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Original paper

Evaluation of some traditional medicinal plants: phytochemical profile, antibacterial and antioxidant potentials

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Abstract

Bioassays provide an authentication of the traditional knowledge of medicinal plants. Antibacterial, antioxidant and phenolic content of nine medicinal plants (*Achillea millefolium, Prunella vulgaris, P. laciniata, Lythrum salicaria, Epilobium angustifolium, E. hirsutum, Pedicularis comosa, Agrimonia eupatoria* and *Verbena officinalis*) were investigated in connection with folkloric usages. Aerial parts of nine plant species were extracted with water and methanol (MeOH). Disc diffusion method was performed to evaluate the antibacterial activity of the extracts against ten pathogenic bacteria. Antioxidant activity was determined by using 2,2-diphenyl-1-picrylhydrazil (DPPH) radical photometric assay. Total phenolic and flavonoid content were investigated by using Folin-Ciocaltaeu and aluminum chloride ($AlCl_3$) colorimetric method, respectively. Quantitative analysis of phenolic constituents of nine plants species were performed by high performance liquid chromatography-diode array detector (HPLC-DAD) via chosen ten phenolic standards (gallic acid monohydrate, caffeic acid, rutin hydrate, luteolin-7-O-β-D glucoside, kaempferol, rosmarinic acid, myricetin, quercetin, coumarin and apigenin). *E. hirsutum* showed the best antibacterial activity against gram-positive bacteria (*S auerus, S. epidermidis* and *S.* pyogenes). *L. salicaria* also demonstrated strong and broad spectrum antibacterial activity. *P. laciniata, L. salicaria* and *E. angustifolium* showed potent antioxidant activity. The highest phenolic and flavonoid content was observed with *E. hirsutum* and *A. millefolium,* respectively. *L. salicaria* also had very high amount of total phenol and flavonoid content. HPLC-DAD analysis displayed that *P. laciniata* and *A. eupatoria* were the best sources of rosmarinic acid. Furthermore, *V. officinalis* and *P. vulgaris* also had remarkable amount of rutin. This study revealed the scientific rationale behind the traditional knowledge of the tested plants. Consistent with traditional usages, the most prominent plants were *L. salicaria, E. hirsutum* and *E. angustifolium* in regard to strong antibacterial and antioxidant potentials. These plants may be proper natural sources with potential applications in pharmaceutical and food industry.

Keywords Antibacterial; Antioxidant; Flavonoid; HPLC; Phenol.

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Introduction

Natural products have been the source for the treatment of diseases since the dawn of human civilization, and through scientific and observational efforts from traditional medicine, modern medicine has slowly developed over the years. Besides the obvious use of raw plant preparations or plant extracts, plant-based natural product research has become a common tool in the production of drugs (MUKHERJEE & al [1]). Scientific validations can be developed with bioassays on natural products originating from the traditional usages of medicinal plants.

A. millefolium has ethnobotanical records for the treatment of diarrhea, common cold, migraine, stomachache, headache, toothache, cough, hemorrhoids, uroclepsia, lung cancer, rheumatism, backache, nephritis, cardiopathy, migraine, dizziness, gynecological diseases, epistaxis and eye strain in traditional medicine. It possess vasodilator, antiseptic, wound cleaner, antiemetic, menstrual regulator, weight allowance, antitussive, diuretic, carminative, stomachic, urinary antiseptic, tonic, astringent, vulnerary, hemostatic, anticancer and analgesic properties (KÜLTÜR [2]; ALTUNDAG and OZTURK [3]; POLAT and SATIL [4]; GULER & al [5]).

P. vulgaris has been used in the treatment of cystitis, female disorders, wounds, hemorrhoids, senses disorders, respiratory and gastrointestinal system ailments, blood system disorders, bleeding, anthrax, sore throat, headache, heart diseases, difficult breathing, gastric ulcer and weakness of eyesight due to overage in traditional medicine (ALTUNDAG and OZTURK [3]; GUARINO & al [6]; KOYUNCU & al [7]; ŠARIĆ-KUNDALIĆ & al [8]). It has also used as a refreshing drink, and substitute of tobacco (KUJAWSKA & al [9]). *P. vulgaris* has folkloric usage as expectorant, antiseptic, cicatrisant, intestinal antispasmodic, hepatoprotective, choleretic, antiulcer, antidiarrheal, antipyretic, laxative, tonic, diuretic and animal fodder (ALTUNDAG and OZTURK [3]; KOYUNCU & al [7]; ŠARIĆ-KUNDALIĆ & al [8]; REDZIC [10]; MUMCU and KORKMAZ [11]).

In traditional medicine, *P. laciniata* has been used for making infusions for the cough treatment, gall and skin diseases. It is used externally for cleaning open sores and wounds (REDZIC [12]). It is a wound healer (KOYUNCU & al [7]) and has been used in the diseases of the respiratory and gastrointestinal systems (GUARINO & al [6]).

According to ethnobotanical records, *L. salicaria* has been used as a treatment in dysentery, diarrhea, intestinal inflammation, hematuria, leucorrhea, epistaxis, dysmenorrhea, lupus, eczema, impetigo, skin diseases, anemia, female urogenital inflammation, hemorrhoids, internal bleeding, stomach disorders, high blood pressure, gastrointestinal tract ailments, blood circulation disorders, dysentery, uterine hemorrhages, colitis and stomatitis. It has astringent, antihemorrhagic, tonic, vulnerary, cleansing and intestinal disinfectant activities (ŠARIĆ-KUNDALIĆ & al [8]; REDZIC [10]; MUMCU and KORKMAZ [11]; TITA & al [13]; DI NOVELLA & al [14]; PIWOWARSKI & al [15]).

E. hirsutum has been recorded in the treatment of gastritis, stomach disease, ulceration, inflammations, prostate tumors, rectal bleeding, constipation, menstrual and gastrointestinal disorders, weakness, nervous debility, stomachache, joint pains, skin allergies, acne, prostatitis, prostate adenoma, hepatitis, ulcer, enteritis, cirrhosis and urinary tract disorders in ethnobotanical studies and it has been used as depurative, cholagogue, astringent, anti-inflammatory, haemostatic, antimicrobial, cytostatic, regenerative, hypertensive and animal fodder in folk medicine (TITA & al [13]; AL-QURA'N [16]; ARNOLD & al [17]; KORKMAZ & al [18]).

E. angustifolium has been used as a remedy in prostate diseases, benign prostate hyperplasia, prostatitis, prostate cancer, urinary disorders, stomachache, intestinal discomfort, enteritis, hepatitis, ulcer, cirrhosis, inflammation of mouth, blister, constipation, cuts, abdominal pain, burns, eye conditions due to asthma and allergies, female diseases, cardiovascular diseases, and mouth, hepatic, stomach, intestinal and renal complaints in traditional medicine. It has depurative, cholagogue, astringent, antidiarrheal, carminative, anti-inflammatory, haemostatic, antimicrobial, cytostatic, regenerative, laxative and blood cleanser properties, and been used as an antiseptic to treat infected wounds (TITA & al [13];) MENALE & al [19]; BALLABH and CHAURASIA [20]; CANSARAN & KAYA [21]; BARTFAY & al [22]; KORKMAZ [23]; AKAN and BAKIR [24]; KORKMAZ & al [25]; SETZER [26]).

Pedicularis spp. have been used in the treatment of cold, cough, fever, sterility, rheumatism, general debility, collapse, poor appetite, chronic hepatitis, pancreatic disease**,** digestive, reproductive and urinary problems in traditional medicine (YATOO & al [27]). Local people consume nectar of *P. comosa* by sucking their flowers in Iğdır, Turkey (ALTUNDAĞ ÇAKIR [28].

A. eupatoria has been used as a treatment in goiter, hernia, sore throat, laryngitis, pharyngitis, diarrhea, enteritis, gastritis, gut, anorexia, gall-bladder ailments, liver diseases, jaundice, fatty liver, stomach ulcers, digestive disorders, respiratory diseases, cardiovascular system disorders, gastrointestinal disorders, renal and biliary lithiasis, chronic cholecystitis rheumatism, headache, dermatitis, skin diseases, hemorrhoids and snake bites. It has constipant, diuretic, astringent, hypotensive, cholagogue, sedative, anti-inflammatory, antiaphonic, antilithiase, cicatrisant, blood purifier, vulnerary, depurative, gastric analgesic, hepatic, antiulcerose, antidiarrheal, anthelmintic and tonic properties. *A. eupatoria* has also records as renal, bladder, bronchial, intestinal and hepatic anti-inflammatory in folkloric medicine (KÜLTÜR [2]; ALTUNDAG and OZTURK [3]; GUARINO & al [6]; TITA & al [13]; KORKMAZ [25]; SETZER [26]; YATOO & al [27]; ALTUNDAĞ ÇAKIR [28]; CAKILCIOGLU and TURKOGLU [29]; CAVERO and CALVO [30]; OZTURK [31]).

V. officinalis has healing property in cough, asthma, cold-fever, constipation, milk secretion, muscular pains, contusions and bruises, herpes zoster, ascaris, snake bite, eczemas, furuncle, boil, sore throat, sinusitis, jaundice, hepatitis, enteritis, convulsion, colitis, migraine, depression, insomnia, nervous headache, urinary problems, menstrual

cramps, and stomach, intestine, liver, spleen, gall bladder and kidney disorders. It has been recorded as antiarthrosic, antirheumatic, antipneumonic, antiechimotic, antihaemastenic, antianemic, astringent, tonic, diaphoretic, antispasmodic, emmenagogue for late menstruation, cardiotonic, febrifuge, stimulant, restorative, stomachache, tranquillizer, hepatoprotective, liver stimulant, choleretic, antiulcer, diuretic against dropsy, antidiarrheal, aphrodisiac, analgesic, anticonvulsant, learning and memory enhancer, antidepressant and sedative in traditional medicine (GUARINO & al [6]; REDZIC [10]; MUMCU and KORKMAZ [11]; CAKILCIOGLU and TURKOGLU [29]; BONET & al [32]).

The objective of this study was to assess antibacterial and antioxidant potentials of nine plant species correlating with their ethnomedicinal knowledge and to determine their phenolic constituents by HPLC-DAD analysis.

Materials and Methods

1. Plant material and extraction

Nine different plant species were collected in Bolu, Turkey and identification was performed using Flora of Turkey and the East Aegean Islands" (DAVIS [33]). Table 1 included family, botanical and common names, and collection number of tested species, besides extraction solvent types and yield for each extract. Above ground parts were used for each extraction of plants. Plant samples were powdered after drying for preparation of extracts. Twenty grams of plant materials were extracted with water or methanol (MeOH) at 40ºC by using water bath for 24 h and then filtered. Methanolic extract was concentrated using rotary evaporator and dissolved in water. Aqueous and methanol extracts were finally lyophilized and powdered crude extract was obtained.

Table 1. Botanical and common names, collection numbers, extraction types and yield (%) of plants.

Family and Species Name	Common names: English	Common names: Turkish	Collection Number	Extraction Solvents	Yield (%)[a]
ASTERACEAE					
Achillea millefolium L. subsp. *millefolium*.	Yarrow, common yarrow, milfoil, bloodwort, carpenter's weed, gordaldo, nosebleed plant, old man's pepper, devil's nettle, plumajillo, sanguinary, soldier's woundwort, thousand-leaf, thousand-seal boreal yarrow, California yarrow, giant yarrow, coast yarrow, western yarrow, Pacific yarrow	Civanperçemi, kurp otu, diş otu, ayvadana, ronagvac, sporis, sporiyis, krannavaz, ayvadana, beyaz ormadere, oymadere, çeren, yavşan	AUT-1956	Water Methanol	8.2 8.1
LAMIACEAE					
Prunella vulgaris L.	Self-heal, common selfheal,	Bahar çiçeği, bumbur otu, yara otu, siğil otu, gelincikleme otu	AUT-1957	Water Methanol	14 7
Prunella laciniata (L.) L.	Heal all, self-heal	Yara otu, horoz ibiği,	AUT-1958	Water Methanol	49.65 3.05
LYTHRACEAE					
Lythrum salicaria L.	Purple loosestrife, red sally, blooming sally, rainbow weed, spiked loosestrife, bouquet violet, black blood, purple willow-herb, water purslane, spatula leaf loosestrife	Tıbbi hevhulma, kançiçeği, kırmızı hevhulma, kırmızı kançiçeği, aklar ot	AUT-1964	Water Methanol	16.05 28.5
ONAGRACEAE					
Epilobium angustifolium L.	Fireweed, tall fireweed, rose way willow herb	Yakı otu, büyük mera gülü, çayır gülü	AUT-1959	Water Methanol	16.85 18.4
Epilobium hirsutum L.	Great willow herb, hirsute willow-herb	Hasan Hüseyin çiçeği	AUT-1960	Water Methanol	9.8 17.1
OROBANCHACEAE					
Pedicularis comosa L. var. *sibthorpii* (Boiss.) Boiss.	Tufted lousewort	Hotozlu bit otu, sorma, sormuk	AUT-1962	Water Methanol	18.8 10
ROSACEAE					
Agrimonia eupatoria L.	Agrimony, aremonia, churchsteeples, stickwort	Koyun otu, guatr otu, fıtık otu	AUT-1961	Water Methanol	12.2 7.2
VERBENACEAE					
Verbena officinalis L.	Common vervain, verbena, berbena, herb of the cross, pigeon's grass	Güvercin otu, sultan otu, mine çiçeği	AUT-1963	Water Methanol	8.5 13.75

[a]Yield (%) = Weight of extract (g)/20 g of plant sample X 100.

2. Antibacterial bioassay

Three gram-positive bacteria [*Streptococcus pyogenes* (ATCC® 19615), *Staphylococcus aureus* (ATCC® 25923) and *Staphylococcus epidermidis* (ATCC® 12228)] and seven gram-negative bacteria [*Escherichia coli* (ATCC® 25922), *Pseudomonas aeruginosa* (ATCC® 27853), *Salmonella typhimurium* (ATCC® 14028), *Serratia marcescens* (ATCC® 8100), *Proteus vulgaris* (ATCC® 13315), *Enterobacter cloacae* (ATCC® 23355) and *Klebsiella pneumoniae* (ATCC® 13883)] were used for disc diffusion assay according to method described by YILDIRIM & al. [34]. Firstly, turbidity of each broth culture of bacteria was adjusted with saline (0.5 McFarland) and then inoculated into Mueller Hinton agar plates using cotton swabs. All extracts were dissolved in dimethyl sulfoxide (DMSO, Sigma®) (100 mg/mL) and then sterile filter paper discs (Glass microfibre filters, Whatman®; 6 mm diameter) including filter-sterilized extracts (0.22 µm filter-Acrodisc®) were placed into inoculated plates. There were two plates containing five replicates for each extract tested for each bacterium. Erythromycin, ampicillin, carbenicillin, tetracycline and chloramphenicol (Bioanalyse®) were used as positive controls. Negative control was DMSO. Incubation period was 24 hours at 37°C and then each disc was measured to evaluate the diameter of inhibition zones. All experiments were repeated three times.

3. Free radical scavenging activity

Free radical scavenging activity of the methanol extracts of plant species was assesed spectrophotometrically by using 2,2-diphenyl-1-picrylhydrazil (DPPH•, Sigma®) according to method described by YILDIRIM & al. [34]. DPPH was mixed with the extracts at different concentrations (12.5, 25, 50, 100 and 200 µg/mL), vortexed and then kept in the dark for 30 min. Decline in the absorbance was measured at 517 nm (Hitachi U-1900, UV-VIS Spectrophotometer 200V) against blank samples. All analyses were made in triplicate.

4. Determination of total phenolic content

Folin-Ciocalteu phenolic reagent (Sigma®) was used to determine total phenolic contents of methanolic extracts according to method described by YILDIRIM & al [34]. Calibration curve of gallic acid (Sigma®) (0, 50, 100, 150, 200, 250 and 500 mg/L) was prepared as standard. Plant extracts, each concentration of gallic acid and blank were mixed with Folin-Ciocalteu reagent and were neutralized with aqueous sodium carbonate (Na_2CO_3). The mixture was incubated at room temperature for 2 hours. The absorbance of each solution was measured at 765 nm against the blank using the spectrophotometer. Total phenolic compounds in plant extracts were calculated according to the calibration curve of gallic acid. Total phenolic was presented as mg/g gallic acid equivalent (GAE) of dry extract. Three replicates were performed.

5. Determination of total flavonoid

Aluminum chloride ($AlCl_3$) colorimetric assay was used to determine the amount of total flavonoids in the methanol extracts according to method described by YILDIRIM & al [34]. Calibration curve of catechol (Sigma®) (20, 40, 60, 80 and 100 mg/mL) was prepared as standard. Extract solution or standard was mixed distilled water and sodium nitrite ($NaNO_2$) and after 5 min, 10 % $AlCl_3$ was added to the test tubes. Thereafter, sodium hydroxide (NaOH) and distilled water was added to the mixture. The absorbance of the mixture was determined at 510 nm. Total flavonoid compounds in plant extracts were calculated according to the calibration curve of catechol. Total flavonoid was presented as mg/g catechol equivalent (CAE) of dry extract. Three replicates were performed.

6. High-Performance Liquid Chromatography (HPLC) analysis

Ten phenolic standards (gallic acid monohydrate, caffeic acid, rutin hydrate, luteolin-7-O-β-D glucoside, kaempferol, rosmarinic acid, myricetin, quercetin, coumarin and apigenin) (Sigma®) were used as reference. All standards were prepared at 1 mg/mL in acetonitrile (ACN) and mixed together to get different concentrations (1, 5, 10, 20, 40, 60, 80, 100 and 200 mg/L) for plotting the standard curve. The chromatogram of the used standards was presented in Figure 1. All methanolic extracts and standards were filtered through a 0.2-µm GHP Acrodisc (25 mm) (Pall Corporation) into 2 mL HPLC vials. HPLC system (VWR-Hitachi LaChrom Elite®) equipped with a Hitachi L-2455 diode array detector (DAD), Hitachi L-2130 Pump, Hitachi L-2200 autosampler was used for analysis. Chromatographic separation was achieved using Hitachi column oven L-2300 and Venusil XBP C18 column (Bonna-Agela Technologies, particle size 5 µm, 4.6 x 250 mm). Flow rate was 1 mL/min with 25ºC oven and injection volume was 20 µL. All solvents were HPLC grade (Merck®) and the mobile phase was composed of solvent (A) acetonitrile (ACN) and solvent (B) 0.1% acetic acid. The gradient program was started with 10% of A and 90% of B at 0 min and changed to obtain 20%, 40%, 60%, 80%, 10% A at 5, 10, 15, 20 and 20.1 min, respectively. Mobile phases and ultrapure water (SG Labostar) were filtered through a 0.45 µm hydrophilic polypropylene membrane filter (47 mm) (Pall Corporation) prior to HPLC injection. Spectra data were recorded from to 200 to 400 nm during the entire run. The chromatograms were obtained at 280 nm

7. Data analysis

All data were analyzed by analysis of variance (ANOVA) and mean values were compared with Duncan's Multiple Range Tests using SPSS vers. 15 (SPSS Inc., Chicago, IL, USA).

Results and Discussion

Nine plant species used in traditional medicine (Table 1) were evaluated for their antibacterial (Table 2) and antioxidant (Table 3) potentials, and phenolic profiles (Table 4 and 5).

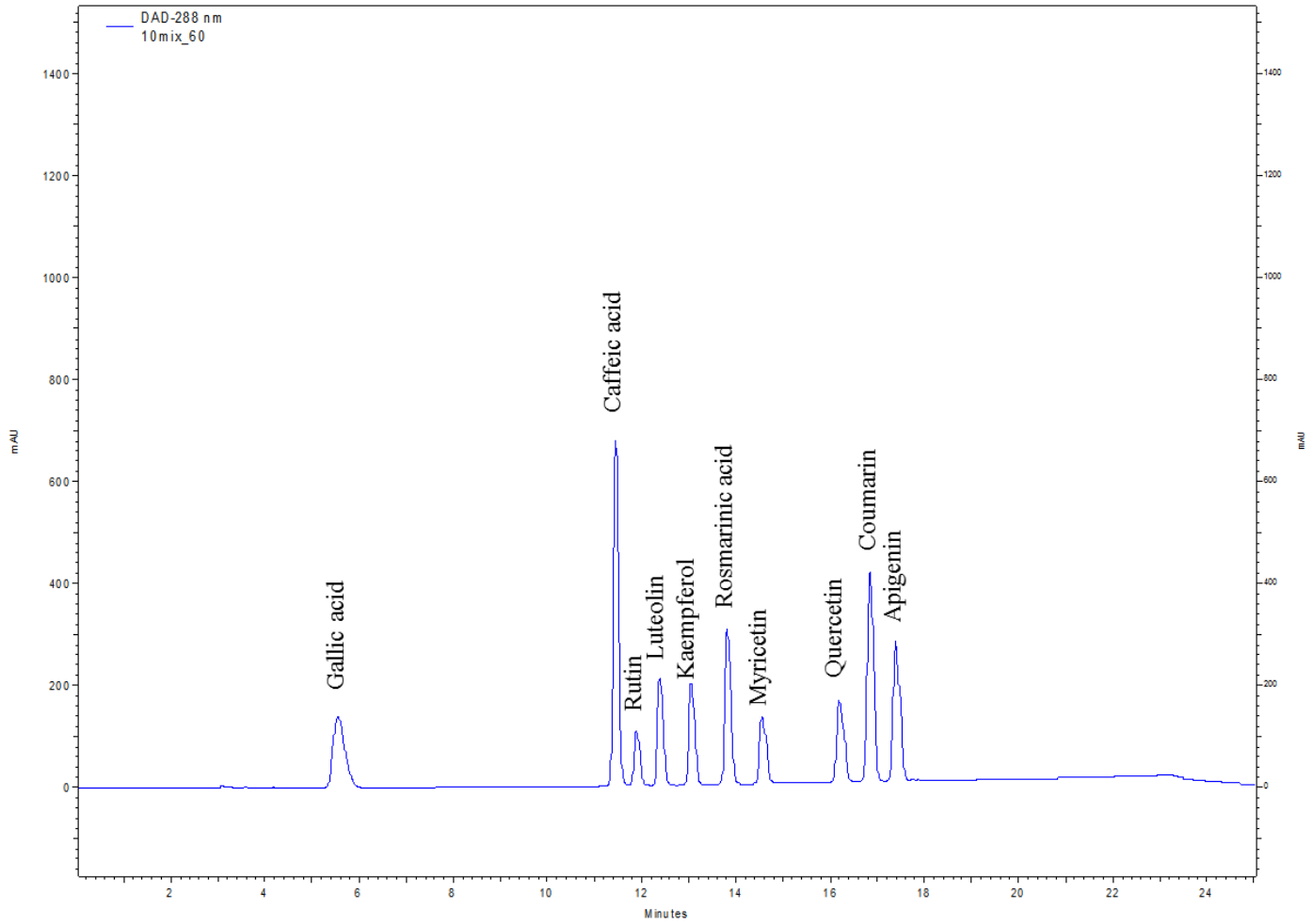

Figure 1. Chromatogram of the standards.

1. Antibacterial potential

Inhibition zone diameters for aqueous and methanol extractions of nine plant species against ten different bacterial strains were reported in Table 2. Plant extracts showed strong antibacterial activity against gram positive bacteria (*S. aureus*, *S. epidermidis* and *S. pyogenes*) in general. It may come from differences in cell wall structure in that gram-positive bacteria have single layer cell wall. On the other hand, gram-negative bacteria have multi-layered cell wall structure and it may provide strength against plant extracts. Tested bacteria were generally susceptible to positive controls (tested reference antibiotic discs). Final concentrations of all extracts were adjusted with DMSO and it was used as negative control. There was no inhibition with DMSO (Table 2).

Best antibacterial activity was observed with *E. hirsutum* against all tested gram positive bacteria. *E. angustifolium* also showed strong inhibition against these bacteria. Folkloric usage record of *E. hirsutum* for the treatment of acne and *E. angustifolium* for the cuts and wound may be explained with the strong inhibitory activity of these plants against gram positive bacteria especially *S. epidermidis*. Moreover, both plants exhibited the best inhibition against *P. vulgaris*, a gram negative bacterium.

Table 2. Antibacterial potential of tested plant extracts and controls. Data were presented as a mean diameter of inhibition zones ± standard error (SE). Means with the same letter within columns are not significantly different at $P > 0.05$. W: Water, M: Methanol, DMSO: Dimethyl sulfoxide.

Treatments		Mean Diameter of Inhibitory Zones (mm ± SE)									
		S. aureus	*S. epidermidis*	*S. pyogenes*	*S. marcescens*	*S. typhimurium*	*P. aeruginosa*	*P. vulgaris*	*K. pneumonia*	*E. cloacae*	*E. coli*
A. millefolium	W	-	-	-	-	-	-	-	-	-	-
	M	9.9 ± 0.4^{i}	-	-	-	-	-	-	-	-	-
P. vulgaris	W	9.4 ± 0.2^{ij}	10.4 ± 0.2^{h}	8.2 ± 1.8^{j}	-	-	-	-	-	-	-
	M	-	10.2 ± 0.2^{h}	-	-	-	-	-	-	-	-
P. laciniata	W	-	-	-	-	-	-	-	-	-	-
	M	-	-	-	-	-	-	-	-	-	-
L. salicaria	W	15.9 ± 0.5^{fg}	17.7 ± 0.8^{g}	14.4 ± 0.8^{h}	-	-	-	11.8 ± 1.0^{gh}	9.3 ± 0.3^{e}	-	-
	M	18.7 ± 0.4^{e}	20.4 ± 0.4^{de}	16.8 ± 1.4^{g}	-	-	11.2 ± 0.5^{c}	14.0 ± 0.7^{fg}	10.4 ± 0.5^{d}	-	-
E. angustifolium	W	13.3 ± 1.3^{h}	18.2 ± 1.3^{fg}	14.0 ± 0.9^{h}	-	-	-	15.2 ± 1.2^{ef}	-	-	-
	M	15.0 ± 0.4^{g}	18.4 ± 0.3^{fg}	16.4 ± 0.9^{g}	-	-	-	17.3 ± 0.5^{de}	-	-	-
E. hirsutum	W	16.7 ± 0.7^{f}	19.3 ± 0.9^{ef}	16.4 ± 1.0^{g}	-	-	-	12.5 ± 2.7^{gh}	-	-	-
	M	18.4 ± 0.5^{e}	20.7 ± 0.4^{d}	20.8 ± 0.5^{f}	-	-	9.3 ± 0.2^{d}	17.8 ± 0.9^{d}	-	-	-
A. eupatoria	W	8.4 ± 0.2^{j}	-	9.6 ± 0.2^{ij}	-	-	-	-	-	-	-
	M	15.4 ± 0.2^{g}	17.6 ± 0.6^{g}	20.0 ± 0.0^{f}	-	-	-	11.4 ± 0.2^{h}	-	-	-
P. comosa	W	-	-	-	-	-	-	-	-	-	-
	M	-	9.4 ± 0.2^{h}	9.2 ± 0.2^{ij}	-	-	-	-	-	-	-
V. officinalis	W	-	-	-	-	-	-	-	-	-	-
	M	-	-	10.7 ± 0.3^{i}	-	-	-	-	-	-	-
Ampicillin		34.5 ± 0.3^{b}	29.0 ± 1.2^{c}	50.5 ± 1.4^{b}	-	27.0 ± 0.0^{b}	-	23.0 ± 0.0^{c}	7.5 ± 0.3^{f}	26.5 ± 0.3^{c}	20.0 ± 0.0^{d}
Carbenicillin		36.0 ± 0.0^{a}	33.0 ± 0.0^{b}	54.0 ± 1.7^{a}	22.5 ± 1.4^{b}	27.0 ± 0.0^{b}	22.0 ± 0.0^{a}	29.0 ± 0.0^{a}	-	32.0 ± 0.6^{a}	28.0 ± 0.0^{a}
Chloramphenicol		24.5 ± 0.9^{d}	32.3 ± 0.8^{b}	36.0 ± 0.6^{e}	24.0 ± 0.6^{a}	28.5 ± 0.9^{a}	8.5 ± 0.3^{e}	26.0 ± 0.0^{b}	23.0 ± 0.0^{b}	26.5 ± 0.9^{c}	27.0 ± 0.0^{b}
Erythromycin		28.5 ± 0.9^{c}	37.0 ± 0.0^{a}	44.5 ± 0.3^{c}	7.0 ± 0.0^{d}	9.0 ± 0.6^{d}	8.0 ± 0.0^{f}	11.0 ± 0.0^{h}	11.5 ± 0.9^{c}	-	11.0 ± 0.0^{e}
Tetracycline		28.5 ± 0.9^{c}	-	41.5 ± 0.9^{d}	18.0 ± 1.2^{c}	21.0 ± 0.6^{c}	12.5 ± 0.3^{b}	28.5 ± 0.3^{a}	24.5 ± 0.3^{a}	28.5 ± 0.9^{b}	26.5 ± 0.3^{c}
DMSO		-	-	-	-	-	-	-	-	-	-

Strong antibacterial activity of these plants against *P. vulgaris* may explain why *Epilobium* species are used in traditional medicine to treat urinary tract disorders. *E. angustifolium* was recorded as antibacterial against *S. aureus*, *P. aeruginosa* and *E. coli* (BARTFAY & al [22]; RAUHA & al [35]). Similar to previous studies, both *E. angustifolium* and *E. hirsutum* exhibited strong anti-bacterial activity against *S. aureus*. But, only *E. hirsutum* showed potent inhibition against *P. aeruginosa* and no inhibition was observed against *E. coli* with both *Epilobium* species in our study (Table 2). KUNDUHOGLU & al [36] showed that ethanol extract of *E. hirsutum* flowers had antibacterial activity against *S. aureus*, *S. epidermidis*, *E. coli* and *S. typhimurium*. But, stem of this plant showed an inhibitory effect against only *S. aureus* and *S. epidermidis* but not *E. coli* and *S. typhimurium*. Similar to this study, aerial part of *E. hirsutum* including flowers and stem together demonstrated antibacterial effect against *S. aureus* and *S. epidermidis* but no activity was observed against *E. coli* and *S. typhimurium* in our study. PIRVU & al [37] also reported strong antibacterial activity of *E. hirsutum* against *E. coli* and *S. aureus* (17 mm for both of them).

The broadest spectrum of antibacterial activity was observed with *L. salicaria*. In addition to the inhibition against gram positive bacteria, 3 gram negative bacteria (*P. aeruginosa*, *P. vulgaris* and *K. pneumonia*) were susceptible to methanol extract of *L. salicaria* (Table 2). *L. salicaria* has been used as curative purpose in the skin diseases and inflammations, and as intestinal disinfectant. Broad spectrum antibacterial activity of this plant supports these traditional usages. Vulnerary activity of *L. salicaria* in folkloric usage may be due to its strong antibacterial activity against *S. aureus*, *S. pyogenes*, *S. epidermidis*, *P. vulgaris* and *P. aeruginosa*. Although RAUHA & al [35] reported strong antibacterial activity of *L. salicaria* against *E. coli* and slight activity against *S. aureus*, strong antibacterial activity against *S. aureus* (18.7 mm) and no activity against *E. coli* were observed in our study (Table 2). PIRVU & al [37] also showed weak antibacterial activity of *L. salicaria* against *E. coli* (8 mm) and moderate activity against *S. aureus* (12 mm). In consistent with our study, BECKER & al [38] reported strong antibacterial activity of *L. salicaria* against *S. aureus* and no activity against *E. coli*.

Methanolic extract of *P. vulgaris* was effective against all tested gram positive bacteria and exhibited medium

strength inhibition against these bacteria. However, antibacterial activity was not observed with *P. laciniata* against any bacteria. According to ethnobotanical records, *P. vulgaris* has a remedy in respiratory and gastrointestinal system ailments, sore throat and wounds. Moderate antibacterial activity of *P. vulgaris* against gram positive bacteria may explain and justify these traditional usages. *P. laciniata* has been recorded for the treatment of cough, skin diseases and wounds in traditional medicine. Antibacterial activity of *P. laciniata* was not observed against 10 bacteria in our study and antibacterial results did not support these traditional usages for *P. laciniata*. It was reported in one study that *P. vulgaris* extract inhibited *P. aeruginosa*, *E. coli*, *P. vulgaris*, *S. aureus* and no activity was observed against *K. pneumoniae* (KIRBAG & al [39]). But, only *S. aureus*, *S. epidermidis* and *S. pyogenes* were sensitive to *P. vulgaris* extract and no inhibition was observed with other bacteria in our study (*P. aeruginosa*, *E. coli*, *P. vulgaris* and *K. pneumoniae*) (Table 2).

Only *S. aureus* was sensitive to *A. millefolium* in our study. *A. millefolium* has been used as an antiseptic, wound cleaner and a remedy in common cold and cough in folk medicine. Moderate inhibition of *S. aureus* may explain these uses. One previous study showed inhibition against *E. coli* in addition to *S. aureus* (FAIKU & al [40]). In consistent to our result, BOBIS & al [41] reported that *A. millefolium* exhibited inhibition against *S. aureus* but no inhibition was observed against *S. typhimurium*, *E. coli* and *P. aeruginosa*. On the other hand AFSHARI & al [42] indicated the sensitivity of *E. coli*, *S. epidermidis* and *S. typhimurium* against *A. millefolium* extract. CANDAN & al. [43] determined that water soluble portion of methanolic extract of *A. millefolium* did not show antibacterial activity against *S. aureus*, *E. coli*, *P. aeruginosa* and *K. pneumoniae*. On the other hand, SHAHBAZI and ZADEH [44] exhibited antibacterial activity of *A. millefolium* alcoholic extract against wound pathogen microorganism such as *S. aureus*, *K. pneumoniae*, *P. aeruginosa* and *E. coli*.

Methanolic extract of *A. eupatoria* was very potent on all tested gram positive bacteria. Furthermore, gram negative bacteria *P. vulgaris* was sensitive to this extract in our study (Table 2). According to ethnobotanical records, *A. eupatoria* has therapeutic potential in sore throat, laryngitis, pharyngitis, respiratory diseases, dermatitis, skin diseases and wounds. Very potent inhibitory activity against *S. aureus*, *S. epidermidis* and *S. pyogenes*, and moderate activity against *P. vulgaris* may justify above traditional usages. One study reported that ethanol extract of *A. eupatoria* showed inhibition against *E. coli*, *P. aeruginosa*, *K. pneumoniae* and *S. aureus* (MURUZOVIĆ & al [45]).

Only *S. pyogenes* was susceptible to methanolic extract of *V. officinalis* (Table 2). CAVERO and CALVO [30] highlighted the value of *V. officinalis* in the treatment of respiratory problem after reviewing many ethnobotanical, phytochemical and pharmacological studies. Moderate antibacterial activity of *V. officinalis* against *S. pyogenes* may prove the traditional usage of this plant in the treatment of cough, sore throat, sinusitis and boils. SISAY & al [46] reported that 80% methanol extracts of the leaves of *V. officinalis* displayed antibacterial activity against *S. aureus* and *E. coli*, and no activity against *P. aeruginosa*. AHMED & al [47] investigated ethanolic extract of leaves and stem of *V. officinalis*. They observed that *S. aureus*, *S. epidermidis*, *K. pneumoniae*, *P. aeruginosa* and *E. coli* were susceptible to this ethanolic extract.

2. Antioxidant capacity

Among the tested plant species, *P. laciniata*, *E. angustifolium* and *L. salicaria* showed the highest antioxidant capacities. *A. eupatoria*, *E. hirsutum* and *P. comosa* also had very potent radical scavenging capacity (< 12.5 µg/mL) (Table 3).

Table 3. Free radical scavenging activity of tested plant extracts. IC_{50}: The half maximal inhibitory concentration.

		% DPPH Inhibition Concentrations				
Treatments	**IC_{50} (µg/mL)**	**12.5 µg/mL**	**25 µg/mL**	**50 µg/mL**	**100 µg/mL**	**200 µg/mL**
Ascorbic acid	< 12.5	90.1	91.2	96.0	96.5	99.7
A. millefolium	26.9 ± 3.3	20.8	45.1	78.9	92.5	92.2
P. vulgaris	18.9 ± 2.9	32.8	52.2	85.9	90.5	91.8
P. laciniata	< 12.5	88.9	89.3	90.3	90.4	91.7
L. salicaria	< 12.5	81.0	88.7	88.9	93.7	96.2
E. angustifolium	< 12.5	81.0	92.3	95.4	98.2	98.2
E. hirsutum	< 12.5	71.6	81.2	88.7	88.4	91.1
A. eupatoria	< 12.5	73.3	79.5	80.7	83.9	83.2
P. comosa	< 12.5	68.6	87.5	93.5	95.9	96.8
V. officinalis	33.1 ± 3.2	19.4	23.1	79.9	90.8	90.5

In regard to total phenol-flavonoid content of the tested species, *E. hirsutum* had the highest total phenolic content [331.58 mg gallic acid equivalent (GAE)/g dry extract] and *L. salicaria* had the highest total flavonoid content [346.67 mg catechol equivalent (CAE)/g dry extract] (Table 4).

Table 4. Total phenolic and flavonoid content of tested plant extracts. Data were presented as a mean number ± standard error (SE). GAE: Gallic acid equivalent, CE: Catechol equivalent.

Treatments	Total Phenolics in mg GAE/g dry extract	Total Flavonoids in mg CE/g dry extract
A. millefolium	87.33 ± 0.01	464.38 ± 0.01
P. vulgaris	190.21 ± 0.02	266.00 ± 0.001
P. laciniata	222.64 ± 0.05	220.00 ± 0.001
L. salicaria	311.88 ± 0.04	346.67 ± 0.001
E. angustifolium	215.06 ± 0.04	178.86 ± 0.001
E. hirsutum	331.58 ± 0.03	259.43 ± 0.001
A. eupatoria	260.97 ± 0.12	264.76 ± 0.001
P. comosa	91.12 ± 0.02	115.62 ± 0.001
V. officinalis	102.03 ± 0.02	280.67 ± 0.001

Oxidative stress is an imbalance between reactive oxygen species (ROS) production and their removal through defensive mechanisms that can give rise to chronic inflammation. Oxidative stress-triggered inflammation is the cause of many chronic diseases. Polyphenols, associated with antioxidant activity, are suggested to be useful as booster therapy for their possible anti-inflammatory impact. Phenolic compounds and flavonoids can interfere with ROS and thus stop the chain reaction before seriously affecting cell viability (HUSSAIN & al [48]). Anti-inflammatory properties of *E. angustifolium*, *E. hirsutum*, *L. salicaria* and *A. eupatoria* in the treatment of various diseases have been recorded in traditional medicine. Strong free radical scavenging activities and higher total phenol-flavonoid content of these species support this property.

Similar to our results, *E. angustifolium* showed high radical scavenger activity in one previous study (TÓTH & al [49]). DENG & al [50] showed that ethanol extract of *E. angustifolium* had potent antioxidant activities in DPPH radical scavenging activity with EC_{50} of 25.53 µg/mL that is lower than our result (< 12.5 µg/mL). PIRVU & al [37] exhibited very strong scavenger potency of *L. salicaria* and *E. hirsutum* (IC_{50} = 2.83 and 4.66 µg/mL, respectively) that is higher than our result (< 12.5 µg/mL). MANAYI & al [51], LOPEZ & al [52] and TUNALIER & al [53] reported strong antioxidant activity of *L. salicaria* as IC_{50} of 13.52 µg/mL, 4.84 µg/mL, and 0.3 mg/mL, respectively. Our results were in agreement with those reported by WOJDYŁO & al. [54] in that *E. angustifolium* had high total phenolic content (4.03 mg of GAE/100 g of dry weight). Furthermore, DENG & al. [50] reported higher phenolic and flavonoids compounds (16.81 g GAE/100 g extract and 4.95 g quercetin equivalent (QE)/100 g extract, respectively) in ethanol extract of *E. angustifolium* than our results (Table 4). MANAYI & al [51] indicated high total phenol and flavonoid content of *L. salicaria* as 331 µg GAE/mg and 5.8 µg QE/mg, respectively. TUNALIER & al [53] demonstrated high total phenol and flavonoid content of *L. salicaria* as 191.35 mg GAE/g extract and 37.57 mg rutin equivalents (RE)/g extract, respectively. Ethanol extract (70%) of *L. salicaria* was recorded having total phenolic and flavonoid compounds as 17.5% tannin equivalent/g dried plant and 0.594% QE/g dried plant, respectively (HUMADI & al [55]). On the other hand, methanolic extract of *L. salicaria* had total phenolic and flavonoid compounds as 31.1% GAE/g dry extract and 34.6% CAE/g dry extract, respectively in our study (Table 4).

The highest total flavonoid content and the lowest total phenolic content was obtained with *A. millefolium* having 464.38 mg CAE/g dry extract and 87.33 mg GAE/g dry extract, respectively (Table 4). On the other hand, BOBIS & al [41] demonstrated that *A. millefolium* had total phenolic and flavonoid content as 134.65 mg GAE/g and 42.6 mg QE/g dry weight of plant, respectively. Total phenolic and flavonoid content of *A. millefolium* were recorded by AFSHARI & al [42] as 48.10 mg tannic acid equivalent/g DW and 10.9 mg QE/g DW, respectively. Also, they found IC_{50} of *A. millefolium* as 854.1 µg/mL showing lower antioxidant activity than our result (IC_{50} = 26.9 µg/mL) (AFSHARI & al [42]). Conversely, CANDAN & al [43] determined the IC_{50} of *A. millefolium* as 45.60 µg/mL that is very close to our result.

Higher total phenolic content (222.64 and 190.21 mg GAE/g, respectively) and antioxidant capacity (IC_{50} as < 12.5 µg/mL and 18.9 µg/mL, respectively) were obtained for *P. laciniata* and *P. vulgaris* in our study than previous studies. For example, ŞAHIN & al [56] showed that total phenolic content of *P. laciniata* and *P. vulgaris* were 93.4 and 97 mg GAE/g dried plant, respectively. AHN & al. [57] also reported that *P. vulgaris* had IC_{50} as 50.35 µg/mL and total phenolic content as 90.53 mg GAE/g.

CORREIA & al. [58] determined IC_{50} value of aqueous alcoholic extract of *A. eupatoria* as 18.12 µg/mL and total phenol as 15.78% that is to say lower antioxidant capacity and phenolic content than our study (<12.5 µg/mL and 26% total phenol, respectively) (Table 3 and 4). Also, SANTOS & al. [59] indicated IC_{50} value of *A. eupatoria* infusion as 12.8 µg/mL. MURUZOVIĆ & al [45] indicated total phenol and flavonoid content of *A. eupatoria* as 123.9 mg GAE/g extract and 46.5 mg RE/g extract, respectively and they obtained 59.59% inhibition of DPPH at 31.25 µg/mL that means lower total phenol, flavonoid

and antioxidant capacity than our results (260.97 mg GAE/g, 264.76 mg CE/g and IC_{50} < 12.5 µg/mL, respectively)

IC_{50} value and total phenolic content of *V. officinalis* aerial part containing stem, leaves and flowers were 33.1 µg/mL, 10.2 g GAE % and 28.0 g CAE % in our study (Table 3 and 4). In one study it was found that *V. officinalis* aerial part had IC_{50} value as 69.58 µg/mL (LOPEZ & al [52]). Hydroalcoholic extract of *V. officinalis* aerial part was investigated by REHECHO & al [60]. IC_{50} value, total phenolic and flavonoid contents were recorded as 1.25 g GAE %, 0.76 g RE % and 21.04 µg/mL, respectively. KHALAF & al [61] indicated moderate antioxidant potential of *V. officinalis* leaves (IC_{50} = 23.63 µg/mL) and total phenolic content as 652.5 mg GAE %.

Strong radical scavenging capacity of *P. comosa* was revealed for the first time with this study. Correlation was not observed between antioxidant activity and total phenol-flavonoid content for *P. comosa* (Table 3 and 4).

3. HPLC Analysis

Results obtained from the HPLC-DAD analysis presented that rosmarinic acid was the most dominant compound in *P. laciniata* and *A. eupatoria*. Rosmarinic acid was proved to have strong antioxidant activity (HOLZMANNOVÁ & al [62]). Strong antioxidant potency of them may be related to high content of rosmarinic acid in the extract. In addition to rosmarinic acid, *A. eupatoria* contained rutin, apigenin, kaempferol and quercetin in the order of decreasing phenolic quantity (Table 5). KUBÍNOVÁ & al [63] demonstrated HPLC profile of the aqueous extract of *A. eupatoria* containing quercetin, apigenin, luteolin and kaempferol glycosides.

Table 5. Quantitative analysis of tested plant extracts by HPLC-DAD. Data were presented as a mean number of phenol amount ± standard error (SE). RT: Retention time.

STANDART COMPOUNDS			**PLANT EXTRACTS (mg/g dry extract)**								
Names	**Peak number**	**RT (min)**	*A. millefolium*	*P. vulgaris*	*P. laciniata*	*L. salicaria*	*E. angustifolium*	*E. hirsutum*	*A. eupatoria*	*P. comosa*	*V. officinalis*
Gallic acid	1	5.51	1.02±0.10	-	-	1.24±0.02	2.47±0.03	2.30±0.23	-	0.65±0.00	0.38±0.00
Caffeic acid	2	11.4	2.05±0.02	-	-	0.91±0.06	-	0.53±0.02	0.03±0.01	-	4.39±0.04
Rutin	3	11.84	1.23±0.25	84.15±0.05	14.94±0.03	-	4.27±0.38	-	11.97±0.06	30.27±0.41	126.06±0.14
Luteolin	4	12.33	5.43±0.13	0.17±0.06	-	0.27±0.04	14.09±0.45	-	-	5.52±0.05	0.31±0.04
Kaempferol	5	13	0.44±0.04	-	2.14±0.01	-	2.48±0.26	0.99±0.02	0.91±0.01	0.73±0.05	0.26±0.05
Rosmarinic acid	6	13.77	-	2.01±0.01	114.72±0.06	-	0.97±0.01	-	111.49±0.07	0.88±0.01	0.28±0.00
Myricetin	7	14.51	1.10±0.00	-	0.15±0.01	0.15±0.01	0.43±0.03	0.33±0.01	-	0.14±0.02	0.27±0.01
Quercetin	8	16.15	7.46±0.03	0.10±0.04	0.13±0.02	-	0.19±0.07	0.02±0.01	0.19±0.02	0.94±0.03	0.55±0.01
Coumarin	9	16.81	-	-	-	-	-	-	-	0.12±0.01	-
Apigenin	10	17.34	1.06±0.06	1.54±0.01	1.80±0.02	2.13±0.02	2.14±0.01	2.13±0.00	1.78±0.01	1.96±0.01	1.59±0.02

V. officinalis and *P. vulgaris* were the best rutin source. In consistent with the result of ŞAHIN & al [56], *P. laciniata* and *P. vulgaris* were the best sources of rosmarinic acid and rutin, respectively. Also, FENG & al. [64] presented that main compounds in ethanol extract of *P. vulgaris* were caffeic acid, rosmarinic acid, rutin and quercetin, respectively. However, caffeic acid was not detected in our study (Table 5). Caffeic acid and apigenin were also abundant besides rutin in *V. officinalis* in our study (Table 5). In one record, hastatoside, verbenalin, luteolin, apigenin and verbascoside were identified as major components of *V. officinalis* in 50% MeOH extract (CALVO & al [65]).

Phenolic content of *E. angustifolium* followed the order of decreasing amount as luteolin, rutin, kaempferol, gallic acid, apigenin, rosmarinic acid, myricetin and quercetin (Table 5). Similar to our result, TÓTH & al [49] reported that quercetin and kaempferol were dominant in *E. angustifolium*. Also, gallic acid, ellagic acid, quercetin and myricetin were found in high amount in ethanol extract of *E. angustifolium* (DENG & al [50]). However, luteolin and rutin were found in high concentrations in *E. angustifolium* in our study (Table 5).

HPLC analysis for *A. millefolium* showed that phenolic content followed the decreasing order of quercetin, luteolic, caffeic acid and rutin (Table 5). BOBIS & al [41] identified rosmarinic acid, rutin, luteolin and caffeic acid with descending order in this plant. Rosmarinic acid was not identified in our study. 1,3-dicaffeoylquinic acid, rutin, apigenin, caffeic acid and luteolin were identified as the major phenols with HPLC analysis in *A. millefolium* by AFSHARI & al [42].

It was found for the first time that *P. comosa* was the good source of rutin, luteolin and apigenin in the order of descending amount. Additionally, phenolic profile of *L. salicaria* was first revealed using tested phenolic standards with this study (Table 5).

Conclusion

Bioevaluation of the effectiveness of traditional medicines is gaining importance in recent years. Verification of traditional usage of medicinal plant opens new doors for the discovery of different drugs. In this study, nine different traditional plants were evaluated in terms of antibacterial and antioxidant potential relating with their

traditional usages. Some traditional usages of *L. salicaria*, *E. angustifolium*, *E. hirsutum* and *A. eupatoria* were strongly confirmed with our antibacterial results. Furthermore, strong antioxidant potential of these plants scientifically justified the ethnobotanical records of them as anti-inflammatory. On the other hand, folkloric usages of *P. laciniata* as a remedy in skin diseases, wounds and cough were not proved with our tested 10 pathogenic bacteria. The best antioxidant activity was determined with *L. salicaria*, *P. laciniata* and *E. angustifolium*. Moreover, the highest total phenolic and flavonoid contents were obtained with *E. hirsutum*, *A. millefolium* and *L. salicaria*. The most remarkable plants in terms of antibacterial and antioxidant potentials were *L. salicaria*, *E. hirsutum* and *E. angustifolium* when evaluated together. These plants may be appropriate natural sources for drugs that can be used for the treatment of infections and also they can be a source of food additives as antioxidant ingredient to prevent many diseases.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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