http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 12 (12): 934-938, 2009
ISSN 1028-8880

Correlation Between the *in vitro* Iron Chelating Activity and Poly Phenol and Flavonoid Contents of Some Medicinal Plants

M.A. Ebrahimzadeh, S.M. Nabavi and S.F. Nabavi
Pharmaceutical Sciences Research Center, School of Pharmacy,
Mazandaran University of Medical Sciences, Sari, Iran

Abstract: Iron chelating activity of 16 extracts from 11 medicinal plants has been determined to find alternative sources with lower side effects in thalassemic patients. Thalassemia is characterized by iron overload and chelation therapy reduces iron-related complications and thereby improves quality of life and overall survival. Because of poor oral bioavailability, short plasma half-life and severe side effects of available chelators, this screening may be useful in this area. Extracts were prepared by soaking dry material of the selected plant in appropriate solvent. Phenol and flavonoid content of the extract were measured by Folin Ciocalteu and $AlCl_3$ colorimetric assays. Phenol content of the extracts varied between 8.4-194.9 mg g^{-1} of extract. The highest chelating activity was found in aerial parts of *Leonurus cardiana* and *Grammosciadium platycarpum* which had high amount of phenol and flavonoid contents. All extracts contained various amount of flavonoids from 5.9 to 90.9 mg g^{-1} of extract. Weak correlations were found between phenolic and flavonoids contents and iron chelatory activity with $R^2 = 0.40$. Extracts with high phytochemicals and chelating activity can be candidate as a good source of new agents for thalassemic patients.

Key words: Antioxidant activity, iron chelating, phenols, thalassemia

INTRODUCTION

Patients with chronic anemias such as thalassemia, require regular blood transfusions in order to improve both quality of life and survival. Humans are unable to eliminate the iron released from the breakdown of transfused red blood cells and the excess iron is deposited as hemosiderin and ferritin in the liver, spleen, endocrine organs and myocardium. The accumulation of toxic quantities of iron causes tissue damage and leads to complications such as heart failure, endocrine abnormalities like diabetes, hypothyroidism, liver failure and ultimately early death (Taher *et al*., 2006; Rund and Rachmilewitz, 2005; Loukopoulos, 2005). Thalassemia major is characterized by anemia, iron overload, further potentiation of ROS and damage to major organs, especially the cardiovascular system. Oxidative stress is ultimately involved in endothelial dysfunction, a condition which is evident in adults suffering from various cardiovascular diseases including thalassemia (Shinar and Rachmilewitz, 1990; Hebbel *et al*., 1990; Grinberg *et al*., 1995). Antioxidant and other supportive therapies protect RBC against oxidant damage (Kukongviriyapan *et al*., 2008; Filburn *et al*., 2007). Also, a higher rate of LDL oxidation in thalassemia patients is due to a lower concentration of vitamin E and C in the LDL particles. Enrichment with vitamins E and C was effective in preventing LDL oxidation in patients with thalassemia (Rachmilewitz *et al*., 1979; Livrea *et al*., 1996). Iron chelators mobilize tissue iron by forming soluble, stable complexes that are then excreted in the feces and/or urine. Chelation therapy reduces iron-related complications and thereby improves quality of life and overall survival (Shinar and Rachmilewitz, 1990; Hebbel *et al*., 1990). In addition, brain iron dysregulation and its association with amyloid precursor protein plaque formation are implicated in Alzheimer's Disease (AD) pathology and so iron chelation could be considered a rational therapeutic strategy for AD (Reznichenko *et al*., 2006). The poor oral bioavailability, short plasma half-life and severe side effects of available chelators are still not optimal (Hebbel *et al*., 1990; Grinberg *et al*., 1995; Kukongviriyapan *et al*., 2008; Filburn *et al*., 2007; Rachmilewitz *et al*., 1979; Livrea *et al*., 1996). Within this context and taking in consideration the relative paucity of iron chelating agents it is not surprising that clinical scientists put a great effort towards finding any potentially useful sources in order to obtain the maximum possible benefit with the least possible harm (Loukopoulos, 2005; Ebrahimzadeh *et al*., 2006, 2007; Mahmoudi *et al*., 2007; Pourmorad *et al*., 2007). For thousands of years, mankind has known about the benefit of drugs from nature. Plant extracts, for the treatment of various ailments, were highly regarded by the ancient

Corresponding Author: Mohammad Ali Ebrahimzadeh, Department of Medicinal Chemistry, School of Pharmacy, Mazandaran University of Medical Sciences, Postal Code 48189, Sari, Iran

civilizations. Even today, plant materials remain an important resource for combating illnesses. In this study, some medicinal plants traditionally used for management of diseases were selected and their phenol and flavonoid content and iron chelating activities were evaluated.

MATERIALS AND METHODS

This study was performed from the spring and the end of autumn 2008 in Pharmaceutical Sciences Research Center, School of pharmacy, Mazandaran University of Medical Sciences, Sari, Iran.

Chemicals: Gallic acid, quercetin, EDTA and other necessary agents were purchased from Merck and Fluka companies. All other chemicals and reagents used were of the highest commercially available purity.

Preparation of extracts: A brief description of the plants can be found in Table 1. Plant parts were collected from Sari forest, Iran, in 2008 and identified by Dr. Bahman Eslami. vouchers have been deposited in the Sari School of Pharmacy herbarium. Hundred grams each of the dried specific part of plant was soaked in desired solvent for 3 days in room temperature. The solvent was evaporated under reduced pressure and then lyophilized. The resulting solid masses were preserved in 4°C.

Determination of total phenolic compounds and flavonoid contents: Total phenolic compound contents were determined by the Folin-Ciocalteau method according to our recently published paper (Ebrahimzadeh *et al*., 2008a, b). The standard curve was prepared using 50 to 250 mg mL^{-1} solutions of gallic acid in methanol-water (1:1, v/v). Total phenol values are expressed in terms of gallic acid equivalent (mg g^{-1} of dry mass), which is a common reference phenolic compound.

Flavonoid content of each extract was determined by following colorimetric method (Ebrahimzadeh *et al*., 2008c; Nabavi *et al*., 2008). The calibration curve was prepared by preparing quercetin solutions at concentrations 12.5 to 100 μg mL^{-1} in methanol.

Metal chelating activity: The chelation of ferrous ions by extracts was estimated by method of (Ebrahimzadeh *et al*., 2008b; Nabavi *et al*., 2009). The percentage inhibition of ferrozine-Fe^{2+} complex formation was calculated as:

$$[(A_0 - A_s)/ A_s] \times 100$$

Table 1: The studied plants and their medicinal uses

Plant	Common name	Part of plant tested	Medical use/disease treated
Umbelliferae *Grammosciadium platycarpum* Boiss	----------	Aerial parts	Antibacterial Activity
Umbelliferae *Froriepia subpinnata*		Aerial parts	Antioxidant
Umbelliferae *Ferula assa-foetida*	Asafetida giant fennel	Aerial parts	Antispasmodic, aromatic, carminative, digestive, expectorant, laxative, sedative, nervine, analgesic, aphrodisiac and antiseptic properties, anthelminitic, anticoagulant, Hypotensive and pregnancy interceptive activity
Apiaceae *Eryngium caucasicum Trautv*	----------	Leaves (at Flowering stage) and Inflorescence Flowers	Flavouring agent.
Caprifoliaceae *Sambucus ebulus*	Danewort, Dwarf Elder,		Antinociceptiv, anti inflammatory activity, Antiphlogistic,Cholagogue, Diaphoretic, Diuretic,Expectorant, Homeopathy, Poultice, Purgative.
Lamiaceae *Leonurus cardiaca*	Motherwort	Aerial parts	Sedative, Hypotensive, Cardiotonic and Antioxidant activity. In nervous disorders for facilitating child-birth.
Boraginaceae *Onosma demawendicum*	----------	Aerial parts	AntiCholinesterase activity
Liliaceae *Ornithogalum sintenisii Freyn*	----------	Bulbs and Aerial parts	It is known to be poisonous.
Solanaceae *Hyoscyamus squarrosus*	----------	Fruits and Leaves	Rich source of tropane alkaloids
Malvaceae *Alcea hyrcana*	----------	Leaves, Flowers and Seeds	----------
Compositae *Artemisia absinthium*	Wormwood	Aerial parts	Antioxidant, anthelmintic, choleretic, antiseptic, balsamic, depurative, digestive, diuretic, emmenagogue and in treating leukaemia and sclerosis, as muscle relaxant, mild sedative, as a flavouring agent in alcoholic beverages and some foods

where A_0 was the absorbance of the control and A_s was the absorbance of the extract/standard. Na_2 EDTA was used as positive control.

Statistical analysis: Results are presented as Mean±SD. Statistical analysis were performed by Student's t-test. The values of $p<0.05$ were considered significant.

RESULTS AND DISCUSSION

Total phenols measured by Folin Ciocalteu reagent in terms of gallic acid equivalent. Phenol content of the extracts varied between 8.4-194.9 mg g^{-1} of extract. The highest amounts were found in aerial parts of *Artemisia absinthium* (194.9±14.51) and fruits extract of *Hyoscyamus squarrosus* (178.90±9.02). They showed good iron chelatory activity (Table 2). The flavonoid content of *Ferula assa-foetida* aerial parts extract in terms of quercetin equivalent was 90.9±6.3. Leaves extract (at Flowering stage) of *Eryngium caucasicum Trautv* also contained high amount of flavonoids (60.12±5.31). Both of them showed good iron chelatory activity (Table 2). The chelating of Fe^{2+} by extracts was estimated by the method of Dinis *et al.* (Ebrahimzadeh *et al*., 2008b). The highest chelating activities were found in aerial parts of *Leonurus cardiana* and *Grammosciadium platycarpum* with IC_{50} = 0.02±0.00 and 0.079±0.00, respectively. Weak correlations were found between phenolic and flavonoids contents and iron chelatory activity with $R^2 = 0.40$ (Fig. 1, 2).

Table 2: Total phenol and flavonoid content and iron chelating IC_{50} of the herbs studied.

Name of the plant	Total phenol content*	Flavonoid content **	Fe^{2+} chelating activity (IC_{50} mg mL^{-1})
Grammosciadium platycarpum			
Aerial parts	66.90±3.24	32.80±2.16	0.079±0.00
Froriepia subpinnata			
Aerial parts	75.70±3.43	35.20±1.88	0.952±0.05
Ferula assa-foetida			
Aerial parts	94.80±5.9	90.90±6.3	0.570±0.02
Eryngium caucasicum Trautv			
Leaves (at Flowering stage)	37.60±2.27	60.12±5.31	0.250±0.01
Inflorescence	63.13±3.07	18.25±1.10	1.470±0.08
Sambucus ebulus			
Flowers	56.30±3.28	14.50±0.64	1.780±0.04
Leonurus cardiaca			
Aerial parts	54.30±2.12	35.20±1.63	0.020±0.00
Onosma demawendicum			
Aerial parts	47.20±3.03	13.70±1.14	0.268±0.01
Ornithogalum sintenisii Freyn			
Bulbs	8.40±0.2	5.90±0.3	0.689±0.04
Aerial parts	28.90±1.92	23.50±1.81	0.340±0.01
Hyoscyamus squarrosus			
Fruit	178.90±9.02	16.40±0.09	0.104±0.01
Leaves	98.95±7.02	44.94±2.91	0.886±0.06
Alcea hyrcana			
Leaves	14.70±0.09	28.30±1.12	0.10±0.01
Flowers	48.10±2.36	24.30±1.87	2.900±0.17
Seeds	68.97±4.51	24.70±1.02	0.402±0.02
Artemisia absinthium			
Aerial parts	194.90±14.51	12.40±1.02	0.419±0.03
EDTA			0.017±0.00

*mg gallic acid equivalent g^{-1} extract, **mg quercetin equivalent g^{-1} extract, Data presented as Mean±SD

It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable. The mechanisms of action of flavonoids are through scavenging or chelating process (Kessler *et al*., 2003; Cook and Samman, 1996). Phenolic compounds are a class of antioxidant compounds which act as free radical terminators (Shahidi and Wanasundara, 1992). The compounds such as flavonoids, which contain hydroxyl functional groups, are responsible for antioxidant effect in the plants (Das and Pereira, 1990; Younes, 1981). According to our study, the content of these phytochemical compounds in studied extracts have direct relation with iron chelating activity.

The chelating of Fe^{2+} by extracts was estimated by the method of (Ebrahimzadeh *et al*., 2008b). Ferrozine can quantitatively form complexes with Fe^{2+}. However, in the presence of chelating agents, the complex formation is disrupted with the result that the red color of the

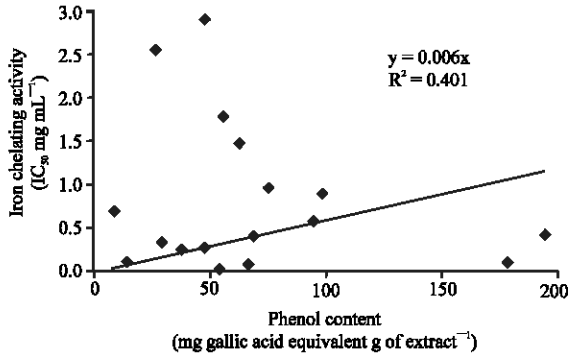

Fig. 1: Correlation between phenol contents and iron chelating activity in 16 tested extracts

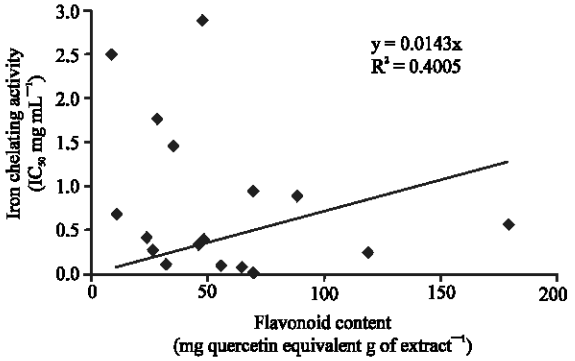

Fig. 2: Correlation between flavonoids contents and iron chelating activity in 16 tested extracts

complex is decreased. Measurement of colour reduction, therefore, allows the estimation of the chelating activity of the coexisting chelator. The transition metal ion, Fe^{2+} possess the ability to move single electrons by virtue of which it can allow the formation and propagation of many radical reactions, even starting with relatively non-reactive radicals (Aboul-Enein *et al*., 2003). The main strategy to avoid ROS generation that is associated with redox active metal catalysis involves chelating of the metal ions. *Leonurus cardiana* and *Grammosciadium platycarpum*, the most active extracts interfered with the formation of ferrous and ferrozine complex, suggesting that it has chelating activity and captures ferrous ion before ferrozine. The highest chelating activities was found in aerial parts of *Leonurus cardiana* with IC_{50} = 0.02±0.00 which was comparable to the reference compound EDTA (IC_{50} = 0.017 mg mL^{-1}). A weak direct relation between this activity and the content of active compounds, phenols and flavonoids were found in persent study (Fig. 1, 2).

The interaction between flavonoids and iron ion has been published previously (Mira *et al*., 2002). It is suggested that this chelating activity depends on the flavonoid structures and may be a proposal explanation for antioxidant activity of flavonoids (Mira *et al*., 2002). In our recently published paper, in spite of some relation between chelating activity and phenol and flavonoids, no direct correlation was found between these contents and chelating activity in tested plants ($p>0.001$). Some plants with high phenol and flavonoid contents showed very weak chelating activity but some with high phenol and flavonoid contents showed good chelating activity. This lack of relationship is in agreement with other literature (Ghasemi *et al*., 2009; Heinonen *et al*., 1998; Assimopoulou *et al*., 2006; Nickavar *et al*., 2007). It is known that o nly flavonoids with a certain structure and particularly hydroxyl position in the molecule can act as proton donating and show radical scavenging activity (Mensor *et al*., 2001; Hou *et al*., 2003). Furthermore, the extracts are very complex mixtures of many different compounds with distinct activities (Mensor *et al*., 2001; Hou *et al*., 2003). In this study, a weak direct relation was found (R^2 = 0.40). So the chelating activity of these extracts may be a function of these compounds.

CONCLUSION

All extracts showed a variety of activity and phytochemical compounds in this study, but *Leonurus cardiana* can be observed as a potent iron chelating source for further investigation.

ACKNOWLEDGMENT

This research was Financial supported by a grant from the research council of Mazandaran University of Medical Sciences, Iran.

REFERENCES

Aboul-Enein, A.M., F.K. El Baz, G.S. El-Baroty, A.M. Youssef and H.H. Abd El-Baky, 2003. Antioxidant activity of algal extracts on lipid peroxidation. J. Medical Sci., 3: 87-98.

Assimopoulou, A.N., D. Boskou, M.A. Anagnostopoulou, P. Kefalas and V.P. Papageorgiou, 2006. Radical scavenging activity of various extracts and fractions of sweet orange peel (*Citrus sinensis*). Food Chem., 94: 19-25.

Cook, N.C. and S. Samman, 1996. Flavonoids-chemistry, metabolism, cardioprotective effects and dietary sources. J. Nutr. Biochem., 7: 66-76.

Das, N.P. and T.A. Pereira, 1990. Effects of flavonoids on thermal autooxidation of palm oil: Structure-activity relationship. J. Am. Oil Chem. Soc., 67: 255-258.

Ebrahimzadeh, M.A., M. Mahmoudi and E. Salimi, 2006. Antiinflammatory activity of *Sambucus ebulus* hexane extracts. Fitoterapia, 77: 146-148.

Ebrahimzadeh, M.A., M. Mahmoudi, M. Karami, S. Saeedi, A.H. Ahmadi and E. Salimi, 2007. Separation of active and toxic portions in *Sambucus ebulus*. Pak. J. Biol. Sci., 10: 4171-4173.

Ebrahimzadeh, M.A., S.J. Hosseinimehr, A. Hamidinia and M. Jafari, 2008a. Antioxidant and free radical scavenging activity of *Feijoa sallowiana* fruits peel and leaves. Pharmacologyonline, 1: 7-14.

Ebrahimzadeh, M.A., F. Pourmorad and A.R. Bekhradnia, 2008b. Iron chelating activity screening, phenol and flavonoid content of some medicinal plants from Iran. Afr. J. Biotechnol., 7: 3188-3192.

Ebrahimzadeh, M.A., F. Pourmorad and S. Hafezi, 2008c. Antioxidant activities of Iranian corn silk. Turk. J. Biol., 32: 43-49.

Filburn, C.R., R. Kettenacker and D.W. Griffin, 2007. Bioavailability of a silybin-phosphatidylcholine complex in dogs. J. Vet. Pharmacol. Ther., 30: 132-138.

Ghasemi, K., Y. Ghasemi and M.A. Ebrahimzadeh, 2009. Antioxidant activity, phenol and flavonoid contents of 13 *Citrus* species peels and tissues. Pak. J. Pharm. Sci., 22: 277-281.

Grinberg, L.N., E.A. Rachmilewitz, N. Kitrossky and M. Chevion, 1995. Hydroxyl radical generation in ß-thalassemia red blood cells. Free Radic. Biol. Med., 18: 611-615.

Hebbel, R.P., A. Leung and N. Mohandas,1990. Oxidation-induced changes in microheological properties of the red cell membrane. Blood, 76: 1015-1020.

Heinonen, I.M., P.J. Lehtonen and A.I. Hopia, 1998. Antioxidant activity of berry and fruit wines and liquors. J. Agric. Food Chem., 46: 25-31.

Hou, W.C., R.D. Lin, K.T. Cheng, Y.T. Hung and C.H. Cho *et al*., 2003. Free radical scavenging activity of *Taiwanese native* plants. Phytomedicine, 10: 170-175.

Kessler, M., G.C. Ubeaud and L. Jung, 2003. Anti-and pro-oxidant activity of rutin and quercetin derivatives. J. Pharm. Pharmacol., 55: 131-142.

Kukongviriyapan, V., N. Somparn, L. Senggunprai, A. Prawan, U. Kukongviriyapan and A. Jetsrisuparb, 2008. Endothelial dysfunction and oxidant status in pediatric patients with hemoglobin E-beta Thalassemia. Pediatr., Cardiol., 29: 130-135.

Livrea, M.A., L. Tesoriere, A.M. Pintaudi, A. Calabrese and A. Maggio *et al*., 1996. Oxidative stress and antioxidant status in ß-thalassemia major: Iron overload and depletion of lipid-soluble antioxidants. Blood, 88: 3608-3614.

Loukopoulos, D., 2005. Combined therapy with deferiprone and desferrioxamine in thalassemia major. Haematologica, 90: 1304-1305.

Mahmoudi, M., M.A. Ebrahimzadeh, F. Pourmorad and S. Yasini, 2007. Antinociceptive and locomotor impairment by methanolic extract of *Urtica dioica*. Int. J. Biol. Biotech., 4: 181-185.

Mensor, L.L., F.S. Menezes, A.S. Leitao, A.S. Reis and T.C. Santos *et al*., 2001. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother. Res., 15: 127-130.

Mira, L., M.T. Fernandez, M. Santos, R. Rocha, M.H. Florêncio and K.R. Jennings, 2002. Interactions of flavonoids with iron and copper ions: A mechanism for their antioxidant activity. Free Radical. Res., 36: 1199-1208.

Nabavi, S.M., M.A. Ebrahimzadeh, S.F. Nabavi, A. Hamidinia and A.R. Bekhradnia, 2008. Determination of antioxidant activity, phenol and flavonoid content of *Parrotia persica* MEY. Pharmacologyonline, 2: 560-567.

Nabavi, S.M., M.A. Ebrahimzadeh, S.F. Nabavi, M. Fazelian and B. Eslami, 2009. *In vitro* antioxidant and free radical scavenging activity of diospyros lotus and pyrus boissieriana growing in Iran. Phcog. Mag., 4: 122-126.

Nickavar, B., M. Kamalinejad and H. Izadpanah, 2007. *In vitro* free radical scavenging activity of five salvia species. Pak. J. Pharm. Sci., 20: 291-294.

Pourmorad, F., M .A. Ebrahimzadeh, M. Mahmoudi and S. Yasini, 2007. Antinociceptive activity of methanolic extract of *Epilobium hirsutum*. Pak. J. Biol. Sci., 10: 2764-2767.

Rachmilewitz, E.A., A. Shifter and I. Kahane, 1979. Vitamin E deficiency in ß-thalassemia major: Changes in hematological and biochemical parameters after a therapeutic trial with á-tocopherol. Am. J. Clin. Nutr., 32: 1850-1858.

Reznichenko, L., T. Amit, H. Zheng, Y. Avramovich-Tirosh, M.B.H. Youdim, O. Weinreb and S. Mandel, 2006. Reduction of iron-regulated amyloid precursor protein and [beta]-amyloid peptide by (-)-epigallocatechin-3-gallate in cell cultures: Implications for iron chelation in Alzheimer's disease. J. Neurochem., 97: 527-536.

Rund, D. and E. Rachmilewitz, 2005. β-thalassemia. New Engl. J. Med., 353: 1135-1146.

Shahidi, F. and P.K.J.P.D. Wanasundara, 1992. Phenolic antioxidants. Crit. Rev. Food Sci. Nutr. 32: 67-103.

Shinar, E. and E.A. Rachmilewitz, 1990. Oxidation denaturation of red blood cells in thalassemia. Semin. Hematol., 27: 70-82.

Taher, A., H. Isma'eel and MD. Cappellini, 2006. Thalassemia intermedia: Revisited. Blood Cells Mol. Dis., 37: 12-20.

Younes, M., 1981. Inhibitory action of some flavonoids on enhanced spontaneous lipid peroxidation following glutathione depletion. Planta Med., 43: 240-245.