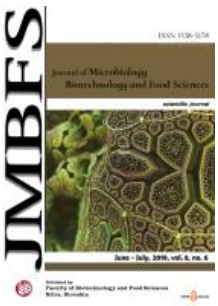

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IN VITRO SCREENING OF ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF MEDICINAL PLANTS GROWING IN SLOVAKIA

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ABSTRACT

Traditional medicine usage at the preventive medicine level is comprehensive and plant-based remedies are being prescribed for the treatment of a large scale of diseases by indigenous medical practitioners around the world. The phytochemicals being observed in the fruits and vegetables as well as herbal plants are getting investigated intensively as their important role in the avoidance of diverse human diseases is undoubtful. Antioxidant and antimicrobial activities of selected medicinal herbs were evaluated in this study. Aerial parts of plants from *Hypericaceae*, *Lamiaceae*, *Fabaceae*, *Plantaginaceae*, *Rubiaceae* growing wild in Slovakia (locality Zobor) were investigated to characterize their polyphenol content, flavonoid content, as well as antioxidant and antimicrobial activity. Inspection of the metabolic profile combined with HPLC data showed that the ethanol extract contained mainly eight polyphenols with apigenin, daidzein, kaempferol, rutin, quercetin, vitexin, and cynaroside as the main chemicals. Among them, neochlorogenic acid, chlorogenic acid, *trans-p*-caffeic acid, *trans-p*-coumaric acid, *trans-p*-sinapic acid, *trans-p*-ferulic acid, rosmarinic acid were identified. The antioxidant capacity of ethanol extracts was evaluated by employing DPPH radical scavenging and phosphomolybdenum assays. All samples were also tested against Gram-positive (*Staphylococcus aureus*, *Bacillus thuringiensis*, *Micrococcus luteus*, *Staphyloccocus epidermidis*) and Gram-negative (*Citrobacter koseri*, *Escherichia coli*, *Pseudomonas proteolytica*, *Hafnia alvei*, *Salmonella enterica*, *Yersinia enterocolitica*) bacterial species using the disk diffusion and minimum inhibitory concentration assays.

Keywords: polyphenols, phenolic acids, flavonoids, chemical profile, herbs

INTRODUCTION

Medicinal plants have been used extensively as alternative agents for the treatment of various infections and diseases for thousands of years **(Jamshidi-Kia *et al.*, 2018)**. Traditional herbs have received much attention as a source of novel drugs since they are considered safe for human use **(Yixuan *et al.*, 2007)**. Plant-based medicines are widely used for primary health care in many developing countries **(Abu-Irmaileh *et al.*, 2003)**. As a result, it is found that about 60–80% of the world population relies on traditional treatment. The contribution of medicinal plants and natural products as drugs or as sources of using drugs in medicine is unquestionable **(WHO, 2002)**. Numerous secondary metabolites such as alkaloids, tannins, and flavonoids extracted from different medicinal plants have shown antioxidant **(Rauf *et al.*, 2013)**, anticancer **(Yizhong *et al.*, 2004; Costa-Lotufo *et al.*, 2005)**, anti-inflammatory **(Latifi *et al.*, 2015)**, antibacterial potential **(Mahboubi *et al.*, 2012)**.

The Slovak Republic is situated in Central Europe. It is placed in the climatically welcoming mild zone of Northern Hemisphere. Since time traditional medicinal plants have proved their important part in the content of therapeutic and various preparations used in popular human health medicine in Slovak. In past centuries about 600 to 800 species were used for curative reasons **(Salamon, 2014)**. Nowadays about 200 medicinal plants are used in the official therapy and in popular doctoring, respectively **(Salamon, 2015)**. It is approved by the years of positive experience of people carrying explicit contact with nature. Over the years this experience has been validated in practice, improved and categorized, folk herbalist knowledge got formed and passed through generations. This study focused on 7 plants form 5 plant family as useful in traditionally managing various human diseases. We have summarized the data obtained from published literature and the uses of chosen studied plants in folk medicine in Table 1. Infectious diseases are a major cause of death and disability in humans as they prevail constant and rapid change. With microorganisms emerging new preventions and treatments evolve as well. It has been reported that almost 25% of the population experienced 2-3 episodes of infection every year **(Shuman *et al.*, 2018)**. Many foods primarily deteriorate because of microbes that give rise to the loss of quality and safety, on which people worldwide are concerning more because foodborne diseases are subject to outbreak due to pathogenic and spoilage microorganisms in foods **(Rawat, 2015)**. Moreover, for a long time considered to be non-pathogenic to humans, some bacteria have been sporadically identified as responsible for various infectious diseases in humans. Numerous diseases, even those that were once easily healed, are becoming a huge problem. Noteworthy, the frequency of sporadically infections can also be underestimated. Hence, search for novel antimicrobial compounds or alternative therapy for these resistant infectious agents is inevitable.

Antibacterial compounds provided widely by herbal species may have crucial applications in the nearest future as native antimicrobial components not only in the food industry but also for the medicinal purpose. The antimicrobial activity mechanisms of important herbal species demonstrate the process of bacteria growth inhibition that may involve cytoplasmic membrane destabilization and further excessive permeabilization, extracellular microbial enzymes inhibition, etc. Antimicrobial mechanism of action of herbs may also be connected with antiadherence of bacteria to epithelial cells, being essential means for colonization and infection of many pathogens **(Davidson *et al.*, 2015)**.

In the present study, we used 10 strains of Gram-negative and Gram-positive bacteria, with known and/or recently found pathogenesis against humans and animals. Data summarized in Table 2.

Table 1 Short summary of traditional medicinal uses of studied plants

Common name	Botanical name	Family	Use in traditional medicine	References
Yellow toadflax	*Linaria vulgaris L.*	*Plantaginaceae*	To treat coughs and asthma. Possesses uterine stimulatory activity, expectorant, antiseptic, antiperiodic and anthelminthic properties	**(Hua *et al.*, 2002, Bruhn, 1982)**
Yellow bedstraw	*Galium verum* L.	*Rubiaceae*	Used as preventive and/or a concomitant therapeutic approach in head and neck cancer	**(Schmidt *et al.*, 2013)**
Perforate St John's-wort	*Hypericum perforatum* L.	*Hypericaceae*	The popular treatment for anxiety, depression, cuts, and burns.	**(Gaster *et al.*, 2000)**
Black locust	*Robinia pseudoacacia* L.	*Fabaceae*	Demonstrates laxative, antispasmodic, diuretic effect	(**Subramoniam, 2016)**
Hungarian thyme	*Thymus pannonicus* L.	*Lamiaceae*	Popular against coughs and other respiratory complaints, as well as some cases of gastrointestinal disorders	**(Maksimović *et al.*, 2008)**
Lemon balm	*Melissa officinalis* L.	*Lamiaceae*	Menstrual problems, hypertension, migraines, vertigo and fever, depression and melancholy, bronchitis and asthma, eczema and gout, epilepsy, paralysis, Bell's palsy, and arthritis. Advanced researches showed its neuroprotective, anxiolytic, antispasmodic, antihyperlipidemic and hepatoprotective effects.	**(Lopez *et al.*, 2009, Kennedy *et al.*, 2004, Cases *et al.*, 2011)**
Sage	*Salvia officinalis* L.	*Lamiacea*	Used in the treatment of digestive and circulation disturbances, bronchitis, cough, asthma, angina, mouth and throat inflammations, depression, excessive sweating, skin diseases, and many other diseases	**(Rami *et al.*, 2011, Walch *et al.*, 2011, Khan *et al.*, 2011)**

Table 2 Studied bacteria strains and their reported pathogenic effects

Bacteria	Gram test	Family:	Pathogenesis	References
Bacillus thuringiensis	+	*Bacillaceae*	Nongastrointestinal infections in mammals	**(Celandroni *et al.*, 2014)**
Micrococcus luteus	+	*Micrococcaceae*	Septic arthritis, meningitis, and prosthetic valve endocarditis	**(Miltiadous *et al.*, 2011)**
Staphyloccocus epidermidis	+	*Staphylococcaceae*	Devastating effects on certain organs such as kidney, liver, intestine, stomach, and spleen which, depending on their severity, could be fatal	**(Akinkunmi *et al.*, 2014)**
Staphyloccocus aureus	+	*Staphylococcaceae*	Bacteremia and infective endocarditis as well as osteoarticular, skin and soft tissue, pleuropulmonary, and device-related infections.	**(Tong *et al.*, 2015)**
Citrobacter koseri	-	*Enterobacteriaceae.*	Neonatal meningitis and brain abscess with high mortality rates	**(Lin *et al.*, 2011)**
Escherichia coli	-	*Enterobacteriaceae*	Urinary tract infections, meningitis, pneumonia, septicemia, and other types of infections	**(Russo and Johnson, 2003, Smith *et al.*, 2007, Fratamico, 2016)**
Pseudomonas proteolytica	-	*Pseudomonadaceae*	Not found in the literature	**(Chauhan *et al.*, 2015)**
Hafnia alvei	-	*Enterobacteriaceae*	Gastroenteritis, meningitis, bacteremia, pneumonia, nosocomial wound infections, endophthalmitis, and a buttock abscess	**(Günthard and Pennekamp, 1996)**
Salmonella enterica	-	*Enterobacteriaceae*	Gastroenteritis, bacteremia, enteric fever, and an asymptomatic carrier state	**(Ryan and Ray, 2004)**
Yersinia enterocolitica	-	*Enterobacteriaceae*	Diarrhea in the inoculated animals followed by lethality in guinea pigs and mice, but negative for autoagglutination test, calcium dependency, conjunctivitis, and positive for heat-stable enterotoxin production	**(Igumbor *et al.*, 1993)**

The aims of the present study were (1) to evaluate total polyphenol, phenolic acid and flavonoid content in ethanolic extract of selected medicinal herbs (2) to assess the antioxidant activity of these samples; (3) to detect the antibacterial activities of samples against gram-positive and gram-negative bacteria.

MATERIAL AND METHODS

Chemicals and Reagents

The HPLC phenolic standards were apigenin, daidzein, kaempferol, resveratrol, rutin, quercetin, vitexin, neochlorogenic acid, chlorogenic acid, *trans-p*-caffeic acid, *trans-p*-coumaric acid, *trans-p*-sinapic acid, *trans-p*-ferulic acid, rosmarinic acid. All other chemicals and reagents were of analytical grade and were purchased from Reachem (Slovakia) and Sigma Aldrich (Germany and/or Switzerland). Cynaroside as a standard was kindly provided by Prof. Peter Rapta, DSc (Institute of Physical Chemistry and Chemical Physics, Bratislava, The Slovak Republic).

Plant material

Mature plant samples were harvested in May 2016 from the spontaneous flora in Slovak Republic (locality Zobor, DMS N 48° 19' 46.676"E 18° 6' 2.003") and identified. Aerial parts of the collected plants were separated from undesirable materials and dried in open air under shade for 2 weeks. The dried plants were powdered with a mechanical grinder to obtain a coarse powder, stored in an airtight container and kept in a cool, dark, and dry place until analysis commenced.

Preparation of extracts

Content of selected polyphenolic compounds (apigenin, cynaroside, daidzein, kaempferol, resveratrol, rutin, quercetin, vitexin, neochlorogenic acid, chlorogenic acid, *trans-p*-caffeic acid, *trans-p*-coumaric acid, *trans-p*-sinapic acid, *trans-p*-ferulic acid, rosmarinic acid) was performed by HPLC method (according to **Novakova *et al.* 2010**), as described below.
Preparation of calibration solutions: the standard stock solution was prepared by dissolving 0.5 mg each of them with methanol in 10 ml volumetric glass flasks.

All standard solutions were kept at 6°C in the dark for a maximum of 3 h. prior to injection, the solutions were filtered through syringe filter Q-Max (0.22 µm; Frisenette ApS, Knebel, Denmark).
Sample preparation: Dried plants (2.5 g) after homogenization in a mortar were extracted with 25 ml of 80% ethanol (v/v) at laboratory temperature for 8 h (Unimax 2010, Heidolph Instrument, Schwabach, Germany). The extract was filtered through Munktell No 390 paper (Munktell & Filtrac, Barenstein, Germany) and stored in closed 20 ml vial tubes. Prior to injection, the extracts were filtered through syringe filter Q-Max (0.22µm; Frisenette ApS, Knebel, Denmark).
HPLC analyses: The phenolic compounds were analyzed using an Agilent 1260 Infinity high-performance liquid chromatography (Agilent Technologies, Waldbronn, Germany). Separation was achieved on a Purosphere reverse-phase C18 column (4 mm×250mm×5µm) Merck KGaA, Darmstadt, Germany). The solvents were: (A) acetic acid in methanol (50/1000ml), (B) acetic acid in HPLC grade water (50/1000ml). the following gradient program was employed: 0-5 min isocratic elution (20% A and 80 % B), 5-11 min linear gradient elution (60% A and 40% B), and 80% A and 20 % B 11-20 min. the initial flow rate was 1 ml/min. column oven temperature was set up to 30°C and the samples were kept at 4°C in the sample manner. The PDA detection was conducted at 330nm for quantitative purposes with data acquisition rate of 5 Hz. Detection was carried out in a diode array detector (DAD), within 210-400 nm as the preferred wavelengths.

Radical scavenging activity

Radical scavenging activity of extracts was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) **(Sanchéz-Moreno *et al*., 1998).** The sample (0.4 ml) was mixed with 3.6 ml of DPPH solution (0.025 g DPPH in 100 ml methanol). An absorbance of the reaction mixture was determined using the spectrophotometer Jenway (6405 UV/Vis, England) at 515 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2carboxylic acid) (10-100 mg/L; R^2=0.989) was used as the standard and the results were expressed in mg/g Trolox equivalents.

Reducing power

Reducing power of extracts was determined by the phosphomolybdenum method of **Prieto *et al*. (1999)** with slight modifications. The mixture of sample (1 ml), monopotassium phosphate (2.8 ml, 0.1 M), sulfuric acid (6 ml, 1 M), ammonium heptamolybdate (0.4 ml, 0.1 M) and distilled water (0.8 ml) was incubated at 90°C for 120 min, then rapidly cooled and detected by monitoring absorbance at 700 nm using the spectrophotometer Jenway (6405 UV/Vis, England). Trolox (10-1000 mg/L; R^2=0.998) was used as the standard and the results were expressed in mg/g Trolox equivalents.

Total polyphenol content

Total polyphenol content of extracts was measured by the method of **Singleton and Rossi (1965)** using Folin-Ciocalteu reagent. 0.1 ml of each sample was mixed with 0.1 ml of the Folin-Ciocalteu reagent, 1 ml of 20% (w/v) sodium carbonate, and 8.8 ml of distilled water. After 30 min. in darkness the absorbance at 700 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Gallic acid (25-300 mg/L; R^2=0.998) was used as the standard and the results were expressed in mg/g gallic acid equivalents (mg GAE/g).

Total flavonoid content

Total flavonoids were determined using the modified method of **Willett (2002)**. 0.5 ml of sample was mixed with 0.1 ml of 10% (w/v) ethanolic solution of aluminium chloride, 0.1 ml of 1 M potassium acetate and 4.3 ml of distilled water. After 30 min. in darkness the absorbance at 415 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Quercetin (0.520 mg/L; R^2=0.989) was used as the standard and the results were expressed in µg/g quercetin equivalents (mg QE/g).

Total phenolic acid content

Total phenolic acids content was determined using the method of **Farmakopea Polska (1999)**. A 0.5 mL of sample extract was mixed with 0.5 mL of 0.5 M hydrochloric acid, 0.5 mL Arnova reagent (10% $NaNO_2$ + 10% Na_2MoO_4), 0.5 mL of 1 M sodium hydroxide (w/v) and 0.5 mL of water. Absorbance at 490 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Caffeic acid (1-200 $mg.L^{-1}$, R^2=0.9996) was used as a standard and the results were expressed in mg/g caffeic acid equivalents (mg CAE/g).

Microbial strains

Ten strains of microorganisms were tested in this study, Gram-negative bacteria: *Escherichia coli* CCM 3988, *Salmonella enterica* subsp. *enterica* CCM 3807, *Yersinia enterocolitica* CCM 5671, *Citrobacter koseri* CCM 2535, *Pseudomonas proteolytica* *CCM 7690, Hafnia alvei CCM 2636* and Gram-positive bacteria: *Bacillus thuringiensis* CCM 19^T, *Stapylococcus aureus subsp*. aureus CCM 2461, *Micrococcus luteus* CCM 732, *Staphylococcus epidermidis* CCM 4684. All tested strains were collected from the Czech Collection of microorganisms. The bacterial suspensions were cultured in the nutrient broth (Imuna, Slovakia) at 37°C.

Disc diffusion method

Antimicrobial activity of each plant extract was determined by a disc diffusion method. Briefly, 100 µl of the test bacteria were grown in 10 ml of fresh media until they reached a count of approximately 10E5 colony-forming units (cfu)/ml. Then 100 µl of the microbial suspension was spread onto Mueller Hinton agar plates. The extracts were tested using 6 mm sterilized filter paper discs. The diameters of the inhibition zones were measured in millimetres. All measurements were to the closest whole millimetre. Each antimicrobial assay was performed in at least triplicate. Filter discs impregnated with 10 µl of distilled water were used as a negative control.

Microbroth dilution method

MICs were determined by the microbroth dilution method according to the Clinical and Laboratory Standards Institute recommendation **(CLSI, 2015)** in Mueller Hinton broth (Biolife, Italy). Briefly, the DMSO plant extracts solutions were prepared as serial two-fold dilutions obtaining a final concentration ranging between 0.5-512 µg/ml. After that, each well was inoculated with the microbial suspension at the final density of 0.5 McFarland. After 24 h of incubation at 37°C, the inhibition of microbial growth was evaluated by measuring the well absorbance at 450 nm in an absorbance microplate reader Biotek EL808 with a shaker (Biotek Instruments, USA). The 96 microwell plates were measured before and after an experiment. Differences between both measurements were evaluated as growth. Measurement error was established for 0.05 values of absorbance. Wells without plant extracts were used as negative controls of growth. Pure DMSO was used as negative control. This experiment was done in eight-replicates for a higher accuracy of the MICs of used medical plant extracts.

Statistical analysis

All experimental measurements were carried out in triplicate and are expressed as an average of three analyses ± standard deviation. The correlations coefficient (R^2) between the parameters established by regression analysis.

RESULTS AND DISCUSSION

Total polyphenolic, flavonoids and phenolic acids content

Total polyphenolic content, total flavonoid content and total phenolic acids content of the seven extracts considered in this study are presented in Table 3. Total polyphenolic content under the applied conditions varied from 60,94±6.68 (*L.vulgaris*) to 10.05±0.17 (*R.pseudoacacia*) mg GAE per g of sample. According to the Table 3, the polyphenolic content of 3 species from *Lamiaceae* family (*M.officinalis*, *S.officinalis*, and *T.pannonicus*) were quite similar (20.90 ± 1.06, 20.01 ± 0.71 and 23.98 ± 1.37 mg GAE per g of sample respectively).
The flavonoid content of studied extracts varied from 83.72 ± 1.29 (*L.vulgaris*) to 11.56 ± 0.15 (*R.pseudoacacia*) mg QE per g of sample. The flavonoid contents of species from *Lamiaceae* family varied lightly but all were less than 20 mg QE per g of sample – *M.officinalis* (11.56±0.15), *S.officinalis* (14,35±0.49) and *T.pannonicus*. (19,35±1.22) mg QE per g of sample.
The total phenolic acids content of the studied ethanolic extracts varied within the samples, from 2.50 ± 0.74 (*R.pseudoacacia*) to 52.25 ± 2.61 *(L.vulgaris*) mg CAE per gram of sample. *Lamiaceae* family species namely lemon balm, sage and thyme had the values (24.24±2.80, 18.52±7,73 and 16.74±7.56 mg CAE per gram of sample) respectively.
In general, *L.vulgaris* and *H.perforatum* had the highest content of polyphenols, flavonoids, and phenolic acids while *R.pseudoacacia* had the lowest.

Antiradical and antioxidant activity

DPPH is a widely used free radical for simple and fast estimation of the antiradical capacity due to its stability, reliability and the simplicity of the assay **(Ali, 2013)**. DPPH radical scavenging capacity of ethanolic extracts is presented in Table 4.
The total antioxidant assay based on the reduction of phosphate-molybdenum (VI) to phosphate-molybdenum (V) gives the information on the presence of antioxidant components in the sample predicting the antioxidant activity of crude extracts on the total basis. The total antioxidant activity of ethanolic extracts was evaluated, and the results were expressed as mg Trolox equivalents/g (Table 4).

Table 3 Total polyphenolic, flavonoids and phenolic acids content of studied samples

Ethanolic extracts	Total polyphenolic content (mg GAE/g)	Total flavonoid content (mg QE/g)	Total phenolic acids content (mg CAE/g)
Linaria vulgaris L.	60.94±6.68	83.72±1.29	52.25±2.61
Galium verum L.	37.53±1.90	48.83±0.81	29.41±4.84
Thymus pannonicus L.	23.98±1.37	19.35±1.22	16.74±7.56
Hypericum perforatum L.	50.79±2.02	72.40±2.45	46.06±7.26
Robinia pseudoacacia L.	10.05±0.17	15.27±0.97	2.50±0.74
Melissa officinalis L.	20.90±1.06	11.56±0.15	24.24±2.80
Salvia officinalis L.	20.01±0.71	14.35±0.49	18.52±7.73

GAE – gallic acid equivalent; QE – quercetin equivalent; CAE –caffeic acid equivalent; mean ± standard deviation

Table 4 DPPH scavenging effect vs total antioxidant capacity of studied ethanolic extracts. Values are expressed as mean ± standard deviation (n=3), the correlation between total antioxidant capacity and total antiradical activity R^2 = 0.23.

Ethanolic extracts	Reducing power (mg TEAC/g)	Radical scavenging activity (mg TEAC/g)
Linaria vulgaris L.	8.38±0.08	134.49±3.63
Galium verum L.	8.00±0.46	63.61±7.62
Thymus pannonicus L.	8.23±0.19	52.71±4.08
Hypericum perforatum L.	8.56±0.07	135.47±42.21
Robinia pseudoacacia L.	3.96±0.40	17.92±3.36
Melissa officinalis L.	8.99±0.04	51.43±4.46
Salvia officinalis L.	8.96±0.04	48.08±2.07

TEAC – Trolox equivalent antioxidant capacity; mean ± standard deviation

The correlation between the antioxidant, antiradical activities and total polyphenolic, phenolic acids and flavonoid contents of the studied medicinal plants ethanolic extracts were calculated (Table 5).

Table 5 the correlation between antioxidant and antiradical capacity and phenolic, phenolic acids and flavonoid contents of studied ethanolic extracts

Ethanolic extracts	Reducing power (mg TEAC/g)	Radical scavenging activity (mg TEAC/g)
total phenolic content (mg GAE/g)	R^2 = 0.92	R^2 = 0.34
total flavonoid content (mg QE/g)	R^2 = 0.86	R^2 = 0.05
total phenolic acids content (mg CAE/g)	R^2 = 0.93	R^2 = 0,19

GAE – gallic acid equivalent; QE – quercetin equivalent; CAE –caffeic acid equivalent; TEAC – Trolox equivalent antioxidant capacity

The total flavonoid, polyphenol and phenolic acids content correlated with the phosphomolybdenum assay (R2 = 0.86, 0.92 and 0.93, respectively). The results suggested that the phenolic compounds contributed significantly to the antioxidant capacity of the medicinal herbs. However, a direct correlation between radical scavenging activity and total flavonoid, polyphenolic, and phenolic acids contents failed to demonstrate linear regression.

Quantification of polyphenolic compounds by HPLC

Targeted metabolic profiling of studied ethanolic extracts using HPLC has resulted in the characterization of seven phenolic acids namely neochlorogenic acid, chlorogenic acid, trans-caffeic acid, *trans-p*-coumaric acid, *trans-p*-sinapic acid, *trans-p*-ferulic acid and rosmarinic acid and eight flavonoids namely apigenin, cynaroside, daidzein, kaempferol, rutin, quercetin and vitexin in various quantities (Table 6).

Of them, cynaroside, neochlorogenic and *trans-p*-coumaric acid were found in all the samples. Resveratrol was not observed in any extracts.

The persistent presence of cynaroside was observed in all the strains and the maximum content was 1434.14±2.40 in extracts of *G.verum* followed by 1385.55±1.57 in *T.pannonicus* and 1360.19±1.51 in *R.pseudoacacia*. \ *L.vulgaris* sample contained the least amount of cynaroside (55.36±0.49) in comparison to its content in other samples.

Table 6 The content of standard samples in mg per kg of the total dry extracts or the dry plant material. Values are expressed as mean ± standard deviation (n=3)

Extract	*Linaria vulgaris* L.	*Galium verum* L.	*Thymus pannonicus* L.	*Hypericum perforatum* L.	*Robinia pseudoacacia* L.	*Salvia officinalis* L.	*Melissa officinalis* L.
Apigenin	5.06 ±0.03	–	–	–	–	58.00 ±5.47	41.71 ±20.6
Cynaroside	55.36 ±0.49	1434.14 ±2.40	1385.55 ±1.57	429.25 ±0.57	1360.19 ±1.51	567.97 ±19.4	408.13 ±30.0
Daidzein	24.28 ±0.47	15.30 ±0.02	–	–	–	-	51.25 ±8.07
Kaempferol	–	–	–	15.54 ±0.19	–	43.89 ±2.20	–
Resveratrol	–	–	–	–	–	–	–
Rutin	220.06 ±17.13	491.93 ±1.65	27.06 ±3.83	176.41 ±0.44	23.63 ±0.57	130.68 ±6.63	–
Quercetin	–	4.69 ±0.16	139.56 ±0.48	4.84 ±0.06	39.50 ±0.04	24.50 ±0.66	–
Vitexin	–	8.14 ±3.32	–	–	26.77 ±0.15	64.85 ±3.24	–
Neochlorogenic acid	138.09 ±8.19	228.15 ±6.35	316.36 ±9.65	179.11 ±9.79	612.98 ±0.71	136.44 ±4.11	113.14 ±8.07
Chlorogenic acid	1010.07 ±8.81	2886.92 ±11.82	698.99 ±0.32	–	677.95 ±0.98	-	–
***Trans-p*-caffeic acid**	–	–	2.17 ±0.05	7.73 ±0.06	11.76 ±0.07	472.0.5 ±62.5	220.15 ±5.88
***Trans-p*-coumaric acid**	0.14 ±0.06	3.79 ±0.03	0.08 ±0.02	1.69 ±0.02	1.34 ±0.04	285.62 ±4.58	13.37 ±2.84
***Trans-p*-sinapic acid**	110.91 ±0.09	9.01 ±0.03	2.58 ±0.01	15.13 ±0.08	3.69 ±0.09	-	–
***Trans-p*-ferulic acid**	–	12.10 ±0.05	39.84 ±0.04	86.34 ±0.05	36.98 ±0.15	24.68 ±2.70	–
Rosmarinic acid	184.34 ±1.07	196.02 ±0.55	–	13.38 ±0.17	–	2906.73 ±150	6914.1 ±779

The content of neochlorogenic acid varied from minimum (113.14±8.07) in *M.officinalis*. to maximum (612.98±0.71) in *R.pseudoacacia*.
The minimum content of *trans-p*-coumaric acid was as low as 0.08±0.02 in *T.pannonicus* and 0.14±0.06 in *L.vulgaris.* More significant contribution of the phenolic acid was in Salvia officinalis Mill. (285.62±4.58).
Rutin was found in all the extracts except *M.officinalis*. The content was as high as 491.93±1.65 (in *G.verum*) and 220.06±17.13 (in *L.vulgaris*), an as low as 27.06±3.83 in *T.pannonicus* and 23.63±0.57 in *R.pseudoacacia*.
S.officinalis and *M.officinalis* contained the highest content of rosmarinic acid - 2906.73±150 and 6914.1±779 correspondingly.
The maximum content of chlorogenic acid (2886.92±11.82) was found in *G.verum*, *trans-p*-caffeic acid (472.0.5±62.5) in *S.officinalis*, *trans-p*-sinapic acid (110.91±0.09) in *L.vulgaris*, *trans-p*-ferulic acid (86.34±0.05) in *H.perforatum.*
Among other polyphenols, the presence of quercetin was detected in various quantities in 5 strains, apigenin, daidzein and vitexin in 3 strains, and kaempferol in 2 strains.

Antimicrobial activity

The serial microdilution results were analyzed using the Analysis of Variance (ANOVA). Single factor statistical tool indicated that there is a significant difference in the sensitivity of the tested microorganisms to the various extracts. The MIC_{50} ranged from 1.33 to 204.80, MIC_{90} from 2.92 µg/mL to 304.16 µg/mL (Table 7). The inhibition zone was as high as 12 mm (by *G.verum* against *Yersinia enterocolitica* CCM 5671). In general, there was not observed remarkable correlation between MICs and the inhibition zone parameters. Gram-positive bacteria seemed to be more resistant to all the extracts that gram-negative strains – MIC_{50} was not lower than 25.58 µg/mL and MIC_{90} was not lower than 27.20 µg/mL.
Among them *Yersinia enterocolitica CCM 5671* was the most succeptible, it was inhibited by *G.verum* with MIC_{50} (1.33 µg/mL) and MIC_{90} (2.92 µg/mL) and the highest inhibitory zone (12.80mm). *S.officinalis* had a bit higher MICs (5.44 µg/mL and 8.59 µg/mL correspondingly), followed by *T.pannonicus* and *H.perforatum* (MIC_{50} = 12.80 µg/mL, MIC_{90} = 13.64 µg/mL).
Ps.proteolytica was also effectively inhibited by *G.verum*, *T.pannonicus* and *H.perforatum* (MIC_{50} = 0.80 µg/mL, 12.80 µg/mL,12.80 µg/mL and MIC_{90} = 0.86 µg/mL, 13.64 µg/mL, 13.64 µg/mL) recpectively. Among Gram –negative bacteria *H.alvei* was the most succeptible to *S.officinalis* (MIC_{50} = 10.09 µg/mL, MIC_{90} = 28.00 µg/mL).
Gram-positive species were less affected by studied extracts. *Stapylococcus aureus subsp. aureus CCM 2461* showed succeptibility to *H.perforatum* (MIC_{50} = 12.80 µg/mL, MIC_{90} = 13.64 µg/mL). In general, the microorganisms of the species *Ps.proteolityca and Yersinia enterocolitica CCM 5671* were the most susceptible to most plant extracts tested in this work (with MICs <25 µg/mL).

Table 7 The antimicrobial activity of medical plant extracts expressed in MICs (µg/mL) and inhibition zones (mm)

Microorganism	Extracts effect	*Linaria vulgaris* L.	*Galium verum* L.	*Thymus pannonicus* L.	*Hypericum perforatum* L.	*Robinia pseudoacacia* L.	*Salvia officinalis* L.	*Melissa officinalis* L.
***Bacillus thuringiensis* CCM 19^T**	Zone (mm)	3,67 ±0,58	4,00 ±0,01	3,00 ±0,01	9,00 ±0,01	1,00 ±0,01	4,50 ±0.40	0,50 ±0,01
	Mic50	102.4	25,58	51,07	25,58	25,58	144.24	158.02
	Mic90	102,4	27,20	54,30	27,20	27,20	232.55	304.16
***Micrococcus luteus* CCM 732**	Zone (mm)	8,00 ±0,01	11,00 ±0,01	4,33 ±1,15	5,00 ±1,73	1,00 ±0,01	1,33 ±1,44	0,17 ±0,29
	Mic50	102,4	25,58	25,58	25,58	25,58	102.40	76.73
	Mic90	102,4	27,20	27,20	27,20	27,20	114.30	81.59
***Staphylococcus epidermidis* CCM 4684**	Zone	3,00 ±0,01	1,00 ±0,01	4,67 ±0,58	4,67 ±0,58	2,33 ±0,58	0,50 ±0,01	0,50 ±0,01
	Mic50	102,4	25,58	25,58	25,58	25,58	51.20	173.79
	Mic90	102,4	27,20	27,20	27,20	27,20	57.24	265.97
***Stapylococcus aureus subsp.* aureus CCM 2461**	Zone	2,00 ±0,01	6,00 ±0,01	1,67 ±0,58	3,00 ±0,01	1,00 ±0,01	0,17 ±0,29	1,00 ±0,50
	Mic50	102,4	25,58	73,80	12,80	25,58	120.06	144.24
	Mic90	102,4	27,20	123,73	13,64	27,20	186.06	232.55
***Citrobacter koseri* CCM 2535**	Zone	2,00 ±0,01	11,00 ±0,01	2,00 ±0,01	6,00 ±0,01	1,00 ±0,01	1,33 ±1,44	0,50 ±0,01
	Mic50	102,4	25,58	25,58	25,58	25,58	38.88	102.40
	Mic90	102,4	27,20	27,20	27,20	27,20	67.51	114.3
***Escherichia coli* CCM 3988**	Zone	1,00 ±0,01	5,00 ±0,01	2,00 ±0,01	3,33 ±0,58	0,67 ±0,58	0,00 ±0,01	0,83 ±1,04
	Mic50	102,4	25,58	25,58	25,58	25,58	76.73	156.28
	Mic90	102,4	27,20	27,20	27,20	27,20	81.59	265.44
Pseudomonas proteolytica CCM 7690	Zone	1,67 ±0,58	3,00 ±0,01	2,00 ±0,01	3,00 ±0,01	6,00 ±0,01	4,67 ±0,58	6,00 ±1,00
	Mic50	25,57	0,80	12,80	12,80	22,74	51.20	42.96
	Mic90	27,20	0,86	13,64	13,64	43,90	57.24	190.83
Hafnia alvei CCM 2636	Zone	2,33 ±0,58	1,00 ±0,01	1,00 ±0,01	6,00 ±0,01	0,00 ±0,01	1,00 ±0,01	5,00 ±0,01
	Mic50	60,25	25,57	46,18	25,57	11,32	60.04	10.09
	Mic90	118,72	27,20	87,68	27,20	102,4	93.49	28.00
***Salmonella enterica* subsp. *enterica* CCM 3807**	Zone	1,00 ±0,00	1,00 ±0,00	1,00 ±0,00	1,00 ±0,00	1,00 ±0,00	0,00 ±0,00	2,17 ±3,33
	Mic50	102,4	25,58	25,58	25,58	25,58	51.20	204.80
	Mic90	102,4	27,20	27,20	27,20	27,20	57.24	228.59
***Yersinia enterocolitica* CCM 5671**	Zone	2,00 ±0,01	12,00 ±0,01	1,67 ±0,58	6,00 ±0,01	1,00 ±0,01	1,33 ±0,58	5,00 ±0,01
	Mic50	102.4	1.33	12.80	12.80	25.58	45.51	5.44
	Mic90	102.4	2.92	13.64	13.64	27.20	85.74	8.59

mean ±standard deviation

It has been reported that the wild plants are the most abundant and cheapest source of food for the human community and they are also used for the medicinal purpose as a source of antioxidant compounds. Antioxidants like phenolic acids, polyphenols, and flavonoids scavenge free radicals inhibiting the oxidative stress

that leads to a number of human diseases (asthma, inflammatory arthropathies, diabetes, Parkinson's and Alzheimer's diseases, cancers as well as atherosclerosis). Due to **Armatu *et al*. (2010)** low content of polyphenols proves a weak or no antioxidant activity and vice versa. Moreover there is no universal rule for expressing the antiradical and antioxidant capacity so they are reported either as IC_{50} (the antioxidant concentration required to reduce the DPPH absorbance by half), % loss or original absorbance or Trolox/ascorbic acid equivalent (e.g. mg TEAC/100 g FW, mg/g TE, etc). The latter is just a number for comparison, the former at least considers some concentration dependence. On one hand, this incompatibility makes it difficult to compare the results on the other hand however indirect comparison enables to determine the health benefits and public health relevance of polyphenols promising future for the powerful antioxidants and those who consume them.

DPPH and power reducing assays were used in our work to evaluate the antiradical and antioxidant activity of ethanolic plant extracts. In agreement with other authors the results varied depending on the test applied.

The antioxidant and antiradical activities of *Linaria vulgaris* were studied were poorly. Thus, **Vrchovska *et al*. (2008)** have investigated the ability of *L. vulgaris* lyophilized infusion to act as a scavenger of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, reactive oxygen species (superoxide radical, hydroxyl radical, hypochlorous acid) and nitric oxide and proposed the potent antioxidant activity present in the infusion to be ascribed by flavonoid derivatives. Our results show that among other extracts *L.vulgaris* demonstrated comparatively high antioxidant and antiradical activity.

G.verum samples collected at the flowering period at various parts of Europe, and the extracts prepared in ethanol at different temperatures and different time of extraction exhibit very potent antioxidant activities. Our findings of the antiradical ability of *G.verum's* ethanolic extract are in agreement with those reported by **Lakič *et al*. (2010)**, **Vlase *et al*. (2014), Al-Snafi (2018)**. In addition **Lakič *et al*. (2010)** observed quite high scavenging ability of *G.verum* ethanolic extract against hydroxyl radical and hydrogen peroxide, as well as potency to inhibit lipid peroxidation.

Different antioxidant assays were utilized to evaluate free radical scavenging activity and antioxidant activity of *Hypericum perforatum L*. The plant was reported to be an effective scavenger in quenching DPPH and superoxide radical;, metal-chelating capacity was proposed to attribute to its antioxidant mechanisms **(Zou *et al*., 2004)**. **Mašković *et al*. (2011)** report that the ethanolic extract of *H. perforatum* (from central Serbia) possesses higher antioxidant activity in comparison to the *H.perforatum* acetonic extract, as well as to such standards as ascorbic acid and butylated hydroxytoluene (BHT). In our work, *St John's-wort* ethanolic extract showed very high antiradical activity while the total antioxidant assay based on the reduction of Phosphate-Molybdenum (VI) to Phosphate-Molybdenum (V) showed a low presence of antioxidant components in the extract. In addition, **Radulovic *et al*. (2007)** showed that the antioxidant capacity of *H. perforatum* methanolic extract was highest in the case of the flowers (in comparison to leaves and stems) and also was the highest with the comparison to eight other local (South Serbia) *Hypericum* species.

According to literature *Black Locust* possesses a very good melliferous potential (class IV-VI): it represents an excellent source of nectar for honey production **(Persano-Oddo *et al*., 2004)**. Furthermore, this plant's wood is widely used in the wine industry as it was found to increase antioxidant activity of media and is a very effective means of enriching wines and other beverages in functional phytochemicals. The researches show that at higher concentrations (0.016 mg/mL), the values of antioxidant activity for ethyl acetate fractions of heartwood and bark of *R. pseudoacacia* from Iran were very close to those observed for very important physiological antioxidant - vitamin C while leaves had the lowest **(Hosseinihashemi *et al*., 2016)**. On the other hand, **Marinas *et al*. (2014)** compared the 70% ethanol extracts obtained from *Robinia pseudoacacia's* leaves, seeds and sheaths. The highest content of polyphenols was found in the leaf extract followed by seed extract. Sheath extract showed the lowest content of polyphenols. In accordance with polyphenolic content leaf extracts also showed the strongest antioxidant capacity. On the contrary, our work shows that ethanolic extract of flowers of *R. pseudoacacia* contains a very little amount of polyphenols, phenolic acids and flavonoids (10,05±0,17 mg QE/g) and shows the lowest antioxidant and antiradical capacity compared to other studied plants.

All *Lamiaceae* family plants possess high amounts of polyphenols, high antioxidant, and antiradical activities. The three studied species, namely *S. officinalis*, *M.officinalis*, and *T.pannonicus*, confirmed the good antioxidant and antiradical potential of the *Lamiaceae* species exhibiting almost similar records for radical scavenging (48.08±2.07, 51.43±4.46 and 52.71±4.08 mg/g Trolox equivalent, correspondingly) and antioxidant (8.96±0.04, 8.99±0.04 and 8.23±0.19 /g Trolox equivalent, correspondingly) capacities probably due to a similar quantity of polyphenols, flavonoids and phenolic acids,

Also, HPLC fingerprints of the extracts were analysed in order to determine phenolic compounds from plant material.

Cheriet *et al*. (2015) reviewed the data on different *Linaria* species including the data published as early as 1907 **(Klobb, 1907),** and the phytochemical content of *Linaria* species was revised very carefully.. In addition to a long list of flavonoids that have already been detected, we have found some other compounds not mentioned before, that are apigenin, cynaroside (luteolin-7-O-glucoside), daidzein and rutin. Quercetin was also found in agree with **Pethes *et al*. (1974)**. Vitexin seemed to be not typical to *Linaria* species as it was not mentioned to be observed as *Linaria* species constituent and was also not detected in our experiments. Some other polyphenols reported in the bibliography were identified by reversed-phase HPLC analysis as protocatechuic acid, gallic acid, *p*-hydroxybenzoic acid, vanillic acid and salicylic acid, caffeic acid, *p*-coumaric acid, ferulic acid, homoprotocatechuic acid, *O*-hydroxyphenylacetic acid, gluco-syringic acid and *p*-methoxybenzoic acid **(Cheriet *et al*., 2015)**. We were the first to detect also rosmarinic, sinapic and chlorogenic and neochlorogenic acids in *L. vulgaris* ethanolic extract. On the other hand, ferulic and caffeic acids were not detected in contrast to works by **Sokolowska-Wozniak *et al*. (2003)**.

In recent years, the consumption of products derived from *Hypericum perforatum* L., the plant being one of the most popular of medicinal plants worldwide, has increased dramatically,. *H.perforatum*-derived products are available as phytopharmaceuticals, nutraceuticals, teas, tinctures, juices, and oily macerates **(Gaedcke, 2003)**. The constituents of St John's wort *(Hypericum perforatum* L.*)*, compiled from several sources **(Barnes *et al*., 2001, Ganzera *et al*., 2002, Rusalep *et al*., 2016)**, may let us consider hyperforin (a prenylated phloroglucinol) and hypericin (a naphthodianthrone) to be the major active constituents of the plant. Besides them, *H.perforatum* contains additional biologically active compounds such as rutin, quercetin, and chlorogenic acid **(Hans, 1998)**. The chromatographic analysis of *H.perforatum* extracts also confirmed the presence of kaempferol, luteolin, quercitrin glycosides (hyperoside, quercitrin, and rutoside) **(Stuart, 2014)**. In addition to the above-mentioned constutuents, we also observed cynaroside (luteolin-7-O-glycoside). Some amount of phenolic acids such as neochlorogenic and *trans*-*p*-ferulic acids were detected, rosmarinic, *trans*-*p*-sinapic, *trans*-*p*-coumaric and *trans*-*p*-caffeic acids were detected in minority.

HPLC analysis of 70% ethanolic extracts of *R. pseudoacacia* revealed the presence of catechin, rutin, resveratrol and quercetin in the leaf extract, and catechin, epicatechin and rutin in the seed extract; None of these compounds were identified in the sheath extract. **(Marinas *et al*., 2014)**. In flowers extract we detected rutin and quercetin, as well as a quite high amount of cynaroside and traces of vitexin which were not mentioned to be present in Black locust before. Resveratrol, however, was not detected. For the first time, the presence of chlorogenic and neochlorogenic acids was detected, as well as a little amount of *trans*-*p*-ferulic, *trans*-*p*-caffeic, *trans*-*p*-sinapic and *trans*-*p*-coumaric acids.

Lamiaceae species are ones of the oldest and still the most popular medicinal plants. Although previous studies also reported chemical constituents of the extracts investigated herein, the results presented now may show novel aspects of the plants' composition. Thus *S.officinalis* and *M.officinalis* were not reported to contain sinapic acid which is in correspondence with other researches **(Hernandez-Saavedra *et al*., 2015)**. Furthermore, **Roby *et al*. (2013)** showed the chemical profile of *S.officinalis* from Shambolia farm located at Fayoum area in Egypt to contain *p*-coumaric, caffeic, ferulic and rosmarinic acids. *M.officinalis* was also reported to contain caffeic, ferulic, chlorogenic, rosmarinic acids, the latter being the major constituent **(Arceusz and Wesolowski, 2013)**. On the contrary, chlorogenic acid s was not observed in our samples. The analysis of published literature proves that the presence of neochlorogenic acids observed in *S.officinalis* and *M.officinalis* seems to be reported for the first time by us **(Zheng and Wang, 2001)**. On the other hand we showed that another species of *Lamiaceae* family – *Thymus pannonicus* – had the highest amount of sinapic acid after *Linaria vulgaris*. Rosmarinic acid naturally occurring in plants of the *Lamiaceae* family had the highest content in sage and lemon balm while not being observed in thyme. Contrarily, **Boros *et al*. (2010)** showed that *T. pannonicus* grown and sampled at Soroksár, Hungary showed the highest content of rosmarinic acid than other phenolic acids.

There is renewed interest in antimicrobial activities of herbal plants as potential source of polyphenols reported to be effective antimicrobial substances against a wide variety of microorganisms. Seven species from five popular medicinal herbs were tested against Gram-positive and Gram-negative bacteria in terms of the size of inhibition zone (mm), MIC50 (μg/mL) and MIC90 (μg/mL).

In our work *L. vulgaris* ethanolic extract did not show any significant effect against neither Gram-positive nor Gram-negative bacteria. Publications screening did not reveal data on *L.vulgaris* antimicrobial studies. However the biological study on antibacterial activity was reported to be observed for *Linaria corifolia*, endemic to Irano-Turanian region **(Gonuz *et al*., 2005)**. Assuming inhibitory zone assay they showed that the ethanolic extracts of aerial parts of the plant were more effective against Gram-positive bacteria (especially *St. epedermidis* ATCC 12228 and *St.aureus* ATCC 6538P) in comparison to Gram-negative bacteria. On the other hand, **Gul *et al*. (2017)** reported that ethanolic crude extract of *L.corifolia* aerial parts was only effective against *B.cereus* as well as yeast *Candida albicans* while ethanolic extracts of undersoil parts had an antimicrobial effect against *St.aureus*.

Antibacterial studies of *Galium verum* was not reported to possess very strong antibacterial activity against both Gram-positive and Gram-negative bacteria. However, it is likely that the nature of solvent might play important role in antimicrobial properties of *G.verum*. Thus **Vlase *et al*. (2014)** compared antimicrobial properties of four ethanolic (70%) extracts of *Galium*. Their results

show that the antimicrobial activity of *G.verum* was lower than the effect of *G.odoratum* or *G.mollugo*. However, *G.verum* demonstrated some activity against Gram-negative bacteria (*S.typhimurium*, *E.coli*) and a moderate antibacterial activity against Gram-positive *L.monocytogenes* and *St.aureus*. Extract of *G. verum* in our work was based on 80% ethanol and possessed significant activity against *Y.enterocolitica* with broad inhibition zone and low MICs. High inhibition zone was also observed for *M.luteus* and *C.koseri*, and there were very low MICs for *Ps.proteolitica*. In addition to other works, *Galium verum* extract (in 96% ethanol) exhibited high activity in relation to *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Bacillus subtilis* **(Shynkovenko *et al*., 2017)**. Furthermore in **Ilyina *et al*. (2016)** work lipophilic (chloroform) extract of *G. verum* showed a significant level of antimicrobial activity. It has given the basis for a further search of antimicrobial substances among chloroform complexes obtained from different species. Summarizing our results and bibliography *G.verum* displays more efficiency in reference to Gram-negative microorganisms and slightly less efficiency in reference to Gram-positive strains.

A number of studies are available in the literature regarding *in vitro* antibacterial activity of *Hypericum perforatum* and the extracts are reported to be more active than decoctions **(Kolesnikova, 1986)**. The aerial parts of *H. perforatum* are reported to exhibit more pronounced activity against Gram-positive bacteria than Gram-negative bacteria **(Reichling *et al*., 2001, Avato *et al*., 2004)**. The antibacterial activity of ethanolic extract of *H.perforatum* tested in our work showed variations in activity against tested strains. *Ps.proteolytica* and *Y. enterocolytica* were more susceptible with terms of MICs among Gram-positive bacteria, *B.thuringiensis* had the highest inhibition zone, and *St.aureus* had a moderate susceptibility. According to the review by **Saddiqe *et al*. (2010)**, St.John's wort's antibacterial effect varied significantly depending on solvents, proposing that organic solvents were more suitable for extracting antibacterial plant components. Thus the water extract was active only against *S.oxford* while petroleum ether, chloroform, and methanolic extracts had high MICs and were active against on *P.aeruginosa*, *S.aureus*, *S.oxford*, *S.mutans*, *S.sanguis*, *E.coli*, *P.vulgaris*. On the other hand, even 30% ethanol solution **(Lasik *et al*., 2007)** evaluated antagonistic properties against four bacteria - *Enterococcus faecium*, *Bifidobacterium animalis*, *Lactobacillus plantarum* and *E.coli* isolated from the human large intestine.

Literature data reports the antibacterial effect of aqueous extracts (in contrary to methanol) of black locust flowers against *P.putida*, *B. subtilis*, *E.coli*, *S.cerevisiae* and *P.myxofaciens* **(Cioch *et al*., 2017).** In addition**Marinas *et al*. (2014)** showed that the alcoholic extract of the *R. pseudoacacia* leaves potentiated the antimicrobial activity against the nine tested bacterial strains of *E.coli*, *K. pneumoniae*, *B.subtilis*, *S.aureus*, and *P.aeruginosa* while seeds and sheaths extract possessed very poor effect. Oppositely, the studied flower alcoholic extract did not evaluate any significant antimicrobial effect against test strains. Acetone extract from *S.officinalis* was active against *St.aureus*, *E.coli*, *P.aeruginosa*, *B.subtilis*, *E.cloacae*, *K.pneumoniae*, *Pr.mirabilis*. *St.aureus* strains were found to be the most sensitive bacteria to aqueous ethanolic and aqueous methanolic sage extracts **(Kozłowska *et al*., 2015)**. The distinct antibacterial activity of aqueous ethanolic and aqueous methanolic extracts of *S.officinalis* was also observed against *S.epidermidis* and *B.bronchiseptica* with a MIC value of 0.5 mg/mL, and *B.subtilis* and *G.stearothermophilis* with MIC value of 0.25 mg/mL. Recently, the antimicrobial activity of *S.officinalis* was shown against vancomycin-resistant enterococci **(Horiuchi *et al*., 2007)**. On the contrary, our work did not show any significant antibacterial effect of sage.

Ehsani *et al*. (2017) revealed a significant antimicrobial effect of *M.officinalis* against *S.typhimorium*, *E.coli*, *L.monocytogenes* and *S.aureus*. According to our results, the *M.officinalis* ethanolic extract possesses moderate antibacterial activity against gram-negative *Ps.proteolyticca*, *H.alvei*, and *Y.enterocolitica*. Our results, however, are not supported by some other works like **Rabbani *et al*. (2015)** showing that lemon balm extract had significant antibacterial activity against Gram-positive bacteria such as *S. aureus* and *St. epidermidis*.

The previous researches have shown that most aspects of thyme medicinal applications are related to the various levels of thymol and/or carvacrol, phenolic derivatives with strong and wide-spectrum of antimicrobial activity **(Maksimović *et al*., 2008, Nabavi *et al*., 2014)**. Though the publications on a crude extract of *T.pannonicus* are scarce, the works on its volatile oils' antimicrobial activity revealed noteworthy antimicrobial potential against bacteria and yeasts. Our work, however, showed that among all the studied plants *T.pannonicus* had the highest antibacterial effect against *Ps.proteolitica* and *Y.enterocolitica* with regard to inhibition zone that correlated well with MICs.

CONCLUSION

We have examined the antioxidant, antiradical and antimicrobial activities for seven plant ethanolic extracts and the results complete the lack of literature data with new information concerning the polyphenolic compounds and their bioactivity. Our data demonstrate the difference in antioxidant activities of the reference antioxidants and selected phenolic and flavonoid compounds in different assays. This may be due to the fact that the different antioxidant capacity determining methods have different specificities for different solvents, reagents, pH conditions, or hydrophilic and hydrophobic substances. There seems to be no rule as to the variation of the antioxidant capacity, with the activity also being dependent on the identity of the species and also the site and date of collection. The observed antimicrobial activity confirms evidence of the effectiveness of the traditional use of these herbs drug against various pathogens. In general, the differences in the antiradical, antioxidant and antimicrobial activities may be due to different geographical environment, an age of the plant, the different method followed for isolation, cultivar type, seasonality, etc. Furthermore, the extracts are very complex mixtures of many variable compounds with distinct activities. So we believe that carefully designed studies to standardize methods of extraction and in vitro testing would be advantageous so that the search could be more systematic and interpretation of results would be facilitated.

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