African Journal of Biotechnology Vol. 7 (18), pp. 3188-3192, 17 September, 2008
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB08.476
ISSN 1684–5315 © 2008 Academic Journals

\

Full Length Research Paper

Iron chelating activity, phenol and flavonoid content of some medicinal plants from Iran

Mohammad Ali Ebrahimzadeh, Fereshteh Pourmorad* and Ahmad Reza Bekhradnia

Pharmaceutical Sciences Research Center, School of Pharmacy, Medical Sciences University of Mazandaran, Sari, Iran.

Accepted 8 August, 2008

Thalassemia major is characterized by anemia, iron overload, further potentiation of reactive oxygen species (ROS) and damage to major organs, especially the cardiovascular system. Antioxidant and other supportive therapies protect red blood cells (RBC) against antioxidant damage. Chelation therapy reduces iron-related complications and thereby improves quality of life and overall survival. The poor oral bioavailability, short plasma half-life and severe side effects of available chelators are still not optimal. In this study, iron chelating activity of some medicinal plants was determined to find alternative sources with lower side effects in thalassemic patients. 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 $AlCl_3$ assays. Phenol content of the extracts varied between 9 - 290 mg/g. The largest amount of phenolic compounds and highest chelating activity were found in *Mellilotus arvensis*. All extracts contained various amount of flavonoids from 10 to 60 mg/g. Extracts with high phytochemicals and chelating activity can be observed as a good source of new agents for thalassemic patients.

Key words: Iran herbs, Iron chelating, thalassemia, phenol, flavonoid.

INTRODUCTION

Patients with chronic anemia 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 reactive oxygen species (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 red blood cells (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). The poor oral bioavailability, short plasma half-life and severe side effects makes available chelators suboptimal (Hebbel et al., 1990; Grinberg et al., 1995, Kukongviriyapan et al., 2008; Filburn et al., 2007, Rachmilewitz et al., 1979;

*Corresponding author. E-mail: pourmoradf@yahoo.com. Tel: +98 151 3543081-3. Fax: +98 151 3543084.

Table 1. The studied plants and their medicinal uses.

Plant	Common name	Part of plant tested	Medical use/disease treated
Myrtaceae *Feijoa sellowiana*	Feijoa, Pineapple Guava, Guavasteen	Fruits peels and leaves	Human food
Caprifoliaceae *Sambucus ebulus*	Danewort, Dwarf Elder,	Fruits	Antinociceptiv; anti inflammatory activity Antiphlogistic;Cholagogue; Diaphoretic; Diuretic; Expectorant; Homeopathy; Poultice; Purgative.
Rosaceae *Crataegus pentagyna*	-	Fruits	Hypotensive; cardiotonic
Juglandaceae *Pterocarya fraxinifolia*	Caucasian wingnut, Pterocarya caucasica	Fruits and stem barks	Diaphoretic
Anacardiaceae *Pistacia lentiscus*	Mastic gum	Gum	Antimicrobial;antioxidant;hepatoprotective; Analgesic; Antitussive; Carminative; Diuretic; Expectorant; Odontalgic; Sedative; Stimulant
Fabaceae *Melilotus arvensis*	Yellow Melilot	Arial parts	Antispasmodic; Aromatic; Carminative; Diuretic; Emollient; Expectorant; Ophthalmic; Vulnerary
Onagraceae Epilobium hirsutum	Great Willowherb, Greater Hairy Willowherb	Leaves	Antimotility;antibacterial; anti- inflammatory; analgesic activity
Graminaceae, Corn silk (Zea mays)	Maize silk, mealie silk and Yu mi shu.	The silk on the cob are used for making the brew	Diuretic; kidney Stones; cystitis; demulcent;anti-inflammatory; tonic;anti diarrhea;anti itching; prostateproblems; blood sugar decreasing; intestinal and liver function regulatory effect
Ebenaceae *Diospyros lotus*	Persimmon	Fruit	Anticeptic, sedative, anti fever, antidiabetic, antitumor
Rosaceae *Pyrus boissieriana*	Pear	Fruit	Antioxidant
Lamiaceae *Salvia glutinosa*	Jupiter's distaff	Arial parts	Antimicrobial

Livrea et al., 1996). Within this context and taking into consideration the relative paucity of iron chelating agents, it is not surprising that clinical scientists are putting 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; Pourmorad et al., 2006; Hosseinimehr 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 civilizations. Even today, plant materials remain an important resource for combating illnesses. Some medicinal plants traditionally used for management of diseases were selected and their phenol and flavonoid content and iron chelating activities were evaluated in this study.

MATERIALS AND METHODS

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. 100 g 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 content

Total phenolic compound contents were determined by the Folin-Ciocalteau method (Ebrahimzadeh et al., 2008 a, b). The extract samples (0.5 ml of different dilutions) were mixed with 2.5 ml of 0.2 N Folin-Ciocalteau reagent (Sigma–Aldrich) for 5 min and 2.0 ml of 75 g/l sodium carbonate were then added. The absorbance of reaction was measured at 760 nm with a double beam Perkin Elmer UV/Visible spectrophotometer (USA) after 2 h of incubation at room temperature. The standard curve was prepared using 50 to 250 mg/ml solutions of gallic acid in methanol-water (1:1, v/v). Total

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

Name of the plant	Total phenol content*	Flavonoid content**	Fe^{2+} chelating activity (IC_{50} mg/ml)
Feijoa sellowiana			
Aqueous fruits	89.07 ± 1.38	18.62 ± 0.75	18.2 % ***
Methanolic fruits	81.09 ± 1.75	43.45 ± 1.75	1.50 ± 0.01
Aqueous leaves	92.09 ± 2.23	59.52 ± 1.03	0.11 ± 0.01
Methanolic leaves	44.04 ± 1.27	55.83 ± 1.29	2.40 ± 0.02
Sambucus ebulus			
Aqueous fruits	41.59 ± 0.28	23.80 ± 0.89	20.8 % ***
Methanolic fruits	27.37 ± 0.93	14.70 ± 0.93	1.5 ± 0.01
Crataegus pentagyna			
Aqueous fruits	92.12 ± 1.72	10.56 ± 0.41	5.16 % ***
Methanolic fruits	85.15 ± 1.65	23.68 ± 1.02	1.83 ± 0.16
Pterocarya fraxinifolia			
Methanolic stem peels	85.93 ± 2.20	24.32 ± 0.98	1.40 ± 0.06
Methanolic leaves	17.78 ± 1.32	11.82 ± 0.27	1.89 ± 0.11
Pistacia lentiscus			
Gum	9.92 ± 0.12	30.52 ± 1.10	0.13 ± 0.01
Mellilotus arvensis			
leaves	289.5 ± 5	57 ± 5.4	0.08 ± 0.01
Epilobium hirsutum			
leaves	92.12 ± 2.12	58.45 ± 1.53	0.49 ± 0.01
Zea mays			
Silk	118.95 ± 2.78	58.22 ± 1.34	1.68 ± 0.14
Diospyros lotus			
Methanolic fruits	10.50 ± 0.02	2.03 ± 0.01	0.61 ± 0.16
Pyrus boissieriana			
Methanolic fruits	16.16 ± 0.02	3.71 ± 0.01	0.78 ± 0.07
Salvia glutinosa			
Methanolic aerial parts	48.82 ± 0.07	45.75 ± 0.12	0.21 ± 0.09
EDTA			0.017 ± 0.00

*mg gallic acid equivalent/g of powder.
**mg quercetin equivalent/g of powder.
***at 3.2 mg/ml.
Data presented as Mean ± SD.

phenol values are expressed in terms of gallic acid equivalent (mg/g of dry mass), which is a common reference phenolic compound.

Flavonoid content of each extract was determined by following colorimetric method (Chang et al., 2002). Briefly, 0.5 mL solution of each plant extracts (at 10% w/v) in methanol were separately mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water, and left at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415 nm with a double beam Perkin Elmer UV/Visible spectrophotometer (USA). 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 Dinis et al. (Dinis et al., 1994). Briefly, 50 µl of 2 mM $FeCl_2$ was added to 1 ml of different concentrations of the extract (0.2, 0.4, 0.8, 1.6 and 3.2 mg/ml). The reaction was initiated by the addition of 0.2 ml of 5 mM ferrozine solution. The mixture was vigorously shaken and left to stand at room temperature for 10 min. The absorbance of the solution was thereafter measured at 562 nm. The percentage inhibition of ferrozine–Fe^{2+} complex formation was calculated as $[(A_0 - A_s)/ A_s] \times 100$, where A_0 was the absorbance of the control, and A_s was the absorbance of the extract/ standard. Na_2EDTA was used as positive control.

Statistical analysis

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

RESULTS AND DISCUSSION

Flavonoid and total phenol contents of the extracts

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). The flavonoid content of extracts calculated as quercetin equivalent. *Epilobium hirsutum,* Corn silk and *Mellilotus arvensis* with 57 - 58.5 mg quercetin equivalent in each g dry powder, contained highest flavonoid content (Table 2). Total phenols measured by Folin Ciocalteu reagent in terms of gallic acid equivalent. *M. arvensis* with 289.5, corn silk with 119 and *E. hirsutum* with 92.1 mg gallic acid equivalent in each g dry powder, contained highest total phenol content (Table 2).

Metal chelating activity

The chelating of Fe^{2+} by extracts was estimated by the method of Dinis et al. (1994). 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 colour of the 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. *M. arvensis*, the most active extract interfered with the formation of ferrous and ferrozine complex, suggesting that it has chelating activity and captures ferrous ion before ferrozine. IC_{50} of the extract for chelating activity was 80 ± 0.01 µg/ml which is lower than the positive standard EDTA (IC_{50} = 17 µg/ml). The IC_{50} of chelating effect of other extracts on Fe^{2+} and ferrozine complex formation is shown in Table 2.

Conclusion

There was a direct relation between chelatory activity and the content of active compounds, phenol and flavonoid in some extracts in this study. Some extracts with high phenol and flavonoid contents showed good chelating of Fe^{2+}. For example, *E. hirsutum and M. arvensis* that contained highest phenol and flavonoid contents showed the best chelating activity. Also, aqueous extract of *F. sellowiana* leaves showed good activity. In spite of some correlation, totally, no correlation was found between phenol and flavonoid content of an extract and its chelating activity ($p > 0.001$). Corn silk with high phenol and flavonoid content showed very weak chelating activity but *P. lentiscus* with low phenol and flavonoid content showed good chelating activity (Table 2).

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

REFERENCES

Aboul-Enein AM, El Baz FK, El-Baroty GS, Youssef AM, Abd El-Baky HH (2003). Antioxidant activity of algal extracts on lipid peroxidation. J. Med. Sci. 3: 87-98.

Chang C, Yang M, Wen H, Chern J (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J. Food Drug Anal. 10: 178-182.

Cook NC, Samman S (1996). Flavonoids- chemistry, metabolism, cardioprotective effects, and dietary sources. Nutr. Biochem. 7: 66-76.

Das NP, Pereira TA (1990). Effects of flavonoids on thermal autooxidation of Palm oil: structure- activity relationship. J. Am. Oil Chem. Soc. 67: 255- 258.

Dinis TCP, Madeira VMC, Almeida MLM (1994). Action of phenolic derivates (acetoaminophen, salycilate and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch. Biochem. Biophys. 315: 161-169.

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

Ebrahimzadeh MA, Mahmoudi M, Salimi E (2006). Antiinflammatory activity of Sambucus ebulus hexane extracts. Fitoterapia, 77: 146-148.

Ebrahimzadeh MA, Pourmorad F, Hafezi S (2008b). Antioxidant Activities of Iranian Corn Silk. Turk. J. Biol. 32: 43-49.

Filburn CR, Kettenacker R, Griffin DW (2007). Bioavailability of a silybin-phosphatidylcholine complex in dogs. J. Vet. Pharmacol. Ther. 30: 132-138.

Grinberg LN, Rachmilewitz EA, Kitrossky N, Chevion M (1995). Hydroxyl radical generation in ß-thalassemia red blood cells. Free Rad. Biol. Med. 18: 611-615.

Hebbel RP, Leung A, Mohandas N (1990). Oxidation-induced changes in microheological properties of the red cell membrane. Blood, 76: 1015-1022.

Hosseinimehr SJ, Pourmorad F, Shahabimajd N, Shahrbandy K, Hosseinzadeh R (2007). *In vitro* antioxidant activity of *Plygonium hyrcanicum, Centaurea depressa, Sambucus ebulus, Mentha spicata* and *Phytolacca americana*, Pak. J Biol. Sci. 10: 637-640.

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

Kukongviriyapan V, Somparn N, Senggunprai L, Prawan A, Kukongviriyapan U, Jetsrisuparb A (2008). Endothelial Dysfunction and Oxidant Status in Pediatric Patients with Hemoglobin E-beta Thalassemia, Pediatr. Cardiol. 29: 130-135.

Livrea MA, Tesoriere L, Pintaudi AM, Calabrese A, Maggio A, Freisleben HJ, D'arpa D, D'anna R, Bongiorno A (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. Hematol. J. 90: 1305-1305.

Pourmorad F, Ebrahimzadeh MA, Mahmoudi M, Yasini S (2007). Anti-

nociceptive activity of methanolic extract of *Epilobium hirsutum*, Pak. J. Biol. Sci. 10: 2764-2767.
Pourmorad F, Hosseinimehr SJ, Shahabimajd N (2006). Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants, Afr. J. Biotechnol. 5: 1142-1145.
Rachmilewitz EA, Shifter A, Kahane I (1979). Vitamin E deficiency in ß-thalassemia major: Changes in hematological and biochemical parameters after a therapeutic trial with α-tocopherol. Am. J. Clin. Nutr. 32: 1850-1858.
Rund D, Rachmilewitz E (2005). β-thalassemia. N. Engl. J. Med. 353: 1135-1146.
Shahidi F, Wanasundara PKJPD (1992). Phenolic antioxidants. Crit. Rev. Food Sci. Nutr. 32: 67-103.
Shinar E, Rachmilewitz EA (1990). Oxidation denaturation of red blood cells in thalassemia. Semin Hematol. 27: 70-91.
Taher A, Isma'eel H, Cappellini MD (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. Plant Med 43: 240-245.