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Full Length Research Paper

Determination of *in vitro* antioxidant activities of different extracts of *Marrubium parviflorum* Fish et Mey. and *Lamium amplexicaule* L. from South east of Turkey

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This study was designed to determine the *in vitro* antioxidant activities of methanol and hexane extracts of *Marrubium parviflorum* Fish et Mey. and *Lamium amplexicaule* L. from Lamiaceae and their total phenolics, flavonoids, flavonols and phenolic acid content. The extracts were screened for their possible antioxidant potentials by β-carotene/linoleic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ABTS, power reducing, metal chelating and DNA nicking assays. The methanol extracts of these plants exhibited significant antioxidant activities determined by different these assays and contained significant levels of phenolics. Total phenolic content were determined by Folin-Ciocalteu assay. Phenolic contents of the methanol extracts were found 332.93 ± 26.90 and 183.36 ± 14.78 mg GAE/g dry weight, respectively. The amounts of phenolic acids in the extracts were characterized by a reverse-phase High Performance Liquid Chromatography (HPLC) method that used in the gradient elution employing diode array detection. The most abundant phenolic acid detected by HPLC-DAD in the methanol extracts was rosmarinic acid in *M. parviflorum* whereas valinic acid was observed in the methanol extract of *L. amplexicaule*. This study shows that methanol extracts of the plants have higher activities than their hexane extract, except DNA protecting activity ($p < 0.05$).

Key words: *Marrubium parviflorum, Lamium amplexicaule*, antioxidant activity, phenolics, high performance liquid chromatography.

INTRODUCTION

Today, many scientists emphasize the harmful effects of free radicals (Asadi et al., 2010; Vichapong et al., 2010). Especially, role of oxygen radicals has been implicated in several diseases, including cancer, diabetes and cardiovascular diseases, ageing (Halliwell and Gutteridge, 1999). In addition, these molecules are considered to induce lipid peroxidation causing the deterioration of foods (Duthie, 1993). Antioxidants scavenge reactive free radicals and protect food and living systems from peroxidative damage, thus have health-promoting effects (Biglari et al., 2008). Moreover, edible plants are primary source of natural antioxidants. Polyphenols are important to dietary of humans and they are available in fruit and vegetable (Vayalil, 2002). Phenolic compounds are known to exhibit a range of biological activities, including antibacterial, antioxidant and antiinflammatory properties (Kamatou et al., 2010; Samec et al., 2010). The antioxidant activities of phenolics are mainly due to their redox properties that allow them to act as a reducing agents and hydrogen donors (Rice-Evans et al., 1996). Plants contain high amounts of the phenolic compounds. The Lamiaceae includes a large number of plants and the plants are well worked in terms of antioxidant activities (Barros et al., 2010). *Marrubium parviflorum* and *Lamium amplexicaule* belong to the Lamiaceae. As far as our survey, there are no detailed reports in literature on antioxidant activities and phenolic compounds of *M. parviflorum* and *L. amplexicaule*. Thus, the aim of the study was to investigate the antioxidant potentials of the methanol and hexane extracts obtained from *M. parviflorum* and

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L. amplexicaule growing naturally in Gaziantep flora's where in South-Eastern of Turkey and their total contents of phenolics, flavonoids and flavonols of the plants using current methods.

MATERIAL AND METHODS

Collection of plant materials

M. parviflorum and *L. amplexicaule* were collected at flowering stage from Gaziantep University Campus Area, Gaziantep-Turkey; 15th June, 2008. The plants were identified by expert Mustafa Pehlivan in Gaziantep University.

Preparation of the extracts

The air-dried and powdered leaves of plant (20 g) were extracted successively with 500 ml of hexane and methanol by using a Soxhlet extractor (Gherart) 48 h at 40°C. The extracts were then concentrated at 40°C using a Rotary evaporator. Then, the extracts were kept in the dark at +4°C until tested.

Antioxidant activities

β-carotene/linoleic acid bleaching assay

In this assay antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation (Dapkevicius et al., 1998). A stock solution of β-carotene/linoleic acid mixture was prepared as following: 0.5 mg β-carotene was dissolved in 1 ml of chloroform (HPLC grade). Twenty five micro liters linoleic acid and 200 mg Tween 40 was added. Chloroform was completely evaporated using a vacuum evaporator. Then 100 ml of oxygenated distilled water was added with vigorous shaking; 2.5 ml of this reaction mixture was dispersed to test tubes and 0.5 ml of the extracts in methanol (2 mg ml^{-1}) were added and the emulsion system was incubated for up to 24 h at 37°C. The same procedure was repeated with the positive control BHT, and a blank. After this incubation period, absorbance of the mixtures was measured at 490 nm. Measurement of absorbance was continued until the color of β-carotene disappeared. The antioxidant activity (AA) was calculated in terms of % inhibition relative to the control using following equivalent:

$$AA = R_{control} - R_{sample} / R_{control} \times 100$$

Antioxidative activities of the extracts were compared with those of BHT at 0.5 mg ml^{-1} and blank consisting of only 0.5 ml methanol.

Scavenging effect on 2,2-diphenyl-1-picrylhydrazyl

The hydrogen atoms or electrons donation ability of the corresponding extracts and some pure compounds was measured from the bleaching of purple coloured methanol solution of stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). DPPH radical scavenging activity of the plant extracts was determined according to the method of Gaulejac et al. (1998) with minor changes (Zhao et al., 2008). 0.1 ml of extracts was added to 2.9 ml of 6×10^{-5} mol/l methanolic solution of DPPH. The absorbance at 517 nm was measured after the solution had been allowed to stand in the dark for 60 min. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The Trolox calibration curve was plotted as a function of the percentage of DPPH radical scavenging activity. The final results were expressed as milimoles of Trolox equivalents (TE) per gram of dry weight (mmol TE/g dw).

ABTS radical cation scavenging activity

The radical scavenging activity of the extracts against ABTS radical cation was measured using the method of Re et al. (1999) with some modifications (Zhao et al., 2008). ABTS was dissolved in water to a 7 mmol/L concentration. ABTS radical caution was produced by reacting ABTS stock solution with 2.45 mmol/L potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12 - 16 h before use. The ABTS radical caution solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm and equilibrated at 30°C. An aliquot of each plant extract (0.1 ml) was mixed with 2.9 ml of diluted ABTS radical cation solution. After the reaction was waited at 30°C for 20 min, the absorbance at 734 nm was measured. The Trolox calibration curve was plotted as a function of the percentage of ABTS radical caution scavenging activity. The final results were expressed as milimoles of TE per gram of dry weight (mmol TE/g dw).

Reducing power activity assay

The determination was carried out as described by Oktay et al. (2003). Briefly, 1 ml of plant extract was mixed with phosphate buffer (2.5 ml, 0.2 mol/l, pH 6.6) and K_3Fe (CN)6 (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid solution (10%) was added to the mixture, which was then centrifuged at 10, 000g for 10 min. The upper layer of solution (2.5 ml) was mixed with deionised water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. The measurement was compared to a standard curve of prepared Ascorbic Acid (AA) solution, and the final results were expressed as milimoles of Ascorbic Acid Equivalents (AAE) per gram of dry weight (mmol AAE/g dw).

Metal chelating activity assay

The chelating activity of the plant extracts for ferrous ions was measured following the ferrozine method with minor modifications (Dinis et al., 1994; Zhao et al., 2008). The reaction mixture contained 0.5 ml of plant extract and 0.05 ml of FeCl2 (2 mmol/L). After 5 min, the reaction was initiated by the addition of 5 mmol/L ferrozine (0.1 ml), and the total volume was adjusted to 3 ml with 80% acetone solution. Then, the mixture was shaken vigorously and incubated at room temperature for 10 min. Absorbance of the solution was measured at 562 nm. The Ethylenediaminetetraacetic acid (EDTA) calibration curve was plotted as a function of the percentage of metal chelating activity. The final results were expressed as milimoles of EDTA equivalents (EDTAE) per gram of dry weight (mmol EDTAE/g dw).

DNA nicking assay

The ability of different fractions to protect super coiled pBR 322 DNA from harmful effects of hydroxyl radicals generated by Fenton's reagent was assessed by the DNA nicking assay described by Lee et al. (2002) with slight modifications. The reaction mixture contained 1 µl of plasmid DNA, 10 µl Fenton's reagent (30 mM H_2O_2, 50 mM ascorbic acid, and 80 mM $FeCl_3$) followed by the addition of extracts and the final volume of the

mixture was brought up to 20 µl using distilled water. The mixture was then incubated for 30 min at 37°C and the DNA was analyzed on 1% agarose gel (prepared by dissolving 0.5 g of agarose in 50 ml of 1X TBE Buffer) followed by ethidium bromide staining. Quercetine was used as a positive control.

Chemical screening

Determination of total phenolic content

Determination of total phenol content Folin–Ciocalteu procedure given by Yu et al. (2002) was used to estimate the total phenol contents in the different fractions of *M. parviflorum and L. amplexicaule*. Following this method, 0.1 ml aliquots of fractions were diluted to 1 ml with distilled water. To this solution 0.5 ml of Folin–Ciocalteu reagent (1:1) and 1.5 ml of 20% sodium carbonate solution was added. The mixture was incubated for 2 h at room temperature. The volume was raised to 10 ml with distilled water and the absorbance of blue coloured mixture was measured at 765 nm (Cintra 202 UV–Vis Spectrophotometer). The total amount of phenolic compounds was calculated as mg/g (Gallic Acid Equivalents) from calibration curve of gallic acid standard solution.

Determination of total flavonoids

The flavonoids content was determined by aluminium chloride method using quercetine as a reference compound (Kumaran and Karunakaran, 2006). This method based on the formation of a complex flavonoid-aluminum having the absorptivity maximum at 415 nm. About 100 µl of plant extracts in methanol (10 mg/ml) was mixed with 100 µl of 20% aluminium trichloride in methanol and a drop of acetic acid, and then diluted with methanol to 5 ml. The absorption at 415 nm was read after 40 min. Blank samples were prepared from 100 µl of plant extracts and a drop of acetic acid, and then diluted to 5 ml with methanol. The absorption of standard quercetine solution (0.5 mg/ml) in methanol was measured under the same conditions. All determinations were carried out in duplicates. The amount of flavonoids in plant extracts in Quercetine Equivalents (QE) was calculated by the following formula:

$X = (A \,.m_0) / (A_0 \,. m)$

where X is the flavonoid content, mg/mg plant extract in QE, A is the absorption of plant extract solution, A_0 is the absorption of standard quercetine solution, m is the weight of plant extract, mg and mo is the weight of quercetine in the solution, mg.

Determination of total flavonols

The content of flavonols was determined by using quercetine as a reference compound. This method also based on the formation of complex with maximum absorption at 440. About 1 ml of each methanolic plant extract (10 mg/ml) was mixed with 1 ml aluminium trichloride (20 mg/ ml) and 3 ml sodium acetate (50 mg/ ml). The absorbance at 440 nm was read after 2.5 h. The absorption of standard quercetine solution (0.5 mg/ml) in methanol was measured under the same conditions (Kumaran and Karunakaran, 2006; Abdel-Hameed, 2009). All determinations were carried out in duplicates. The amount of flavonols in plant extracts in Quercetine Equivalents (QE) was calculated by the same formula of flavonoids:

$X = (A \,. m_0) / (A_0 \,. m)$

Determination of phenolic acids of plants

Phenolic acids analysis was carried out using an equipped with ChemStation software, a model G1322A degasser, a model G1311A quaternary pump, a model G1329 autosampler and a model G1321A fluorescence detector. The separation was carried out with a Zorbax Eclipse XDB-C18 column (150, 4.6 i.d. and 5 mm particle size) (Agilent, Waldbronn, Germany). Chromatographic separation was carried out using two solvents system: (A) methanol:water:formic acid (10:88:2,*v/v/v*); (B) methanol:water:formic acid (90:8:2, *v/v/v*), as reported elsewhere (Ozturk et al., 2007). The analyses were performed by using a linear gradient program. Initial condition was 100% A; 0 - 25 min, changed to 80% A; 25 - 54 min, to 50% A; 55 to 64 min, to 0%; 65 - 70 min, went back to 100% A. The flow-rate was 1 ml/dak and the injection volume was 10 µl. Signals were detected at 280 nm. Besides, IS (propyl paraben) technique was applied to the analysis to increase the repeatability. The relevant extracts were dissolved in a mixture of methanol and water (1:1 v/v) and injected into the HPLC.

Statistical analyses

All tests were conducted in triplicate. Data are reported as means ± SD. Analysis of significant differences among means were tested by one-way ANOVA using SPSS software (version 11.0 for Windows, SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

The antioxidant potentials of the methanol and hexane extracts of leaves of *M. parviflorum* and *L. amplexicaule* were measured. For antioxidant activities of these extracts can be evaluated, six different models were used in this study. For the chemical determination, amounts of the total phenolic, flavanoid, flavonol compounds in polar metanol and non-polar hexane extracts were determined. Also separation and amounts of polyphenols were measured by using HPLC in polar extracts. The phenolic acid amounts were ignored in the hexane extracts because of low amounts.

Antioxidant activities

β-carotene/linoleic acid bleaching assay

β-carotene bleaching method is based on the loss of the yellow-orange color of β-carotene due to its reaction with radicals formed by linoleic acid oxidation in an emulsion. The rate of β-carotene bleaching can be slowed down in the presence of antioxidant (Kulisic et al., 2004). The calculated values of the methanol and hexane extracts are given in Table 1. Inhibition values of the plant extracts on the linoleic acid was observed as 67.3 ± 2.17 and 64.2 ± 2.24% in methanol. Inhibition percent (%) of the hexane extracts was not determined. The composition of methanol extract is very complex and it consists of various classes of organic compound which may exert opposite effects on the process of lipid oxidation. Based on the results obtained, it is highly possible that some constituents of different polarity may contribute to antioxidative activity of the extract (Marimuthu et al.,

Table 1. Antioxidative potentials of the methanol and hexane extracts from *M. parviflorum, L. amplexicaule* and positive controls (BHT and ascorbic acid) in β-carotene/linoleic acid assay.

β-caroten/linoleic acid %	Methanol	Hexane
M. parviflorum	67.3 ± 2,17[a]	N.d
L. amplexicaule	64.2 ± 2,24[a]	N.d
BHT	95.3 ± 1.78[b]	95.3 ± 1.78
Ascorbic acid	94.2 ± 2.0[b]	94.2 ± 2.06

Table 2. DPPH, ABTS, metal chelating and reducing power activities of methanol and hexane extracts of *M. parviflorum and L. amplexicaule.*

	Methanol		Hexane	
Assays	***M. parviflorum***	***L. amplexicaule***	***M. parviflorum***	***L. amplexicaule***
DPPH scavenging activity	22.72 ± 0.11^{acx}	15.26 ± 0.04^{ax}	0.74 ± 0.05^{ax}	1.59 ± 0.06^{ay}
ABTS scavenging activity	34.10 ± 1.80^{abx}	20.08 ±1.48^{ax}	1.77 ± 0.11^{bx}	0.30 ± 0.02^{by}
Power reducing activity	46.34 ± 2.43^{bx}	43.08 ± 6.71^{bx}	0.16 ± 0.01^{cx}	0.51 ± 0.08^{cy}
Metal chelating activity	11.47 ± 0.81^{cx}	4.59 ± 0.16^{cx}	2.30 ± 0.05^{dx}	1.39 ± 0.03^{dy}

2008). Therefore, it can be said that the methanol extracts of the plants can more effect for prevent free radicals existed from the lipid oxidation than the hexane extracts. However, the plant extracts was compared with BHT and ascorbic acid as positive control in parallel assay. Extracts exhibited moderate activity when compared with positive controls. Results are expressed as mean of three experiments ± SD. Data were analysed by ANOVA and within each column different letters indicate statistically different values according to LSD-test at P< 0.05. N.d.: Not determined.

Scavenging effect on 2,2-diphenyl-1-picrylhydrazyl

The methanol and hexane extracts obtained by Soxhlet extraction were screened for their possible antioxidant activity by DPPH free radical scavenging assay. Results are expressed as milimoles of Trolox equivalents per gram of dry weight (mmol TE/g dw). According to the result in the Table 2, free radical scavenging activity of the methanol extract of *M. parviflorum* was the highest found whereas it is the hexane extract exhibited the lowest activity. The activity of the methanol extract of *M. parviflorum* was determined as 22.72 ± 0.11 mmol TE/g dw. Activities of methanol extracts could be attributed to the presence of several types of compounds belong to different classes such as polar thermo labile and/or thermo stable phenolics (Sokmen et al., 1998). The methanol extract of *M. parviflorum* was shown higher activity than other methanol and hexane extracts in β-carotene bleaching and DPPH assays.

ABTS radical cation scavenging activity

ABTS radical cation is another common organic radical that has been used to determine the antioxidant activity of single compounds and other complex mixtures (Zhou et al., 2004). Radical caution scavenging activities of the methanol and hexane extracts were determined and compared with their activities against to ABTS caution radical. Results are expressed as millimoles of Trolox equivalents per gram of dry plants. The methanol extracts of *M. parviflorum* and *L. amplexiacaule* exhibited a strong radical scavenging activity as mmol TE/g dw (Table 2). The hexane extracts of the plants showed lower activities when compared with methanol extracts ($p < 0.05$). The values of scavenging activity of the methanol extracts were found as 34.10 ± 1.80 and 20.08 ± 1.48 mmol TE/g dw. It can be suggested that methanol extracts of these plants might have significant effect as free radical scavenging.

Reducing power assay

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Meir et al., 1995). For the determination of reducing power activity, the Fe^{3+} to Fe^{2+} reduction in the presence of the methanol and hexane extracts of the plants were exhibited. As shown in Table 2, the methanol extracts of plants showed significant reducing power activity. The methanol extract of *M. parviflorum* showed higher activity in value of 46.34 ± 2.43 mmol AAE /g dw than the other

Table 3. Total contents of phenolic, flavonoid and flavonols of the methanol and hexane extracts of *M. parviflorum and L. amplexicaule.*

	M. parviflorum		*L. amplexicaule*	
Total	**Methanol**	**Hexane**	**Methanol**	**Hexane**
Phenolic	332.93 ± 26.90	42.79 ± 2.66	183.36 ± 14.78	75.43 ± 5.66
Flavonoid	65.15 ± 5.23	35.75 ± 1.08	96.06 ± 2.54	37.91 ± 5.62
Flavonol	20.41 ± 1.44	8.87 ± 0.41	17.98 ± 1.58	6.64 ± 0.11

Results are expressed as mean of three experiments ± SD.

extracts. Although the hexane extract of the plants exhibited lower activity compared with methanol extract in this assay ($p < 0.05$).

Metal chelating activity assay

Transition metals such as ion can stimulate lipid peroxidation of generating hydroxyl radicals through fenton reaction and accelerate lipid peroxidation into peroxyl and alkoxyl radicals therefore drive the chain reaction of lipid peroxidation (Zhao et al., 2006). Chelating agents may inhibit radical generations by stabilizing transition metals, consequently reducing free radical damage. In addition, some phenolic compounds exhibit antioxidant activity through the chelation of metal ions (Zhao et al., 2008). Phenolic compounds may be permitting that bond to metal ions due to their chemical structures. The chelating activity of plants was evaluated against Fe^{2+} for estimate the potential antioxidant activities of the methanol and hexane extracts. As can be seen in Table 3, the methanol extracts exhibited higher metal chelating activity than the hexane extracts ($p < 0.05$). The methanol extract of *M. parvifloum* exhibited the highest activity in value of 11.47 ± 0.81 mmol EDTAE/g dw whereas the lowest activity was observed in the hexane extract of *L. amplexicaule*.

In DPPH, ABTS, power reducing and metal chelating tests was found that there is statistically an important difference between antioxidant activities of methanol and hexane extracts of *M. parviflorum* and *L. amplexicaule*. Methanol extracts exhibited higher activities than hexane extracts ($p < 0.05$). However, no difference was found statistically in ABTS and DPPH assays of methanol extracts of *M. parviflorum* and *L. amplexicaule* ($p > 0.05$). The hexane extracts of these plants reflect to statistical difference in value of $p < 0.05$ by using methods (Table 2). Results are expressed as mean of three experiments ± SD. Data were analyzed by ANOVA and within each column and line different letters indicate statistically different values according to LSD-test at $p < 0.05$.

DNA nicking assay

Hydroxyl radicals generated by the Fenton reaction are known to cause oxidatively induced breaks in DNA strands to yield its open circular or relaxed forms. Exposure of plasmid DNA to Fenton's reagent ultimately results in strand breaks formation, mainly due to the generation of reactive species-hydroxyl radical and the subsequent free radical-induced reaction on plasmid DNA. Hydroxyl radical, react with nitrogenous bases of DNA producing base radicals and sugar radicals. The base radicals in turn react with the sugar moiety causing breakage of sugar phosphate backbone of nucleic acid, resulting in strand break formation (Sonntag, 1987). Figures 1 and 2 shows the DNA damage protecting activity of the methanol and hexane extracts of *M. parviflorum and L. amplexicaule*. The concentration dependent (20 - 40 µg/ml) free radical scavenging effect of the methanol and hexane of *M. parviflorum and L. amplexicaule* were studied on plasmid DNA pBR322 damage. As can be seen in Figure 1, the methanol extract (20 - 40 µg/ml) of *M. parviflorum* (lane 5 and 6, respectively) and L. amplexicaule (Lane 7 and 8, respectively) showed significant reduction in the formation of nicked DNA and increased native form of DNA. Also, the hexane extracts (20 - 40 µg/ml) of the plants showed higher protection compared with quercetine and the methanol extracts (Figure 2). Interestingly, the hexane extracts showed weak activities in other assay whereas in this assay were exhibited powerful activitiy. Kaur et al. (2008) were reported that chloroform, ethyl acetate and *n*-butanol extracts of *Chukrasia tabularis* showed higher activity on DNA protecting activity when compared with methanol and water extracts. These chemicals have lower polarity than methanol and water. In our study were supplied similar activities on DNA. Hence, it might be said that available non-polar compounds in the hexane extracts might be contribute to phenolic compounds for protecting of DNA. In biological systems metal binding can occur on DNA leading to partial site-specificity hydroxyl radical formation. Polyphenols are potential protecting agents against the lethal effects of oxidative stress and offer protection of DNA by chelating redox-active transition metal ions (Prakash et al., 2007). According to our study can be said that methanol and hexane extracts of these plants can prevent DNA damages caused of the free radicals.

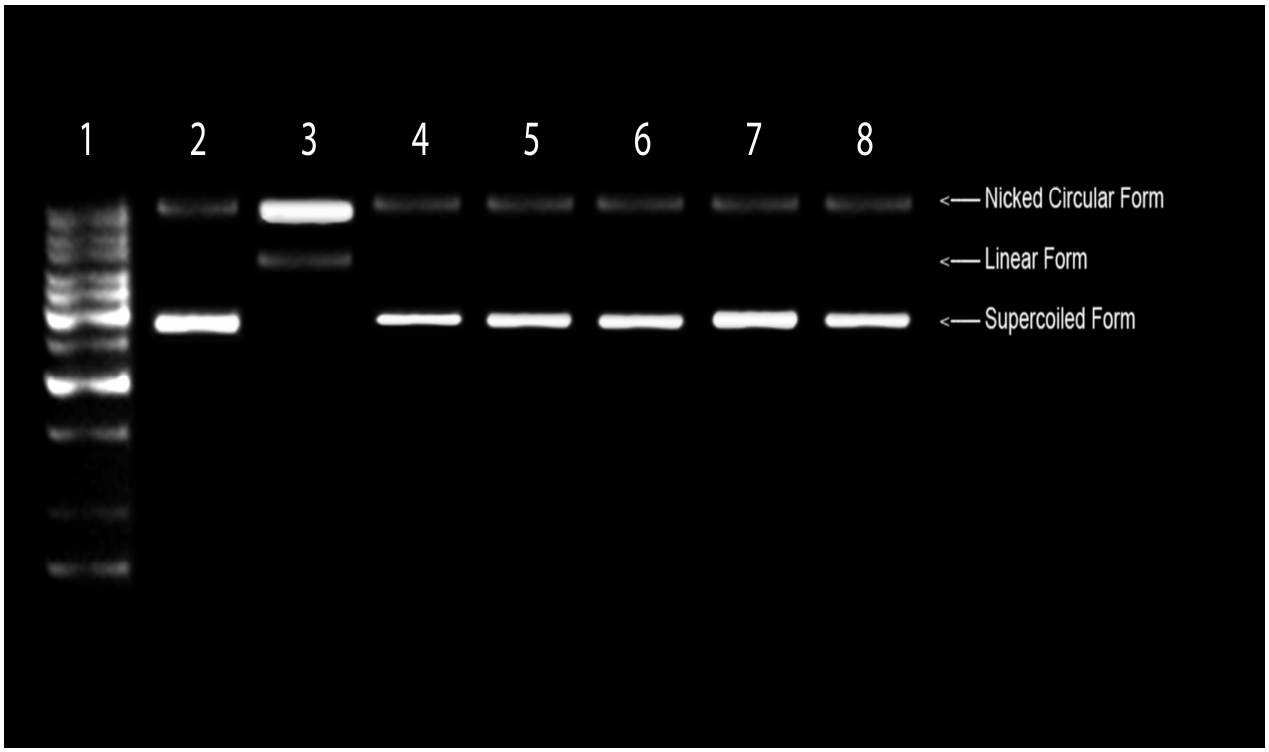

Figure 1. Protective effect of the methanol extracts of *M. parviflorum and L. amplexicaule* in DNA nicking caused by hydroxyl radical. Lane 1: Marker, Lane 2: Negative control (Distilled water+DNA), Lane 3: Control (DNA+Fenton's reagent), Lane 4 and 5: the methanol extract of *M. parviflorum* (20 and 40 mg/ml, respectively) +DNA+Fr, Lane 6 and 7: the methanol extract of *L. amplexicaule* (20 and 40 mg/ml, respectively) +DNA+ Fr, Lane 8: positive control (DNA + Quercetine + Fenton's reagent).

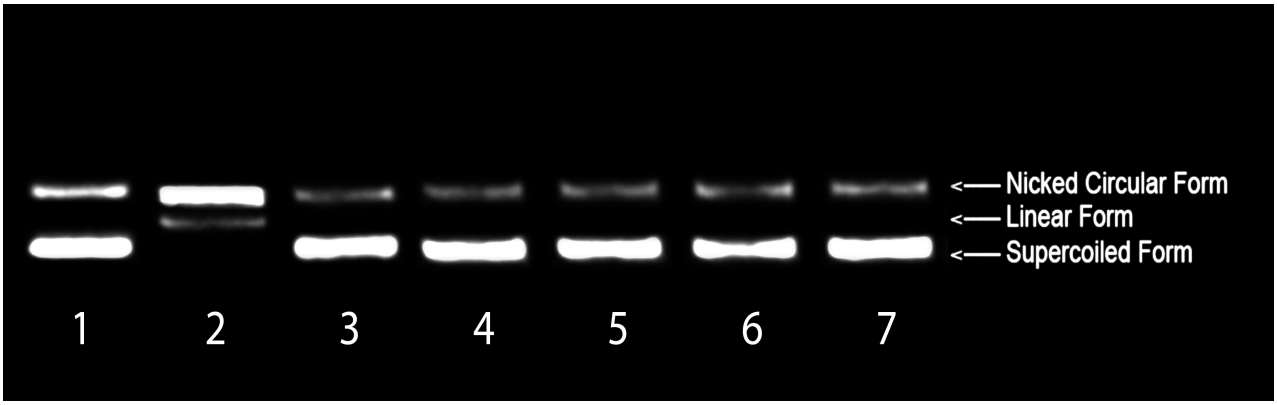

Figure 2. Protective effect of the hexane extracts of *M. parviflorum and L. amplexicaule.* in DNA nicking caused by hydroxyl radical. Lane 1: Negative control (Distilled water + DNA), Lane 2: Control (DNA + Fenton's reagent), Lane 3: positive control (DNA + Quercetine + Fenton's reagent) Lane 4 and 5: the hexane extract of *M. parviflorum* (20 and 40 mg/ml, respectively) +DNA+ Fr, Lane 6 and 7: the hexane extract of *L. amplexicaule* (20 and 40 mg/ml, respectively) +DNA+ Fr.

Chemical screening

The methanol and hexane extracts isolated by Soxhlet from leaves of *M. parviflorum and L. amplexicaule* were obtained in yields 22 and 16% in the methanol extracts and 1.5 and 3.35% in the hexane extracts, respectively.

Determination of total phenolic content

A reagent that Folin–Ciocalteu was used to determine total polyphenol in plant extracts. Folin–Ciocalteu reagent consists of a yellow acidic solution containing complex polymeric ions formed from phosphomolybdic and

phosphotungstic heteropoly acids. This reagent oxidises phenolates, resulting in the production of complex molybdenum-tungsten blue (Singleton and Rossi, 1965). As can be seen Table 1, significant amounts of total phenolics of the methanol extracts were determined in the plant species. Total Phenolic Content (TPC) of *M. parviflorum and L. amplexicaule* was determined in value of 332.93 ± 26.90 and 183.36 ± 14.78 mg GAE/g, respectively. The hexane extracts showed lower activity when compared with methanol extracts (Table 3). The methanol extract of the plant with high amount of phenolics exhibited strong antioxidant activities. The methanol extracts of *M. parviflorum and L. amplexicaule* showed higher activity in DPPH, ABTS, power reducing, metal chelating and β-carotene/linoleic acid assays compared with the hexane extract. Phenolic compounds are commonly found in both edible and inedible plants (Amarowicz et al., 2010), and they have been reported to have multiple biological effects, including antioxidant activity (Albayrak et al., 2010; Guvenc et al., 2010; Nabekura et al., 2010). The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Osawa, 1994).

Determination of total flavanoid and flavonol contents

Flavonoids are the most common and widely distributed group of plant phenolic compounds that are characterized by a benzo-y-pyrone structure, which is ubiquitous in fruits and vegetables. Total flavonoid values of methanol extracts of *M. parviflorum and L. amplexicaule* were determined as 65.15 ± 5.23 and 96.06 ± 2.54 QE/g dw, respectively whereas the hexane extracts exhibited lower flavonoid contents than methanol extracts. The aromatic rings of the flavonoid molecule allow the donation and acceptance of electrons from free radical species (Kanner et al., 1994).

Flavonols are synthesized in plant tissues from a branch of the phenylpraponoid pathway (Hermans, 1988). Flavonols are a class of flavonoids that have the 3-hydroxyflavone backbone. Their diversity stems from the different positions the phenolic -OH groups. Flavonols are present in a wide variety of fruits and vegetables (Cermak et al., 2006). The flavonol values of the methanol extracts of *M. parviflorum and L. amplexicaule* were determined as 20.41 ± 1.44 and 17.98 ± 1.58 mg QE/g dw, respectively. Plant phenolics constitute one of the major groups of compounds acting as primary antioxidant or free radical terminators (Cao et al., 1997). Phenolic compounds such as flavonoids, phenolic acids and tannins are considered to be the major contributor to the antioxidant activity of vegetables, fruits or medicinal plants. The antioxidant activity of the phenolic compounds were attributed to its redox properties, which allow them to act as reducing agents, hydrogen donators, singlet oxygen quenchers and have also metal chelating properties (Rice-Evans et al., 1996).

Determination of phenolic acids of plants

Most of the phenolic acids have absorption maxima in the UV absorption spectra at wavelength of 280 nm and they were identified by matching their retention times (peak normalization, PN) and UV spectra of samples with those of authentic standards, using HPLC-DAD system. All of the phenolic acids were resolved entirely from each other. The original chromatograms of the methanol extracts obtained from studied plants and standard phenolic acids containing IS are demonstrated in Figure 3.

Quantitative determination was performed employing validation tests that has a good repeatability using IS technique in the range of (0.35 - 1.65) RSD; they have LOD values in the range of 2.49×10^{-6} - 9.69×10^{-6} M and LOQ values 1.27×10^{-6} - 2.93×10^{-5} M. The results were found to be by using the calibration equations as reported elsewhere (Ozturk et al., 2007). The amounts of phenolic acid amounts are shown in Table 4.

The results show that Rosmarinic Acid (RA) dominated in *M. parviflorum* and Vanilic Acid (VA) was observed in *L. amplexiacule*. RA and VA were determined in value of 952.7 µg g^{-1} and 4688 µg g^{-1} dry sample, in *M. parviflorum and L. amplexicaule,* respectively. In addition, in leaves of *M. parviflorum,* seven phenolic acids (protocatechic, vanilic, chlorogenic, ferulic, rosmarinic, *tr*-cinnamic and *o*-coumaric acids) and also in leaves of *L. amplexicaule*, nine phenolic acids (gallic, *p*-hydroxybenzoic, vanilic, chlorogenic, *p*-coumaric, ferulic, rosmarinic, *tr*-cinnamic and *o*-coumaric acids) were determined.

Conclusion

Six different antioxidant test systems showed that extracts of *M. parviflorum* and *L. amplexicaule* have significant antioxidant potentials. The extracts are found to have various levels of antioxidant activity in all tests systems. Results showed that especially the methanol extracts of these plants have high linoleic acid oxidation, DPPH and ABTS scavenging, reducing power and metal chelating and have high amounts of phenolic contents. However, the hexane extracts exhibited powerful protecting activity on DNA protecting activity. According to results, the differences in TPC for extracts of plants were significant due to their antioxidant activities. Antioxidant activities of the methanol extract of *M. parviflorum* are higher than the methanol extract *L. amplexicaule* whereas the hexane extracts of these plants

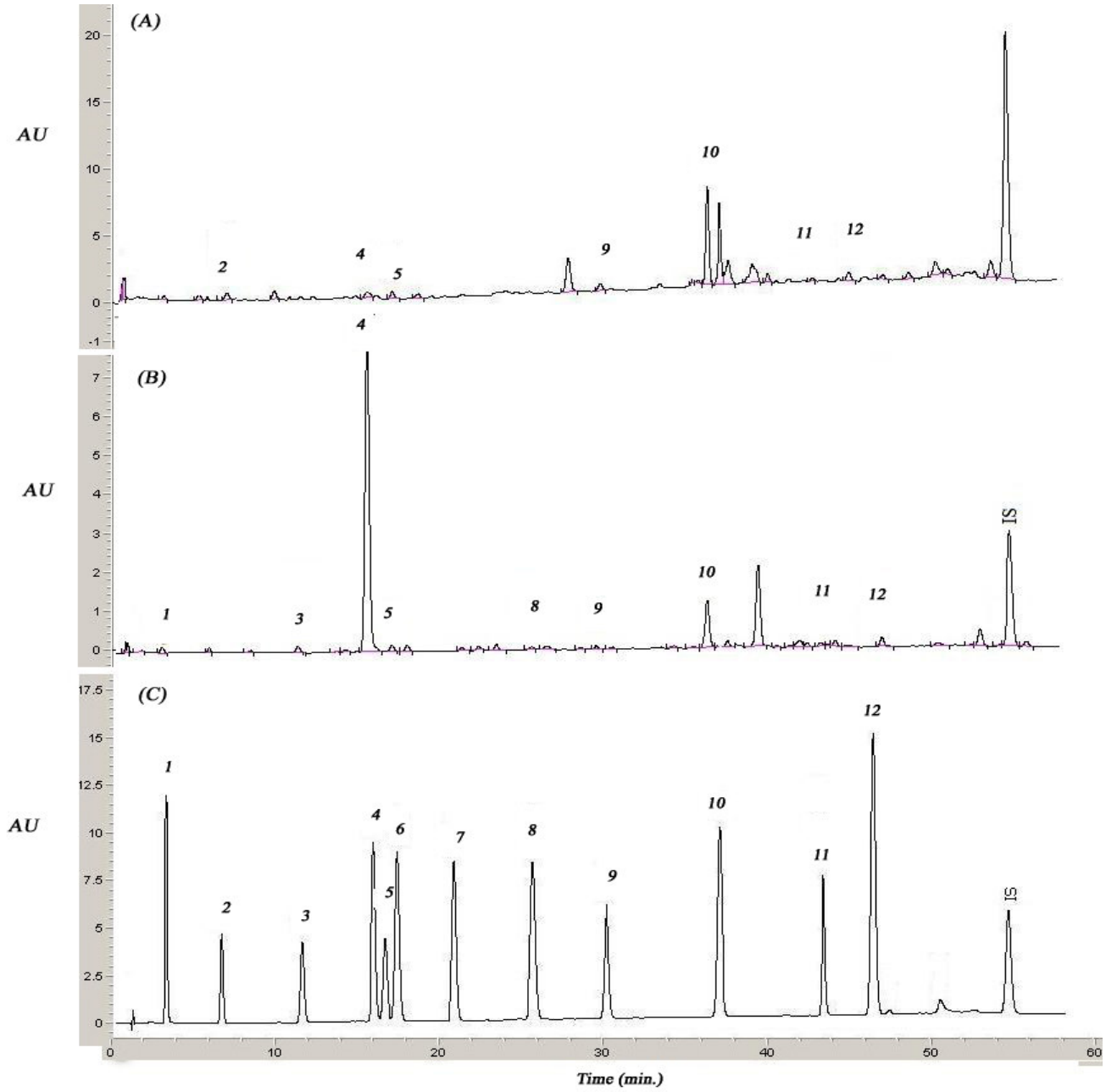

Figure 3. The original chromatograms of the mixture of standard phenolic acid containing IS (C) and methanol extracts obtained from leaves of *M. parviflorum* (A) and *L. amplexicaule* (B). The phenolic acids are symbolized by GA(1), protoCA(2), *p*-hydBA (3), VA(4), CA(5), ChA(6), SA (7), p-COU (8), FA(9), RA(10), o-COU (11), tr-CIN (12) and IS (propylparaben)(13) on the chromatograms.

Table 4. The results of the phenolic acid amounts in the methanol extracts of *M. parviflorum and L. amplexicaule.*

	Phenolic acids									
Plants	**GA**	**proCA**	**p-OH**	**VA**	**ChA**	**p-Cou**	**FA**	**RA**	**o-Cou**	**tr-Cin**
M. parviflorum	-	100.2	-	170.4	154.9	-	565.9	952.7	385.2	83.2
L. amplexicaule	100.1	-	153.6	4688	237.5	95.4	71.9	133.8	155.8	140.1

The values are given as µg mg^{-1}.

antioxidant potentials of phenolic contents of these plants might provide a source for some areas of food industries, health benefits, medicine and pharmacology. As far as our literature survey could ascertain, there is not report detailed study in point of the phytochemical compunds such as phenolic, flavanois and flavonols and phenolic acids. The results of these investigations should be helpful in the better explaining the complex pharmacological activity of some medicinal plants belonging to the Lamiaceae family.

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