Rajput K, Analysis of chemical composition of *Cupressus torulosa* (D.Don) essential oil and bioautography guided evaluation of its antimicrobial fraction. *Indian J Exp Biol*, 56 (2018) 252.

11 Deorankar P, Gangiwale R, Chintamani R &Singh RP, Evaluation of ethanolic and aqueous extract of *Clitoria ternatea* for antimicrobial activity. *Indian J Nat Prod Resour*, 11 (2020) 194.

12 Hussain AI, Anwar F, Sheraziand STH & Przybylski R, Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. *Food Chem*, 108 (2008) 986.

13 Kähkönen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujalaand TS & Heinonen M, Antioxidant activity of plant extracts containing phenolic compounds. *J Agric Food Chem*, 47 (1999) 3954.

14 Dung NT, Kim JM & Kang SC, Chemical composition, antimicrobial and antioxidant activities of the essential oil and the ethanol extract of *Cleistocalyx operculatus* (Roxb.) Merrand Perry buds. *Food Chem Toxicol*, 46 (2008) 3632.

15 Vattem DA, Lin YT, Labbeand RG & Shetty K, Antimicrobial activity against select food-borne pathogens by phenolic antioxidants enriched in cranberry pomace by solid-state bioprocessing using the food grade fungus *Rhizopus oligosporus*. *Process Biochem*, 39 (2004) 1939.

16 Petrovic´ S, Ušjak L, Milenkovic´ M, Arsenijevic´ J, Drobac M, Drndarevic´ A & Niketic´ M, *Thymus dacicus* as a new source of antioxidant and antimicrobial Metabolites. *J Funct Foods*, 28 (2017) 114.

17 Ahmad S, Saba S & Asif HM, Phytochemical analysis, antimicrobial, antioxidant and enzyme inhibitory activities of ethanolic extarct of *Centaurea solstitialis* L. and its different fractions. *Indian J Exp Biol*, 58 (2020) 396.

18 Davis PH, *Flora of Turkey and Aegean island*. Vol. 7. (Edinburgh University Press, Edinburgh), 1982, 479.

19 Erdağ BB, Emek YÇ & Aydoğan SK, Clonal propagation of *Dorystoechas hastata* via axillary shoot proliferation. *Turk J Bot*, 34 (2010) 233.

20 Baytop T, *Türkiye'de Bitkiler ile Tedavi (Geçmişte ve Bugün)*, 2nd Edn. (Nobel Tıp Kitabevi, İstanbul), 1999, 194.

21 Ulubelen A, Meriçli AH & Meriçli F, Diterpenes and norditerpenes from the roots of *Dorystoechas hastata*. *Pharmazie*, 59 (2004) 301.

22 Venturella P, Venturella G, Marino ML, Mericli AH & Cubukcu B, Phytochemical investigation of the Labiate *Dorystoechas hastata. G Bot Ital*, 122 (1988) 291.

23 Baser KHC & Öztürk N, Composition of the essential oil of *Dorystoechas hastata*, A monotypic endemic from Turkey. *J Essent Oil Res*, 4 (1992) 369.

24 Erkan N, Akgonen S, Ovat S, Goksel G & Ayranci E, Phenolic compounds profile and antioxidant activity of *Dorystoechas hastata* L. Boiss et. Heldr. *Food Res Int*, 44 (2011) 3013.

25 Kan A, Günhan RS & Çelik SA, The Chemical composition profile of *Dorystoechas hastata* Boiss. & Heldr. ex Bentham cultivated in Turkey. *Rec Nat Prod*, 9 (2015) 135.

26 Albayrak S, Volatile compounds and bioactivity of *Achillea sieheana* Stapf. (Asteraceae). *Iran J Pharm Res*, 12 (2013) 37.

27 Singleton VL & Rossi JAJr, Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Enol Viticult*, 16 (1965) 144.

28 Mukherjee S, Pal S, Chakraborty R, Koley H & Dhar P, Biochemical assessment of extract from *Oxalis corniculata* L.: Its role in food preservation, antimicrobial and antioxidative paradigms using *in situ* and *in vitro* models. *Indian J Exp Biol*, 56 (2018) 230.

29 Prieto P, Pineda M & Aguilar M, Spectrofotometric quantitation of antioxidant capacity through the formation of a Phosphomolybdenum Complex: specific application to the determination of vitamin. E. *Anal Biochem*, 269 (1999) 337.

30 Lee SK, Mbwambo ZH, Chung HS, Luyengi L, Games EJC, Metha RG., Kinghorn AD & Pezzuto JM, Evaluation of the antioxidant potential of natural products. *Comb Chem High Throughput Screening*, 1 (1998) 35.

31 Cao L, Si JY., Liu Y, Sun H, Jin W, Li Z, Zhao XH & Pan RL, Essential oil composition, antimicrobial and antioxidant properties of *Moslachinensis* Maxim. *Food Chem*, 115 (2009) 801.

32 Apak R, Güçlü K, Özyurek M, Karademir SE & Ercağ E, The cupric ion reducing antioxidant capacity and polyphenolic content of some herbal teas. *Int J Food Sci Nutr*, 57 (2006) 292.

33 Tuberoso CIG, Montoro P, Piacente S, Corona G, Deiana M, Dessi MA, Pizza C & Cabras P, Flavonoid characterization and antioxidant activity of hydroalcoholic extracts from *Achillea ligustica* All. *J Pharm Biomed Anal*, 50 (2009) 440.

34 Peksel A, Arisan-Atac I & Yanardag R, Evaluation of antioxidant and antiacetylcholinesterase activities of the extracts of *Pistacia atlantica* Desf. Leaves. *J Food Biochem*, 34 (2010) 451.

35 Aadil KR, Barapatre A, Sahu S, Jhaand H & Tiwary BN, Free radical scavenging activity and reducing power of *Acacia nilotica* wood lignin. *Int J Biol Macromol*, 67 (2014) 220.

36 Albayrak S, Aksoy A, Sagdic O & Hamzaoglu E, Compositions, antioxidant and antimicrobial activities of *Helichrysum* (Asteraceae) species collected from Turkey. *Food Chem*, 119 (2010) 114.

37 Gulluce M, Sahin F, Sokmen M, Ozer H, Daferera D, Sokmen A, Polissiou M, Adiguzel A & Ozkan H, Antimicrobial and antioxidant properties of the essential oils and methanol extract from *Mentha longifolia* L. ssp. *longifolia*. *Food Chem*, 103 (2007) 1449.

38 Adamczak A, Ożarowski M & Karpiński TM, Antibacterial activity of some flavonoids and organic acids widely distributed in plants. *J Clin Med*, 9 (2020) 109.

39 Özcan MM, Chalchat JC, Figueredo G, Bagci Y, Dural H, Savran A, Al-Juhaimi FY & ÖzcanMM, Chemical composition of the essential oil of the flowers and leaves of Çalba Tea (*Dorystoechas hastata* Boiss & Helder. ex Bentham). *J Essent Oil-Bear Plants*, 19 (2016) 782.

40 Ozturk OM, Aydogmus F, Ozturk O, Duru EM & Topcu G, Antioxidant activity of stem and root extracts of Rhubarb (*Rheum ribes*): an edible medicinal plant. *Food Chem*, 103 (2007) 623.

41 Karagözler AA, Erdağ B, Emek YÇ & Uygun DA, Antioxidant activity and proline content of leaf extracts from *Dorystoechas hastata*. *Food Chem*, 111 (2008) 400.

42 Bozlak ÇEB, Bozhüyük AU, Kordalı Ş & Bozkurt HB, The determination of minimum inhibitory concentration of eighteen essential oils with resazurin method, against a group of yeast and bacteria. *Abant Medic J*, 9 (2020) 72.