Research Journal of Biological Sciences 5 (7): 484-487, 2010
ISSN: 1815-8846

Determination of Total Phenolic and Flavonoid Contents of *Leonurus cardiaca* L. in Compare with Antioxidant Activity

Samineh Jafari, Arman Moradi, Ali Salaritabar, Abbas Hadjiakhoondi and Mahnaz Khanavi
Department of Pharmacognosy and Medicinal Plants Research Center, Faculty of Pharmacy,
Tehran University of Medical Sciences, P.O. Box: 14155-6451, Iran

Abstract: The crude extract and 4 fractions of aerial parts of cultivated motherwort (*Leonurus cardiaca* L.) were investigated for their total phenolic and flavonoid contents and antioxidant activities. The phenolic and flavonoid contents were determined using Folin-Ciocalteu assay and proposed assay of pharmacopeia where as antioxidant activity of samples were measured by 2 tests: DPPH and FRAP assays. Buthanolic fraction showed the highest antioxidant activity with the FRAP value of 1.735±0.07 μmol g^{-1} and DPPH inhibitory percentage of 57.76% μg mL^{-1} as well as the greatest phenolics content (48.37±3.76 mg of gallic acid equivalents g^{-1}). The highest total flavonoid content was revealed in methanolic-aqueous fraction (50.21± 0.65 mg of hyperoside equivalents g^{-1}). There was a direct correlation between total phenol and antioxidant activities which could introduce phenols as the main antioxidants of *L. cardiaca* L. extracts.

Key words: *Leonurus cardiaca* L., antioxidant, phenolic content, flavonoid, methanolic-aqueous, fraction, hyperoside equivalents

INTRODUCTION

In recent years, the possible role of nutrition in prevention of human ailments has been shown to be important. In this context, antioxidants, especially those derived from natural sources, require special attention. These beneficial compounds neutralize the toxic and volatile free radicals that are defined as atoms or groups of atoms having an unpaired electron (Hiramatsu *et al*., 2006). Most antioxidants in plants are phenols which act as chain-breaking antioxidants. Phenols sometimes have additional mechanisms of antioxidant action, e.g., by chelating transition metal ions (Packer and Cadenas, 2002). Flavonoids as the largest group of phenols consist of an aromatic ring, which is condensed to a heterocyclic ring and attached to a second aromatic ring. The abundant phenolic hydroxyl groups on the aromatic ring confer the antioxidant capacity and the 3OH is essential for the iron-chelating activity of these compounds (Hiramatsu *et al*., 2006). Cellular damage induced by oxidative stress, a result of imbalance between the antioxidant defense system and the formation of reactive oxygen species has been implicated in the etiology of a large number of human diseases as well as in the process of aging. The degenerative diseases in which free radicals have beenimplicated include cardiovascular ailments, neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease and multistage process of carcinogenesis (Hiramatsu *et al*., 2006; Dehghan *et al*., 2007). Recently, there have been great efforts in finding safe and potent natural antioxidants from various plant sources to replace synthetic ones.

Motherwort (*Leonurus cardiaca* L.) which originally came from central Europe, has spread to all temperate areas of the world. It has been listed in many pharmacopoeias including the current European Pharmacopoeia. European Motherwort is stated to possess efficient antioxidant properties (Matkowski and Piotrowska, 2006; Matkowski *et al*., 2008) and known to have significant amounts of phenolics and flavonoids (Barnes *et al*., 2007). Traditionally, it has been used for cardiac debility, simple tachycardia, effort syndrome, amenorrhoea and especially for cardiac symptoms associated with neurosis (Gruenwald *et al*., 2000).

In Iran, *L. cardiaca* is cultivated for the first time in order to produce medicinal formulations whereas it has been approved that changing in growth condition can make a variation in the plant main components and consequently its medicinal effects (Urban *et al*., 2009). The present study leads to evaluate, different extracts of cultivated motherwort for their antioxidant activities using two methods: inhibition of DPPH radical and FRAP. Also, the total phenolic and flavonoidcontents of the samples were estimated using the Folin-Ciocalteu assay and proposed method of European pharmacopeia, respectively.

Corresponding Author: Mahnaz Khanavi, Department of Pharmacognosy, Faculty of Pharmacy,
Tehran University of Medical Sciences, P.O. Box: 14155-6451, Iran

MATERIALS AND METHODS

Plant material: The aerial parts of *Leonurus cardiaca* L. were obtained from the experimental plot of the Zardband Pharmaceuticals, Tehran, Iran in July 2009 during the flowering stage. The voucher specimen was deposited in their Herbarium (No. 16 ZA 0526-07).

Chemicals: All reagents and solvents were of analytical grade or of pure quality which were purchased from Merck, Sigma, Aldrich and Fluka.

Extraction: The extract was obtained by percolation using methanol/H_2O (80:20, v/v) at room temperature. The procedure involved 3 consecutive extractions of 48 h using new solvent each time. The solvent was evaporated under vacuum in a rotary evaporator until dryness. The dried extract fractionated using chloroform, ethylacetate, n-buthanol and methanol/H_2O (50:50, v/v) successively. Then dried total extract and fractions were stored at 20°C until use.

Measurement of total phenolics: Total phenolic content was determined spectrophotometrically using Folin-Ciocalteau reagent (Dehghan *et al*., 2007). Folin-Ciocalteau reagent (0.75 mL), previously diluted 10-fold with distilled water were mixed thoroughly with 200 μL of appropriate dilutions of the crude extract or fraction solutions and allowed to stand at room temperature for 5 min. Then 0.75 mL of sodium bicarbonate solution (60 g L^{-1}) was added to the mixture followed by storing at room temperature for 90 min. Subsequently, absorbance was measured at 725 nm using a UV-visible spectrophotometer. Total phenolics were quantified by calibration standard curve obtained using various known concentrations of gallic acid (50-200 μg mL^{-1} in methanol). The concentrations are expressed as mg of Gallic Acid Equivalents (GAE) g^{-1} of dry extract or fraction. The total phenolics content of samples was measured 5 times for each species.

Measurement of total flavonoids: The total flavonoid content of the extract and fractions were determined using proposed method of Council of Europe (2005). Total flavonoid contents were expressed as mg of Hyperoside Equivalents (HE) per g of sample.

DPPH radical scavenging activity: The DPPH radical scavenging activity was measured using the method described by Sanchez-Moreno *et al.* (1999) with some modification. Total extract and its fractions were dissolved in methanol at different concentrations (50, 100, 200 and 500 mg = mL). About 1 mL of these solutions was added to 2 mL of DPPH solution (4×10^{-5} g mL^{-1} in methanol). Absorbance was measured immediately after mixing the solutions at 517 nm using methanol as blank and the absorbance decrease was evaluated every 5 min up to 30 min. About 2 mL of DPPH diluted in 1 mL of methanol was used as control.

The scavenging activity was calculated using the following equation: Inhibition (%) = 100 - [(A0 - As)/A0] ×100 where A0 is the absorbance of the control (The DPPH solution without sample solution) and as is the absorbance in the presence of sample. IC_{50} value was determined from the plotted graph of scavenging activity against the concentrations of the samples which represented the total antioxidant necessary to decrease the initial DPPH radical by 50%. α-tocopherol and BHA were used as positive control and all experiments were carried out at least 3 times.

Frap assay: The ferric-reducing ability of extract and fractions were determined at low pH by Frap assay (Benzie and Strain, 1996; Astaneie *et al*., 2005). Briefly, the FRAP reagent contained 5 mL of a (10 mmol L^{-1}) TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mmol L^{-1} Hcl plus 5 mL of (20 mmol L^{-1}) FeCl3 and 50 mL of (0.3 mol L^{-1}) acetate buffer, pH 3.6 and was prepared freshly and warmed at 37°C. Aliquots of 100 μL sample were mixed with 3 mL FRAP reagent and the mixtures were incubated at 37°C for 10 min. An intense blue color complex was formed when Fe^{3+} TPTZ complex was reduced to the Fe^{2+} form and the absorption at 593 nm was measured. The calibration curve was plotted with absorbance at 593 nm vs. About 5 different concentrations of $FeSO_4.7H_2O$ (125, 250, 500, 750, 1000 μmol L^{-1}). All solutions were used on the day of preparation and the results were expressed in mmol Fe^{2+} g^{-1} of extract or fraction.

Statistical analysis: Values were reported as mean±SD of three parallel measurements. The obtained results were statistically analyzed with one way ANOVA and Tukey post hoc multicomparison tests using a significance level of $p<0.05$. Correlation between phenolic and flavonoid contents and antioxidant activities was established by regression analysis.

RESULTS

Fractionation: Total amounts of crude extract and fractions are shown in Table 1. The amount of Methanolic-aqueous was considerably noticeable among other fractions.

Table 1: Total amounts of the motherwort crude extract and fractions

	Extract				
Yield	Total extract	Chloroform	Ethyl acetate	n-Buthanol	Methanolic-aqueous (50:50, v/v)
Total amount (yielded by 100 g of dry plant)	31.78	1.82	1.13	5.55	22.30
Percentage (relative to crude extract)	100.00	5.73	3.55	17.42	66.83

Table 2: Total phenolic and flavonoid contents and antioxidant activity of motherwort crude extract and fractions

Samples	Phenolics content (mg GAE g^{-1}±SD)	Flavonoid content (mg HE g^{-1}±SD)	[a]DPPH ((%) inhibition±SD)	IC_{50} (µg mL^{-1})	FRAP value (mmol Fe^{2+} g^{-1}±SD)
Chloroform	4.90±0.98	27.25±0.670	Trace	1814.35	1.23±0.05
Ethyl acetate	20.45±2.54	0.42±16.27	47.20±3.87	107.16	0.42±0.03
n-Buthanol	48.37±3.76	0.20±8.750	86.18±5.76	57.76	1.73±0.07
Methanolic-aqueous	42.95±3.55	0.65±50.21	85.84±6.76	53.79	1.52±0.05
Total extract	62.85±3.70	0.05±14.35	80.59±6.98	77.65	1.10±0.06

GAE = Gallic Acid Equivalent; SD = Standard Deviation; HE = Hyperoside Equivalent; IC50 = 50% Inhibitory Concentration; [a]The reported percentages for % inhibition were related to the concentration of 100 µg extract or fraction mL^{-1}

Total phenol estimation: The total phenolic contents of the samples were calculated with a linear equation based on a standard curve using galic acid ($y = 0.0059x+0.0468$, $R^2 = 0.9987$) and shown in Table 2. The buthanolic fraction had the highest phenolic content (48.37±3.76 mg GAE g^{-1}).

Flavonoid content: According to the quantitative results shown in Table 2, there are marked differences between fractions in total flavonoid contents.

The highest amount of flavonoid was found in methanolic-aqueous fraction (50.2±0.65 mg HE g^{-1}) while buthanulic fraction was determined as the lowest (8.75±0.20 mg HE g^{-1}).

DPPH radical scavenging activity: The results of the DPPH assay are shown in Table 2. The higher polar fractions, methanolic-aqueous and buthanolic ones showed high antioxidant activity. Radical scavenger activity of these two fractions at 75 µg mL^{-1} were comparable with α-tocopherol (40 µg mL^{-1}) and BHA (100 µg mL^{-1}), $p>0.05$.

Frap assay: Antioxidant activity of all samples were calculated with a linear equation based on a standard curve using $FeSO_4$ ($y = 0.0007x - 0.0589$, $R^2 = 0.9512$) and shown in Table 2. The buthanolic fraction showed the highest antioxidant activity with the FRAP value of 1.735±0.07 mmol g^{-1}.

DISCUSSION

The amounts of dry extract and fractions obtained from aerial part of *L. cardiaca* L. are shown in Table 1. These results showed that polar solvents could produce massive fractions with most of the constituents. Also, study of estimated total phenolic contents (Table 2) reveals that phenolic components in non polar solvents (chloroform and ethyl acetate) are noticeably less than crude extract and polar fractions (buthanolic and methanolic-aqueous).

So, non polar solvent are not efficient enough for extraction of Motherwort. In previous investigations, the presence of various polyphenol compounds with antioxidant activity were reported in Motherwort (Ali *et al*., 2007; Bernatoniene *et al*., 2009).

High phenolic contents were previously reported in buthanolic (256.8±7.8 GAE mg g^{-1}) and methanolic (150.1±8.9 GAE mg g^{-1}) extracts (Matkowski *et al*., 2008). In another study, IC_{50} of methanolic extract of *L. cardiaca* was reported about 70-80% (Matkowski and Piotrowska, 2006) which is approximately comparable with the results in crude extract whereas buthanolic and methanolic-aqueous fractions of the research were more potent in IC_{50} assay (53.79% and 57.76%, respectively). Total flavonoid content of crude extract was estimated about 0.45% of flavonoids expressed as hyperoside in dried plant (equal to 14.35 mg HE g^{-1} of total extract) which is remarkably higher than minimum approved flavonoid content of Motherwort reported in pharmacopeias (0.2% of dried plant).

The results showed that omission of chloroform fraction reports could lead to a significant correlations between antioxidant activities, phenolic and flavonoid contents in polar and semi-polar fractions of motherwort (Table 3) and phenolic compounds of this plant are probable responsible compounds for antioxidant power. It seems higher antioxidant activity and total phenolic content were exhibited in methanolic-aqueous and buthanolic fractions whereas massive methanolic-aqueous fraction is considerable for further studies.

Table 3: Correlations between antioxidant activities and Phenolic and flavonoid contents of fractions

Sample	Phenolic content (mg GAE g^{-1}) and FRAP value (mmol Fe^{+2} g^{-1})	Phenolic content (mg GAE g^{-1}) and DPPH (inhibitory %)	DPPH (% inhibitory) and FRAP value (mmol Fe^{+2} g^{-1})	Phenolic content and flavonoid content (mg HE g^{-1})	Flavonoid content (mg HE g^{-1}) and FRAP value (mmol Fe^{+2} g^{-1})	Flavonoid(mg HE g^{-1}) content and DPPH (inhibitory %)
Equation	y = 0.0476x - 0.5473	y = 1.4899x+17.564	y = 0.0311x - 1.0483	y = 0.0245x+1.5877	y = 0.0063x+1.0671	y = -0.8194x+109.8
of correlation	R^2 = 0.9991	R^2 = 0.9692	R^2 = 0.9788	R^2 = 0.0269	R^2 = 0.0389	R^2 = 0.3497

CONCLUSION

Motherwort used for a variety of human diseases, especially cardiac disorders. This plant is cultivated in Iran for the first time in order to use in medicinal formulations.

In summary, the current study verifies high amount of flavonoid in total extract and fractions of cultivated *L. cardiaca* which can offer the plant for industrial uses.

ACKNOWLEDGEMENTS

This research has been supported by Tehran University of Medical Sciences and health Services grant (Grant Number 8923-33-02-88). We also thank Zardband Pharmaceuticals for supplying the plant materials.

REFERENCES

Ali, M.S., S.A. Ibrahim, S. Jalil and M.I. Choudhary, 2007. Ursolic acid: A potent inhibitor of superoxides produced in the cellular system. Phytotherapy Res., 21: 558-561.

Astaneie, F., M. Afshari, A. Mojtahedi, S. Mostafalou, M.J. Zamani, B. Larijani and M. Abdollahi, 2005. Total antioxidant capacity and levels of epidermal growth factor and nitric oxide in blood and saliva of insulin-dependent diabetic patients. Arch. Med. Res., 36: 376-381.

Barnes, J., L.A. Anderson and J.D. Phillipson, 2007. Herbal Medicines. Pharmaceutical Press, London.

Benzie, I.F. and J.J. Strain, 1996. The Ferric Reducing Ability of Plasma (FRAP) as a measure of antioxidant power: The FRAP assay. Anal. Biochem., 239: 70-76.

Bernatoniene, J., A. Kucinskaite, R. Masteikova, Z. Kalveniene, G. Kasparaviciene and A. Savickas, 2009. The comparison of anti-oxidative kinetics *in vitro* of the fluid extract from maidenhair tree, motherwort and hawthorn. Acta Pol Pharm., 66: 415-421.

Council of Europe, 2005. European Pharmacopeia. 5th Edn., Council of Europe, Strasbourg, France,.

Dehghan, G., A. Shafiee, M.H. Ghahremani, S.K. Ardestani and M. Abdollahi, 2007. Antioxidant potential of various extracts from ferula szovitsiana in relation to their phenolic content. Pharm. Biol., 45: 691-699.

Gruenwald, J., T. Brendler and C. Jaenicke, 2000. PDR for Herbal Medicines. Medical Economics Company, Montvale.

Hiramatsu, M., T. Yoshikawa and L. Packer, 2006. Molecular Interventions in Lifestyle Related Diseases. Taylor and Francis Group, London, pp: 354.

Matkowski, A. and M. Piotrowska, 2006. Antioxidant and free radical scavenging activities of some medicinal plants from the *Lamiaceae*. Fitoterapia, 77: 346-353.

Matkowski, A., P. Tasarz and E. Szypula, 2008. Antioxidant activity of herb extracts from five medicinal plants from *Lamiaceae*, subfamily *Lamioideae*. J. Med. Plant Res., 2: 321-330.

Packer, L. and E. Cadenas, 2002. Handbook of Antioxidants. Marcel Dekker Inc., Basel, Switzerland.

Sanchez-Moreno, C., J.A. Larrauri and F. Saura-Calixto, 1999. Free radical scavenging capacity and inhibition of lipid oxidation of wines, grape juices and related polyphenolic constituents. Food Res. Int., 32: 407-412.

Urban, L., L. Berti, F. Bourgaud, H. Gautier, M. Lechaudel, J. Joas and H. Sallanon, 2009. The effect of environmental factors on biosynthesis of carotenoids and polyphenolics in fruits and vegetables: A review and prospects. Acta Hort., 841: 339-344.