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Phytochemical screening of antioxidant and antibacterial activities of methanolic extracts of some Lamiaceae

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ABSTRACT

In this study, methanolic extracts of four Lamiaceae (*Ajuga iva*, *Marrubium vulgare*, *Mentha pulegium*, and *Teucrium polium*) were studied for antioxidant and antibacterial activities as well as for polyphenols contents. The antioxidant activity of extracts was evaluated with the use of three assays (reducing power, DPPH assay, and hydrogen peroxide scavenging assay). Generally, the examined plants can be devised in two groups; the first one regrouped plants with high antioxidant activity (*M. pulegium* and *T. polium*) and the second one regrouped plants with low activity (*M. vulgare* and *A. iva*). The antioxidant activity of extracts showed strong positive correlation with total phenolics and total flavonoids. The antibacterial effect of extracts against *Escherichia coli* and *Staphylococcus aureus* has been reported by agar disk diffusion and micro-dilution methods. *Escherichia coli* was more sensitive to the extracts than *S. aureus* and the most effective extract was *M. vulgare*.

1. Introduction

The family of Lamiaceae consists of about 230 genera and 7100 species worldwide (Harley et al., 2004). Many species of the Lamiaceae family are considered of high importance because of their uses in medicine, culinary, and cosmetics (Harley et al., 2004). Some of the major genera belonging to Lamiaceae family are *Ajuga*, *Marrubium*, *Mentha* and *Teucrium*.

The genus *Ajuga* consists of 50 species and is distributed in the temperate and warm temperate zones in the Old World. Several species of the genus *Ajuga* are used in African and Asian folk medicine. *Ajuga iva* L. is used in traditional medicine in Algeria to treat diabetes (Bondi et al., 2000) and gastrointestinal disorders. It is known to have hypoglycaemic (El Hilaly and Lyoussi, 2002), anti-inflammatory, antifungal, antimicrobial, antifebrile, and anthelmintic activities (Bondi et al., 2000; Bellakhdar et al., 1991; Ben Jannet et al., 1999; Stocker et al., 2004). Chemical studies on *A. iva* have revealed the presence of several flavonoids, tannins, terpenes, and steroids (Houghton and Raman, 1998).

Marrubium vulgare L. is native to North Africa, Central and Western Asia, and Southern Europe. It grows wild in dry sandy soils and wastelands. This plant is used in folk medicine for the treatment of a variety of diseases, including inflammatory, gastroenterical, and respiratory disorders (Balmé, 1982; Newall et al., 1996). *Marrubium vulgare* is reported to possess vasorelaxant (El-Bardai et al., 2003), hypoglycemic, antihypertensive (El-Bardai et al., 2004), analgesic (DeSouza et al., 1998), anti-inflammatory (Sahpaz et al., 2002), antispasmodic antinociceptive, hypotensive, insecticidal, and antioxidant properties (Weel et al., 1999). Chemical analysis of this plant has demonstrated the presence of terpenes, sesquiterpenes (Nicholas, 1964; Henderson and McCrindle, 1969), alkaloids (Pandler and Wagner, 1963), and phenolic compounds (De Vicenzi and Maialetti, 1995), diterpenoids were also found, the main one being marrubiin (Knoess, 1994).

Mentha pulegium L. is native to North Africa, Europe and Asia (Chalchat et al., 2000). It is strongly aromatic and has been traditionally used for treatment of cold, sinusitis, cholera, food poisoning, bronchitis, tuberculosis, digestive, liver, and gallbladder disorders (Gruenwald et al., 1998). It is also known for its antispasmodic, carminative, antiseptic, anti-inflammatory (Shirazi et al., 2004), antioxidant (Mata et al., 2007; Jain et al., 2012; Teixeira et al., 2012), and antimicrobial properties (Erhan et al., 2012; Teixeira et al., 2012). Chemical studies on *M. pulegium* have revealed the presence of essential oils (Zwaving and Smith, 1971; Mahboubi and Haghi, 2008), phenolic compounds (Dall Acqua et al., 2008; Teixeira et al., 2012), and flavonoids (Zaidi et al., 1998).

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Teucrium is widely distributed in Europe, Asia, America, and Australia; but the Mediterranean area represents the major area of distribution for the genus. Several plants of this genus are used in folk medicine for the treatment of digestive disorders, hypertension, fever, and parasitic diseases such as amoebicide (Henchiri et al., 2009). *Teucrium* has been used as antispasmodic, diuretic, antidiabetic, tonic, diaphoretic, analgesic as well as antirheumatic, antihelmintic, carminative, and flavoring agents (Gharaibeh et al., 1988). Previous studies demonstrated therapeutic potentialities of some *Teucrium* species such as antibacterial (Djabou et al., 2013), anti-inflammatory (Barrachina et al., 1995; Djabou et al., 2011), antioxidant (Yazdanparast and Ardestani, 2009; Goulas et al., 2012), antiulcer (Fernandez Puntero et al., 1997), and anti-allergic effects (Kim et al., 2009). *Teucrium polium* L. has been used for over 2000 years in traditional medicine for various types of pathological conditions, such as gastrointestinal disorders, inflammations, diabetes, and rheumatism (Abdollahi et al., 2003; Menichini et al., 2009). It is also used as antibacterial, antiulcer, hypotensive, antispasmodic, anorexic, and antipyretic agents. The plant possesses hypoglycaemic, and insulinotropic activities, reduces body weight, lowers high blood pressure, hypolipidemic, antinociceptive, and antioxidant properties, comparable to that of α-tocopherol (Panovska et al., 2007; Menichini et al., 2009).

The genus *Teucrium* is one of the richest sources of essential oils (Djabou et al., 2011, 2012, 2013), flavonoids (Henchiri et al., 2009; Rahim et al., 2013), polyphenols, tannins, sterols (Ben Sghaier et al., 2011), saponins, and phenolic compounds (Rahim et al., 2013). Phytochemical investigations have shown that *T. polium* contains various compounds, such as iridoids, flavonoids, diterpenoids, and essential oils (Piozzi et al., 2005; Menichini et al., 2009).

Research has focused on Lamiaceae species to extract natural antioxidant that can replace synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate and terbutyl hydroquinone (TBHQ), that might have toxic, carcinogenic, and abnormal effects on human (Göktürk Baydar et al., 2007), the synthetic antioxidants (BHA and BHT) have also been suspected of being responsible for liver damage (Gülçin et al., 2005). Moreover, Lamiaceae species have been used widely for their anti-microbial properties. Micro-organisms are causative factors for the pathogenesis of various diseases as well as for the spoilage and deterioration of food, pharmaceutical, and cosmetic products. Furthermore, in the last years, the number of bacteria resistant to current antibiotics has increased dramatically (Theuretzbacher, 2011), thus there is a great need for discovering new antimicrobial agents. Also, the mistrust of antimicrobial agents of synthetic origin due to their potential toxicity and carcinogenicity (Anwar-Mohamed and El-Kadi, 2007; Tsay et al., 2007) has intensified the efforts for discovering natural alternatives. Phenolics in Lamiaceae have been extensively studied by many investigators for their role as antioxidants compounds (Ozgen et al., 2011; Costa et al., 2012; Goulas et al., 2012) that can replace synthetic antioxidants and as antimicrobial agents that can replace synthetic antibiotics.

The objectives of our study were to screen the phenolic compounds and to evaluate the antioxidant and antibacterial activities of methanolic extracts of four Lamiaceae species (*A. iva*, *M. vulgare*, *M. pulegium*, and *T. polium*) and to compare between different parameters of the studied plants.

2. Materials and methods

2.1. Materials

The leaves of *Ajuga iva* L., Schreber., *Marrubium vulgare* L., *Mentha pulegium* L., and *Teucrium polium* L. were collected from plants growing in a natural population in North East of Algeria (Bejaia: Amizour at latitude 36°38′30″ N, longitude 4°55′14″ E) between March and May 2008. Plants were identified at the laboratory of botany (Faculty of Life and Nature Sciences, University of Bejaia) where a collection of voucher specimens has been deposited. The leaves were cleaned and air-dried at room temperature in the dark, ground to a fine powder and passed through sieves, to provide homogeneous powder. All chemicals were purchased from Sigma (represented by Algerian Chemical Society, Setif, Algeria).

2.2. Evaluation of moisture content of the sample

Thermal drying method was used in the determination of moisture content of the sample. 10 g of sample were placed in an oven at 105 °C for 3 h. The moisture content (MC) was calculated by expressing the weight loss upon drying as a fraction of the initial weight of sample used.

$$\mathrm{MC}\,(\%) = \frac{W_0}{W_\mathrm{i}} \times 100$$

where W_0 correspond to the loss in weight (g) on drying and W_i correspond to the initial weight of sample (g).

2.3. Extraction of phenolic compounds

Extraction of phenolics was performed as described by Owen and Johns (1999). 200 mg of dried powder was extracted with 500 mL of methanol 99% containing 0.5% acetic acid. The process of extraction continued for a week at room temperature, using magnetic blender. The extract was filtered (Whatman paper no. 4) and the methanol was evaporated under reduced pressure in rotary evaporation at 40 °C.

2.4. Phytochemical screening

Total phenolics of extracts were estimated using the Folin–Ciocalteu reagent as described by the method of Owen and Johns (1999). Total phenolics were expressed as gallic acid equivalent per gram of powder (GAE/g powder) ($y = 6.19\,x$; $r = 0.99$).

The $AlCl_3$ method, as described by Huang et al. (2004), was used for the determination of total flavonoids content of extracts. Total flavonoids content were expressed as quercetin equivalent per gram of powder (QE/g powder) ($y = 33.43\,x$; $r = 0.99$).

Tannins were estimated according to the protocol developed by Hagerman and Bulter (1978). Tannins were expressed as tannic acid equivalent per gram of powder (TAE/g powder) ($y = 3.19\,x$; $r = 0.99$).

2.5. Antioxidant activity

2.5.1. Reducing power assay

The reducing power of extracts was determined by the method of Oyaizu (1986). Briefly, 1 mL of sample was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide (1%). Reaction mixture was incubated at 50 °C for 20 min and then 2.5 mL of trichloroacetic acid (10%) was added and centrifuged for 10 min. From the upper layer, 2.5 mL was mixed with 2.5 mL of distilled water and 0.5 mL of $FeCl_3$ (0.1%). Absorbance of all solutions was measured at 700 nm. Values are presented as mg of ascorbic acid equivalent per g of powder (mg AscAE/g powder) and as mg quercetin equivalent per g of powder (mg QE/g powder).

2.5.2. DPPH radical scavenging assay

The effect of extracts on DPPH radical was monitored according to the method of Moure et al. (2000). The extracts (0.1 mL) were added to a methanolic solution (1.9 mL) of DPPH radical (final concentration of DPPH radical was 6×10^{-5} M). The mixture was shaken vigorously and left standing at room temperature for 30 min in the dark. The absorbance was then measured at 515 nm. The ability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging effect}\,(\%) = \frac{A_0 - A_1}{A_0} \times 100$$

where A_0 is the absorbance of the control, and A_1 is the absorbance of the sample.

The IC_{50} values were calculated as the concentration of extracts causing a 50% inhibition of DPPH radical, a lower IC_{50} value corresponds to a higher antioxidant activity of sample. The DPPH radical scavenging activity is also expressed as mg BHA equivalent per g of powder (mg BHAE/g powder).

2.5.3. Scavenging of hydrogen peroxide

The ability of extracts to scavenge hydrogen peroxide was determined according to the method of Ruch et al. (1989). Solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (0.1 M, pH 7.4). The crude extracts and their fractions (1.2 mL) were added to the hydrogen peroxide solution (0.6 mL, 40 mM). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging of sample was calculated:

$$\text{Scavenged } H_2O_2\,(\%) = \frac{A_0 - A_1}{A_0} \times 100$$

where A_0 is the absorbance of the control, and A_1 is the absorbance in the presence of the extracts or standards.

2.6. Antibacterial activity

2.6.1. Bacterial strains

Antibacterial activities of extracts and standards were tested against 2 strains of bacteria: Gram-negative (*Escherichia coli* ATCC 25922) and Gram-positive (*Staphylococcus aureus* ATCC 6538).

2.6.2. Agar diffusion method

The agar diffusion method was employed for the determination of antibacterial activities of the solvent extracts, and standards according to the method described by Berghe and Vlietinck (1991). The bacterial strains were cultured in a nutriment broth at 37 °C for 18 h. Then, 1 mL of each suspension bacteria (10^6 colony-forming unit/mL) was spread on plates containing Mueller–Hinton agar. Sterilized paper discs (6 mm) were impregnated with 20 μL of extract (2 mg/mL) prepared in methanol (99%), and placed onto nutrient agar. The plates were incubated at 4 °C for 2 h to allow diffusion of the active compounds in the medium (Tagg and Mcgiven, 1971). Negative controls were prepared using methanol. Gallic and tannic acids are tested in the same conditions as positive controls. Incubation of plates was performed at 37 °C for 24 h. Antibacterial activity was evaluated by measuring the zone of inhibition in millimeters. All experiments were done in triplicates.

2.6.3. Minimum inhibition concentration (MIC)

Minimum inhibition concentration (MIC) was determined as described by Taguri et al. (2004). Different concentrations (between 0.9 and 50 mg/mL) of extract were tested. Briefly, 1 mL of each solution was mixed with 9 mL of Muller Hinton medium and poured into sterilized Petri plates. Immediately after solidification, the plates were spot inoculated with 10 μL of suspension containing 10^6 CFU/mL of each bacterium. The inoculated plates were incubated at 37 °C for 24 h. The MIC values were determined as the lowest extract concentration at which no growth was observed.

Table 1
Moisture, dry mater, and extraction yields of extracts.

Extracts	Moisture (%)	Dry mater (%)	Extraction yield (%)
A. iva	76.61 ± 1.75^c	23.38 ± 1.75^b	22.25
M. vulgare	70.55 ± 2.03^b	29.44 ± 2.03^c	22.50
M. pulegium	81.73 ± 1.54^d	18.27 ± 1.54^a	25.12
T. polium	58.59 ± 0.86^a	41.4 ± 0.86^d	27.50

All the values are mean ± SD; SD: standard deviation.
$^{a–d}$ Column wise values with same superscripts of this type indicate no significant difference (P < 0.05).

2.7. Statistical analysis

All assays were carried out in triplicates and results were reported as mean ± standard error. The statistical significance between phenolic content, antioxidant activity and antibacterial values of the extracts was evaluated with one-way ANOVA followed by LSD test. Values of P less than 0.05 were considered to be statistically significant.

3. Results and discussion

3.1. Moisture, dry mater, and extraction yields

The moisture content, dry mater and extraction yields of extracts are shown in Table 1. Moisture content of leaves ranged from 58.59 to 81.73%, these values indicated that leaves were rich in water. The leaves of *M. pulegium* showed the maximum moisture content while lowest amount was reported in *T. polium*. Yields of dry mater ranged from 18.27 to 41.4%.

The extraction yields were obtained after removal of methanol, which ranged from 22.25% to 27.5% and were influenced by several parameters, including chemical composition and physical characteristics of the plant material (Dai and Mumper, 2010). The highest extraction yields were achieved with *T. polium*. The most widely used solvent for extracting phenolic substances from Lamiaceae are methanol. Çakir et al. (2006) in their study in phenolic compounds of *Teucrium orientale* L., reported that methanol give the higher extraction yield than acetone, chloroform and petroleum ether. Moreover, the use of methanol, water, petroleum ether and chloroform by Sharififar et al. (2009) for the extraction of polyphenols from *T. polium*, showed that methanol give the highest extraction yield. Our results (methanolic extraction yield) were higher than that reported in the literature; Dall Acqua et al. (2008) found that extraction yield of *M. pulegium*, *T. polium*, and *M. vulgare* were 8.3%, 9.5% and 13.8%, respectively. The extraction yield of *T. polium* was 14.9% (Sharififar et al., 2009), that of *M. vulgare* was 16.8% (Matkowski and Piotrowska, 2006). It has been reported that the efficiency of the extraction depends on many parameters, including the extraction time and temperature, the volume and type of the solvents used (Chew et al., 2011; Costa et al., 2012).

3.2. Total phenolic contents (TPC), total flavonoids (TF), and tannins

Phenolic compounds such as tannins, flavonoids are considered to be the major contributors to the antioxidant capacity of plants. Some of diverse biological activities of plants, such as antibacterial activity, may also be related to phenolic compounds (Chung et al., 1998). Thus, the TPC, TF and tannins of the plants extracts were evaluated (Table 2). The amount of TPC of plant extracts, measured by Folin–Ciocalteu method varied significantly (P < 0.05) from 20.07 to 72.84 mg GAE/g powder. The highest level of TPC was found in *M. pulegium*, while the lowest was in *M. vulgare*. Similar results were observed by Dall Acqua et al. (2008), who showed

Table 2
Phenolic compounds of extracts.

Extracts	TPC	TF	Tannins
A. iva	26.86 ± 0.84[b]	7.41 ± 0.21[a]	6.88 ± 1.06[a]
M. vulgare	20.07 ± 0.44[a]	7.03 ± 0.28[a]	10.44 ± 1.45[b]
M. pulegium	72.84 ± 1.46[d]	13.82 ± 0.97[c]	11.54 ± 1.6[b]
T. polium	45.65 ± 1.1[c]	10.98 ± 0.27[b]	21.19 ± 1.71[c]

All the values are mean ± SD; SD: standard deviation.
[a–d] Column wise values with same superscripts of this type indicate no significant difference ($P < 0.05$).
TPC: total phenolic compounds (mg GAE/g powder), TF: total flavonoids (mg QE/g powder).

that extracts contained TPC in the following order: *M. pulegium* > *T. polium* > *M. vulgare*. The TPC of *M. vulgare* is in accordance with the result found by Matkowski and Piotrowska, (2006). Results of other studies reported that extracts of *T. polium, M. pulegium* and *A. iva* exhibited lower TPC (Tawaha et al., 2007; Djeridane et al., 2007; Stocker et al., 2004; Tepe et al., 2011). Stagos et al. (2012) reported that TPC of methanolic extract of *M. pulegium* was 138 mg GAE/g DW.

Previous studies have shown that the amount of polyphenolics in plants depend on biological factors (genotype, organ and ontogeny), as well as edaphic, and environmental (temperature, salinity, waterstress and light intensity) conditions. Besides, the solubility of phenolic compounds is governed by the type of solvent used, the degree of polymerization of phenolics, and their interaction (Ksouri et al., 2008).

The TF of extracts ranged from 7.03 to 13.82 mg QE/g powder. Extract of *M. pulegium* showed the maximum quantity of TF while lowest amount was reported in *A. iva* and *M. vulgare*. Several studies reported that *T. polium* is rich in flavonoids (Stocker et al., 2004; Kadifkova Panovska et al., 2005; Ardestani and Yazdanparast, 2007; Tepe et al., 2011). Chenni et al. (2007) indicated the presence of flavonoids in the extract of *A. iva*. Inomata et al. (2013) have isolated flavones from 14 species of the genus *Ajuga*.

The tannins content ranged from 6.88 to 21.19 mg TAE/g powder. The highest level of tannins was found in *T. polium*, while the lowest was in *A. iva*.

3.3. Antioxidant activity

The diversity of nature and the complexity of phytochemical compounds of plant extracts impose the development of many methods to evaluate the antioxidant activity and to estimate the effectiveness of these substances. Three methods have been used to measure the antioxidant activities of the four plant extracts from Lamiaceae species: reducing power, DPPH radical scavenging assays and H_2O_2 scavenging assay (Table 3).

3.3.1. Reducing power

In the present study, assay of reducing activity was based on the reduction of Fe3+/ferricyanide complex to the ferrous form in presence of reductants (antioxidants) in the tested samples (Aktumsek et al., 2013). Results of reducing power assay, expressed as equivalent ascorbic acid and equivalent quercetin, are shown in Table 3. All extracts showed the presence of the reductive effects. The reducing power of extracts varied significantly ($P < 0.05$) from 182.32 to 427.5 mg AscAE/g powder and from 194.14 to 455.24 mg QE/g powder. *Mentha pulegium* showed the strongest reducing power capacity. In contrast, both extracts of *A. iva* and *M. vulgare* showed the lower reducing power capacity. Furthermore, both reducing power and TF of extracts showed the same order: *M. pulegium* > *M. pulegium* > *T. polium* > *M. vulgare* ≈ *A. iva*. Previous studies reported that Lamiaceae extracts exhibit strong reducing power. *Teucrium polium* has been several times evaluated in terms of reducing power (Ardestani and Yazdanparast, 2007; Tepe et al., 2011). Sarikurkcu et al. (2008) reported the reducing power of species belongs to the *Marrubium* genus (*Marrubium globosum* Montbr. subsp. *globosum*). Teixeira et al. (2012) demonstrated the reducing power of the extracts of *M. pulegium*.

Fig. 1 shows the reducing power of extracts as a function of their concentration. The reducing power of the extracts increased with increasing of concentration. Previous studies showed the same result (Bhandari and Kawabata, 2004; Tepe et al., 2011).

3.3.2. Scavenging activity of DPPH radical

The free radical-scavenging activity was determined by the DPPH test. This test aims to measure the capacity of the extracts to scavenge the stable radical DPPH formed in solution by donation of hydrogen atom or an electron (Tepe et al., 2005).

As can be seen from the Table 3 in DPPH assay, the IC_{50} values of the antioxidant capacity varied significantly ($P < 0.05$) from 0.051 to 1.168 mg/mL. As it is known, the lower the IC_{50} value the higher the antioxidant capacity of the plant extract. The DPPH radical scavenging activity is also expressed as equivalent BHA (Table 3), it was varied significantly ($P < 0.05$) from 35.37 to 780.01 mg BHAE/g powder. *Mentha pulegium* exhibited strong free radical scavenging activity, which is lower than that of synthetic antioxidants quercetin and BHA. In contrast, both extracts of *M. vulgare* and *A. iva* showed weak antioxidant capacity. Furthermore, both antiradical activity and TPC of extracts have the same order: *M. pulegium* > *T. polium* > *M. vulgare* and *A. iva*.

Previous published papers demonstrated that extracts of examined plants have strong ability to act as antioxidant. Extracts of *M. pulegium* showed strong antiradical activity (Mata et al., 2007; Stagos et al., 2012; Teixeira et al., 2012). Kamkar et al. (2010) reported that the IC_{50} of methanolic extract of *M. pulegium* is 6.1 μg/mL. Moreover, extracts of *T. polium* showed higher antiradical activity (Ardestani and Yazdanparast, 2007; Sharififar et al.,

Table 3
Antioxidant activities of extracts.

Extracts	Reducing power		DPPH		Inhibitory power of H_2O_2 (%)
	RPAscAE	RPQE	IC_{50}	AABHAE	
A. iva	182.32 ± 4.005[a]	194.14 ± 4.26[a]	1.168 ± 0.003[e]	35.37 ± 0.53[a]	17.63 ± 0.79[a]
M. vulgare	197.25 ± 8.27[a]	210.05 ± 8.8[a]	0.522 ± 0.001[d]	84.07 ± 3.88[b]	18.81 ± 0.37[a]
M. pulegium	427.5 ± 15.41[c]	455.24 ± 16.4[b]	0.051 ± 0.001[b]	780.01 ± 16.25[d]	39.48 ± 0.66[b]
T. polium	414.65 ± 11.63[b]	441.54 ± 12.38[b]	0.095 ± 0.001[c]	441.33 ± 23.52[c]	41.07 ± 1.59[b]
BHA	ND	ND	0.041 ± 0.001[a]	ND	ND
Quercetin	ND	ND	0.037 ± 0.001[a]	ND	ND
Ascorbic acid	ND	ND	ND	ND	60.46 ± 2.14[c]

All the values are mean ± SD; SD: standard deviation.
[a–e] Column wise values with same superscripts of this type indicate no significant difference ($P < 0.05$).
RPAscAE: reducing power expressed as ascorbic acid equivalent (mg/g); RPQE: reducing power expressed as quercetin equivalent (mg/g); IC_{50} (mg/mL); AABHAE: antiradical activity expressed as BHA equivalent (mg/g). ND: not determined.

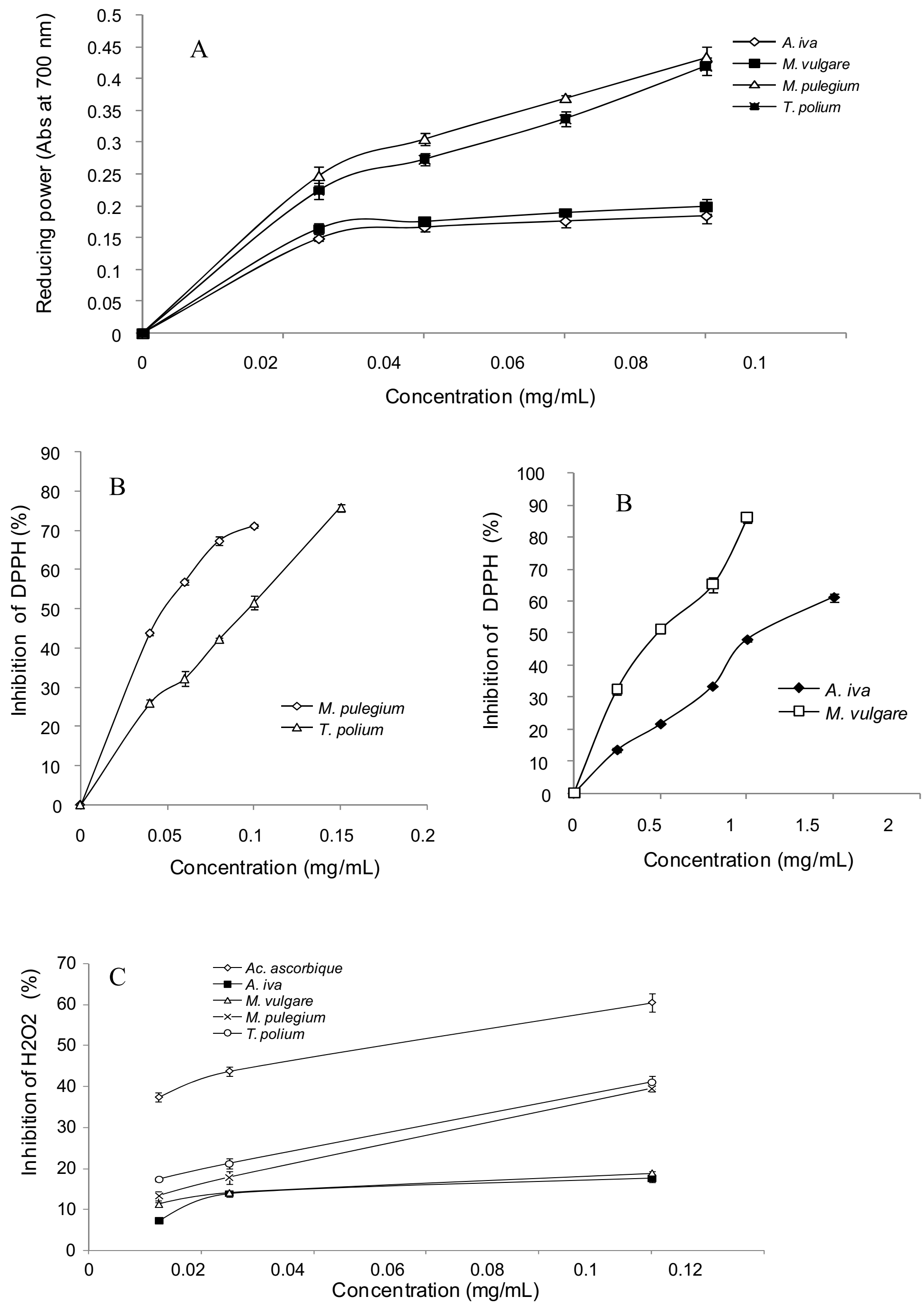

Fig. 1. Dose-dependent antioxidant activities of the studied extracts measured by using (A) reducing power, (B) DPPH assay, (C) H_2O_2 scavenging activity. Each point represents the mean of three experiments and the vertical bars represent the standard error of measurement.

2009). Extract of *M. vulgare* exhibited an IC_{50} value of 36.69 μg/mL (Matkowski et al., 2008), which is below that found in the present study. Also, Pukalskas et al. (2012) reported a good activity of extract of *M. vulgare* in the DPPH assay.

The scavenging activity of the studied extracts increased with increasing of concentration (Fig. 1). Previous studies have showed the same result (Padmashree et al., 2007; Tepe et al., 2011).

3.3.3. Scavenging of hydrogen peroxide

Results of hydrogen peroxide scavenging assay of extracts and ascorbic acid are shown in Table 3, the percentage of inhibition varied significantly ($P < 0.05$) from 17.63 to 41.07%. Extracts of *T. polium* and *M. pulegium* showed the stronger activity, while extracts of *M. vulgare* and *A. iva* have the lower activity.

In the previously published papers, there is only little information about scavenging of hydrogen peroxide assay of some of the examined species. Romero-Jiménez et al. (2005) showed that extract of *M. pulegium* posses the scavenging activity on H_2O_2. The hydrogen peroxide scavenging activity of the studied extracts increased with increasing of concentration (Fig. 1). The same result has been reported in the literature (Gülçin et al., 2004; Rajeshwar et al., 2005).

In general, extracts with a high antioxidant capacity would show a high phenolic content as well. Furthermore, both reducing power

Table 4
Correlation matrix between phenolic compounds and antioxidant activities.

Assays	Correlation coefficient (*r*)			
	TPC	TF	RP	AA
RP	0.884	0.957	1	0.869
AA	0.969	0.985	0.869	1
HPA	0.716	0.826	0.991	0.778

TPC: total phenolic compounds, TF: total flavonoids.
RP: reducing power; AA: antiradical activity; HPA: hydrogen peroxide scavenging activity.

and TF of extracts showed the same order. Also, both antiradical activity and TPC of extracts have the same order. Moreover, polyphenols and flavonoids found in plant extracts are considered the main bioactive compounds with antioxidant activity. Thus, correlation coefficient (*r*) was calculated in order to estimate the correlation between TPC, TF and antioxidant activities (reducing power, DPPH radical or H_2O_2 scavenging activity). Significant positive correlations ($P < 0.05$) has found between antioxidant activities and both TPC and TF (Table 4). These results were in agreement with those reported in previous published papers (Asadi et al., 2010; Zhang et al., 2013).

The comparison between the three antioxidant activities methods showed that there was a significant positive correlations ($P < 0.05$) between the three assays in all tested Lamiaceae (Table 4). Same results are found by Nickavar and Esbati (2012). Our results can be explained by the use of the same mechanisms or same polyphenols were active as antioxidant in the three assays.

3.4. Antibacterial activity

The antibacterial activity of the investigated extracts against Gram positive (*S. aureus*) and Gram negative (*E. coli*) bacteria used by agar disk diffusion and micro-dilution methods were shown in Table 5. The antibacterial activity of all extracts depends largely upon the concentration of extracts, the bacterial strains and the type of plant extract. Antibacterial activity of extracts was compared with gallic acid and tannic acid as standards. Results obtained in the present study relieved that all extracts were found to be active against both *S. aureus* and *E. coli* with zones of inhibition between 13 and 18.5 mm for *E. coli* and between 13.25 and 15.5 mm for *S. aureus*, at the concentration of extracts of 2 mg/mL. Extract of *M. vulgare* had maximum zone of inhibition against *E. coli* and against *S. aureus*. Overall, extract of *A. iva* had minimum zone of inhibition against both *E. coli* and *S. aureus*. The standards (GA and TA) exhibited stronger ($P < 0.05$) antibacterial activities than all extracts. *Escherichia coli* was more sensitive to the extracts than *S. aureus* with the important diameter of the zone of inhibition.

Table 5
Antibacterial activity of extracts (inhibition zone and MIC).

Sample	Inhibition zone (mm)		MIC (mg/mL)	
	E. coli	*S. aureus*	*E. coli*	*S. aureus*
A. iva	13 ± 0.7^{a}	13.25 ± 0.98^{a}	17.1	>50
M. vulgare	18.5 ± 1.04^{d}	15.5 ± 0.7^{bc}	0.9	20
M. pulegium	17.5 ± 0.35^{c}	15.25 ± 0.35^{bc}	1.5	12.2
T. polium	15 ± 1.06^{b}	14.75 ± 0.35^{b}	13	14
Gallic acid	16.33 ± 0.94	37.67 ± 3.30	ND	ND
Tannic acid	19.67 ± 3.77	27.67 ± 3.09	ND	ND

All the values are mean ± SD; SD: standard deviation.
[a–d] Column wise values with same superscripts of this type indicate no significant difference ($P < 0.05$).
Diameter of inhibition zone of extracts measured at the concentration of extracts 2 mg/disk; diameter of inhibition zone of standards measured at the concentration of standards 0.2 mg/disk.

Several studies showed the antibacterial effect of the studied plants against *E. coli* and *S. aureus*. Darabpour et al. (2010) showed that the studied bacterial strains were sensitive to the extracts of *T. polium*.

For *E. coli*, MIC values varied significantly ($P < 0.05$) from 0.9 to 17.1 mg/mL and for *S. aureus*, MIC values were important (≥ 9.6 mg/mL) (Table 5). The most effective extract against *E. coli* was *M. vulgare* with the lowest MIC values (0.9 mg/mL), followed by *M. pulegium*, while lower effective one was *A. iva*. Extract of *A. iva*, at the concentration of 50 mg/mL, exhibited no antimicrobial activity against *S. aureus*.

4. Conclusion

In this study, phenolic compounds and biological properties (antioxidant and antibacterial activities) of methanolic extract of four Lamiaceae (*A. iva, M. vulgare, M. pulegium* and *T. polium*) were determined. The highest levels of TPC and TF were found in *M. pulegium*. *Teucrium polium* showed the highest amounts of tannins, while the lowest amounts of TPC, TF and tannins were found in *A. iva* and *M. vulgare*. The reducing power of extracts showed the same order that TF: *M. pulegium* > *T. polium* > *M. vulgare* ≈ *A. iva*. Furthermore, both antiradical activity and TPC of extracts have the same order: *M. pulegium* > *T. polium* > *M. vulgare* and *A. iva*. The extracts of *T. polium* and *M. pulegium* have the highest capacity to scavenge hydrogen peroxide, while both *M. vulgare* and *A. iva* showed the lowest activity. Significant positive correlations have been found between antioxidant activities and both TFC and TF. Also, strong correlations have been found between the three antioxidants methods. The antibacterial activity of all extracts depends largely upon the concentration of extracts, the bacterial strains and the type of plant extract. *Escherichia coli* was more sensitive to the extracts than *Staphylococcus aureus* and the most effective extract against *E. coli* was *M. vulgare*. Finally, antioxidant and antibacterial activities of the examined plants have been confirmed, so this study can be formed the scientific base of the traditional uses of the tested plants.

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