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Full Length Research Paper

Nitric oxide radical scavenging potential of some Elburz medicinal plants

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Some plants scavenge nitric oxide (NO) with high affinity. For this purpose, forty extracts from 26 medicinal plants, growing extensively in Elburz mountains, were evaluated for their NO scavenging activity. Total phenolic and flavonoid contents of these extracts were also measured by Folin Ciocalteu and $AlCl_3$ colorimetric assays, respectively. *Sambucus ebulus* fruit aqueous extract and *Pterocarya fraxinifolia* leaf methanol extract showed better activity than others with IC_{50} equal to 29 and 65 μg ml^{-1}, respectively. NO is also implicated in inflammation and other pathological conditions, therefore very powerful scavenging activity of *S. ebulus* fruit extract may explain its very good anti-inflammatory activity. Phenolic and flavonoids contents of the extracts varied between 10.2 - 200.4 and 2.1 - 90.9 mg g^{-1} of extract, respectively. Good correlations could be found between total phenolic contents and NO scavenging activity through linear regression analysis (R^2 = 0.63). There was also a better correlation between total flavonoids and NO scavenging activity (R^2 = 0.73). High NO scavenging activity in plants can candidate them for testing their anti-inflammatory property.

Key words: Antiinflammatory, flavonoid contents, medicinal plants, nitric oxide scavenging activity, phenolic contents, *Sambucus ebulus*, *Pterocarya fraxinifolia.*

INTRODUCTION

Nitric oxide (NO) has emerged as one of the more intriguing molecules in vertebrate biology in recent years (Reeves et al., 2008). NO is a lipophilic and highly diffusible solute, forms within the cell and its actions are concentration dependent (Kim et al., 2001). Although NO has been implicated in numerous and extremely diverse biological processes; recently, the role of NO in neuro-transmission (Reeves et al., 2008) has been focused on. It has multiple cellular functions such as regulation of cell growth, differentiation and apoptosis and many physio-logical roles including modulation of blood pressure and synaptic plasticity (Lloyd-Jones and Bloch, 1996). Measurement of exhaled nitric oxide, or fractional exhaled nitric oxide, is a new clinical test which immediately assesses airway inflammation in asthma (Sandrini et al., 2009). It is a potent mutagen in bacterial and mammalian cell culture assays (Inano and Onoda, 2003). NO may regulate hepatic metabolism (Barry, 1998). Its cardioprotective roles include regulation of blood pressure and vascular tone, inhibition of platelet aggregation and leukocyte adhesion and prevention of smooth muscle cell prolife-ration (Naseem, 2005). It is a ubiquitous second messenger in diverse physiological responses in the cardiovascular system (Sessa, 2009). A number of disease states including sepsis and hepatic failure are characterized by abnormally high NO production and removing the excess NO could have salutary effects (Shah et al., 2004). One approach to decrease NO concentrations is to reduce NO synthesis. A large number of NO synthesis inhibitors have been generated, most of which are arginine analogs including isothiourea derivatives. Because of the multiple biochemical roles of arginine, these agents can exhibit other effects (Garvey et al., 1994). Another approach to

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reduce NO levels is making use of NO scavengers. For example, the heme moiety of hemoglobin binds NO with great avidity, but heme and free extracellular hemoglobin can be highly toxic in animals (Broderick et al., 2005). Thus, other NO scavengers have been considered including dithiocarbamate derivatives that chelate iron and thus, bind NO, but these two have some adverse effects (Menezes et al., 1999). Quercetin is a NO radical scavenger, but its carcinogenic activity has been reported (Dunnik and Hailey, 1992). So, using the herbal remediation for nitric oxide scavenging can be useful. In this experiment, some medicinal plants traditionally used for management of various diseases were selected and their NO scavenging activities were evaluated. In addition, their total phenolic and flavonoids contents were also assayed and correlation between these factors was determined.

MATERIALS AND METHODS

Chemicals

Sodium nitroprusside, gallic acid, quercetin, sulfanilamide and N-(1-naphthyl) ethylenediamine dihydrochloride were purchased from Merck and Fluka companies. All other chemicals and reagents used were of the highest commercially available purity.

Plant materials and preparation of the extracts

The plants were collected from Golestanak, Gaduk, Veresk and Dashte-naz areas, Mazandaran, Iran and authenticated by Dr. B. Eslami. Botanical names, common names, plant parts used to obtain the extracts and their medical usages are summarized in Table 1. Plants were dried at room temperature in the dark and soaked in methanol (or water) for 3 days at room temperature. The solvent was evaporated under reduced pressure and then lyophilized. The resulting solid masses were preserved in 4℃.

Determination of total phenolic compounds and flavonoid contents

Total phenolic compound contents were determined by the Folin-Ciocalteau method (Ghasemi et al., 2009; Dehpour et al., 2009). The standard curve was prepared using 50 to 250 $mgml^{-1}$ 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 using colorimetric method (Ebrahimzadeh et al., 2009a,b,e). The calibration curve was prepared by preparing quercetin solutions at concentrations of 12.5 to 250 µg ml^{-1} in methanol.

Nitric oxide radical scavenging activity

The procedure is based on the principle that, sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent (Ebrahimzadeh et al., 2009d). Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions. For the experiment, sodium nitroprusside (10 mM), in phosphate-buffered saline, was mixed with different concentrations of each extracts dissolved in water and incubated at room temperature for 150 min. The same reaction mixture, without extract but with an equivalent amount of water, served as control. After the incubation period, 0.5 ml of Griess reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride) was added. The absorbance of the chromophore which was formed in the reaction was measured at 546 nm. Quercetin was used as positive control (Ebrahimzadeh et al., 2009f; Nabavi et al., 2009).

Statistical analysis

Experimental results are expressed as means ± SD. All measurements were replicated three times. The data were analyzed by an analysis of variance ($p < 0.05$) and the means separated by Duncan's multiple range tests (InStat3 program). The IC_{50} values were calculated from linear regression analysis.

RESULTS

Total phenols was measured by Folin Ciocalteu reagent in terms of gallic acid equivalent. Phenol content of the extracts varied between 10.2 - 200.4 mg/g of extract. Metanol bark extract of *Parrotia persica Mey* and methanol aerial parts extract of *Artemisia absinthium L.* showed highest total phenolic contents with 200.4 ± 9.2 and 194 ± 9.7 mg gallic acid equivalent/g extract, respectively (Table 2). The flavonoid contents of extracts were calculated as quercetin equivalent. Flavonoid contents varied between 2.1 - 90.9 mg/g of extract. Methanol extract of aerial parts of *Ferula assa-foetida (Asafetida)* and methanol extract of leaves in flowering stages in *Eryngium caucasicum trautv* with 90.9 ± 6.3 and 60.0 ± 2.8 mg quercetin equivalent/g of extract showed highest flavonoids contents, respectively (Table 2). Scavenging activity of nitric oxide was estimated by Ebrahimzadeh et al. (2009f) and Nabavi et al. (2009). The IC_{50} of scavenging effect of extracts is shown in Table 2. It varied between 0.029 - 4.37 mg/ml. The highest activities were found in *Sambucus ebulus* fruits aqueous extract and *Pterocarya fraxinifolia* leaves methanol extract with IC_{50} equal to 29 and 65 µg/ml, respectively. Quercetin showed IC_{50} = 17.01 ± 0.03 µg/ml. Good correlations were found between phenolic and flavonoids contents and their nitric oxide scavenging activity with R^2 = 0.63 and 0.73, respectively (Figures 1 and 2).

DISCUSSION

NO has been associated with a variety of physiological processes in the human body since it was identified as a novel signal molecule. It transmits signals from vascular endothelial cells to vascular smooth muscle cells and causes vascular dilation. It also plays an important role in vital physiological functions in respiratory, immune, neuromuscular and other systems. In the nervous system,

Table 1. Botanical name, common name, used part, medical uses of plants used in study.

Medical uses	Voucher specimens	Botanical species (family)
Not reported	274, 275, 276	*Alcea hyrcana Grossh (Malvaceae)*
Anthelmintic, antifungal , antimicrobial, choleretic, digestive, diuretic	345	*Artemisia absinthium L. (Compositae)*
Anti-HIV , acetyl cholinesterase inhibitory, Antioxidant	979	*Buxus hyrcana pojark (Buxaceae)*
Cholinergic ,antileukemia	976	*Colchicum speciosum Steven (Colchicaceae)*
Diuretic; kidney Stones; cystitis; demulcent; antiinflammatory; tonic; antidiarrhea; anti itching; prostate problems; hypoglycemic;	280	*Corn silk (Zea mays) (Graminaceae)*
Hypotensive; cardiotonic	550	*Crataegus pentagyna Waldst* and *kit (Rosaceae)*
Antiseptic, sedative, antipyretic, antidiabetic, antitumor	440	*Diospyros lotus L. (Ebenaceae)*
In menstrual disorders, GI disturbances, in diseases related to prostate also antinociceptive and antimicrobial activities.	76	*Epilobium hirsutum (*Onagraceae)
Aphrodisiac, Nervine	971,972, 973	*Eryngium caucasicum trautv (Umbelliferae)*
Antioxidant	360 , 361	*Fiona sellowiana Berg (Rubiaceae)*
Antidiabetic, lipid lowering , antispasmodic , hypotensive	111	*Ferula assa-foetida L. (Umbelliferae)*
Not reported	974	*Froriepia subpinnata Baill (Umbelliferae)*
Anti bacterial	346	*Grammosciadium platycarpum Boiss.* and *Hauskn. (Umbelliferae)*
Stomachic stimulant, antispasmodic, nervine	370, 371	*Hibiscus esculentus L. (Malvaceae)*
Antimicrobial	846	*Hyoscyamus squarrosus griff (solanaceae)*
Antispasmodic , antifungal ,anti-bacterial , antiplatelet	975	*Hyssopus officinalis L. var. angustifolius (Labiatae)*
Sedative, hypotensive , cardiotonic, antioxidant	342	*Leonurus cardiaca subsp. Persicus (Labiatae)*
Not reported	349	*Onosma demawendicum H.Riedl (Boraginaceae)*
Not reported	79	*Ornithogalum sintenisii Freyn (Liliaceae)*
Antibacterial , Antioxidant	270, 271	*Parrotia persica C.A. Mey (Hamamelidaceae)*
Antioxidant , antiviral , antifungal	443	*Phytolacca americana L. (Phytolaccaceae)*
Diaphoretic , antioxidant	272, 273	*Pterocarya Fraxinifolia Spach (Juglandaceae)*
antioxidant	971	*Pyrus boissieriana Buhse (Rosaceae)*
Antimicrobial	140	*Salvia glutinosa L. (Labiatae)*
Antihemorrhoids, anti Helicobacter pylori, anti-inflammatory, analgesic, insect repellent	75,76	*Sambucus ebulus L. (Caprifoliaceae)*
Digestive, Antipyretic	977	*Viola odorata L. (Violaceae)*

NO works as an atypical neural modulator that is involved in neurotransmitter release, neuronal excitability and learning and memory. Besides its role in physiologic processes, it also participates in pathogenic pathways underlying a large group of disorders including muscle diseases, inflammatory bowel disease, sepsis and septic shock, primary headaches, HIV-associated dementia, multiple sclerosis and stroke. Additionally, increasing evidence shows that NO modulates neurotoxin induced cell damage and is involved in neuronal cell death in Parkinson's disease (PD) and other neurodegenerative disorders such as Alzheimer disease (Aliev et al., 2009; Zhang et al., 2006; Nath and Madri, 2006).

Several studies suggest that NO may modulate iron-catalyzed oxidation reactions such as the O_2^- driven Fenton reaction, which produces powerful oxidants such as the hydroxyl radical (·OH) and metalloxo complexes. The mechanisms by which NO may inhibit lipid peroxidation are not entirely clear, however, one possible mechanism relates to the ability of NO to terminate propagation of lipid peroxidation reactions. Lipid peroxidation is initiated by the formation of potent oxidants such as ·OH or ferryl hemoproteins that produce lipid alkyl radicals (L·) from polyunsaturated fatty acids (LH). This radical is then converted to a hydroperoxyl radical (LOO·) via its interaction with O_2. The LOO· can abstract

Table 2. Total phenolic and flavonoid contents and nitric oxide scavenging IC_{50} of the herbs studied in this paper.

Nitric oxide scavenging [c]	Flavonoid contents [b]	Total phenol contents [a]	Parts used	Sample name *(Common name)*
0.457 ± 0.02	28.3 ± 1.132	14.7 ± 0.441	Leaves	*Alcea hyrcana Grossh*
2.619 ± 0.13	24.3 ± 1.21	48 ± 2.4	flowers	
0.972 ± 0.04	24.7 ± 1.23	68.97 ± 3.4	seeds	
1.77 ± 0.08	12.4 ± 0.62	194 ± 9.7	Aerial parts	*Artemisia absinthium L. (Wormwood , absinthium)*
3.80 ± 0.19	48.2 ± 0.1	55.8 ± 0.54	Leaves	*Buxus hyrcana pojark (Box tree)*
0.248 ± 0.012	39 ± 1.95	48.4 ± 2.42	flowers	*Colchicum speciosum Steven (Autumn Crocus, meadow saffron)*
0.552 ± 0.027	58.22 ± 1.34	118.94 ± 2.78	Corn silk	*Zea mays (Maize silk, mealie silk)*
0.369 ± 0.02 0.347 ± 0.01	23.68 ± 1.02 10.56 ± 0.41	85.15 ± 1.65 92.12 ± 1.72	Fruits Fruits*	*Crataegus pentagyna*
3.66 ± 0.28	2.1 ± 0.05	10.2 ± 0.9	Fruits	*Diospyros lotus L. (Persimmon)*
2.34 ± 0.12	58.45 ± 1.53	92.12 ± 2.12	Aerial parts	*Epilobium hirsutum (great willowherb, hairy willowherb)*
1.26 ± 0.06	60.0 ± 2.8	37.6 ± 1.88	Leaves at flowering stage	*Eryngium caucasicum trautv*
0.210 ± 0.01	25.3 ± 1.26	62.3 ± 3.1	Leaves at pre flowering stage	
2.4 ± 0.12	18.25 ± 0.91	63.13 ± 3.15	inflorescence	
1.843 ± 0.09 0.111 ± 0.005	43.45 ± 1.75 18.62 ± 0.75	69.14 ± 0.39 89.07 ± 1.38	Fruit peel Fruit peel*	*Feijoa sellowiana (Feijoa Pineapple Guava, Guavasteen)*
1.225 ± 0.06 0.135 ± 0.006	55.83 ± 1.29 59.52 ± 1.03	44.17 ± 0.28 92.09 ± 2.23	Leaves Leaves*	
0.270 ± 0.02	90.9 ± 6.3	94.8 ± 5.9	Aerial parts	*Ferula assa-foetida (Asafetida)*
0.370 ± 0.01	35.2 ± 1.76	75.7 ± 3.78	Leaves	*Froriepia subpinnata Baill*
1.196 ± 0.05	32.8 ± 1.64	66.9 ± 3.34	Aerial parts	*Grammosciadium platycarpum*
0.97 ± 0.6	23.9 ± 1.19	58.4 ± 2.92	Seed	*Hibiscus esculentus L. (Okra,Gumbo)*
0.63 ± 0.02	49.3 ± 2.46	68.81 ± 3.44	Leaves	
0.74 ± 0.03	16.4 ± 0.82	178.9 ± 8.94	Fruits	*Hyoscyamus squarrosus griff*
2.83 ± 0.14	44.94 ± 2.24	98.95 ± 4.94	Leaves	
0.27 ± 0.01	30.6 ± 1.53	90 ± 4.5	Aerial parts	*Hyssopus officinalis L. var. angustifolius (hyssop)*
0.15 ± 0.007	35.2 ± 1.76	54.3 ± 2.71	Aerial parts	*Leonurus cardiaca subsp. Persicus (Motherwort)*
0.63 ± 0.03	13.7 ± 0.68	47.2 ± 2.36	Aerial parts	*Onosma demawendicum*
0.34 ± 0.01	23.5 ± 1.17	28.9 ± 1.44	Aerial parts	*Ornithogalum sintenisii Freyn*
3.43 ± 0.171	28.7 ± 0.9	139.2 ± 5.6	Leaves	*Parrotia persica Mey (ironwood)*
4.37 ± 0.21	4.7 ± 0.1	200.4 ± 9.2	Bark	
0.89 ± 0.04	7.24 ± 0.36	102.11 ± 5.1	Fruits	*Phytolacca americana L. (Virginian poke, common pokeberry)*
0.065 ± 0.003	24.32 ± 0.98	88.53 ± 2.20	Leaves	*Pterocarya Fraxinifolia (Caucasian wingnut)*
0.985 ± 0.03	11.82 ± 0.27	17.78 ± 1.32	Bark	
0.65 ± 0.05	3.6 ± 0.07	15.8 ± 0.19	Fruits	*Pyrus boissieriana Buhse* (*Gorganian pear)*
1.21 ± 0.06	44.6 ± 1.2	187.8 ± 11.3	Aerial parts	*Salvia glutinosa L. (Jupiter's distaff)*
0.309 ± 0.01	14.5 ± 0.72	56.3 ± 2.81	Flowers	*Sambucus ebulus L. (Danewort, dwarf elder)*
0.603 ± 0.04 0.029 ±0.01	14.70 ± 0.93 23.80 ± 0.89	27.37 ± 0.93 41.59 ± 0.28	Fruits Fruits*	
2.352 ± 0.117	22.8 ± 1.0	35.4 ± 0.9	Leaves	*Viola odorata L. (Sweet violet)*
0.017	-	-	-	quercetin

[a]mg gallic acid equivalent/g extract. [b] mg quercetin equivalent/g extract. [c] IC_{50} in mg ml^{-1}. All samples were extracted using methanol except the samples marked with an asterisk (*) which were extracted using water. Data presented as Mean ± SD.

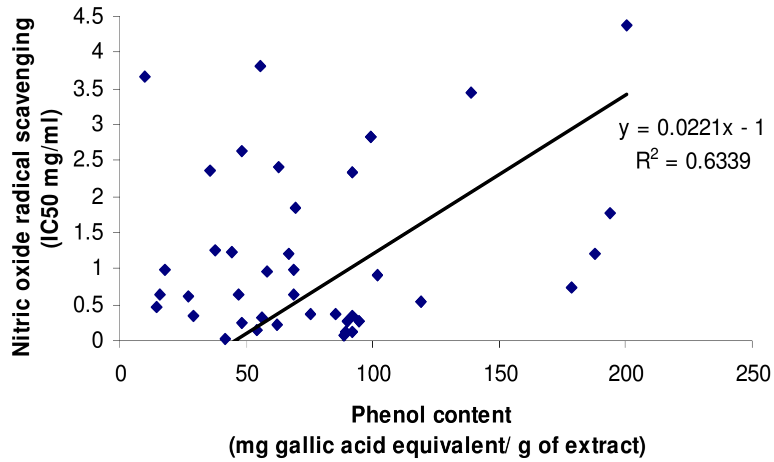

Figure 1. Correlation between phenolic contents and nitric oxide scavenging activity in forty extracts from 26 Elburz medicinal plants.

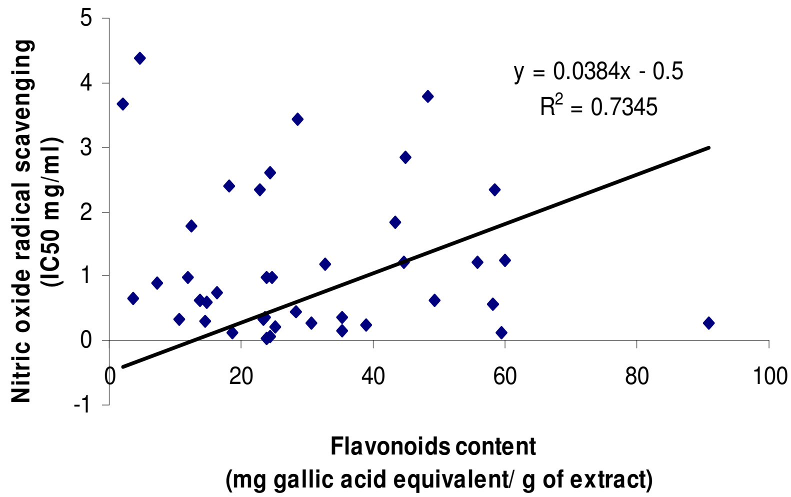

Figure 2. Correlation between flavonoids contents and nitric oxide scavenging activity in forty extracts from 26 Elburz medicinal plants.

an allylic hydrogen atom from another LH, thereby propagating the free radical reaction. NO will rapidly react with these LOO· and/or lipid alkoxyl radicals (LO·), resulting in LOONO or LONO formation, respectively, which in turn leads to chain termination (Rubbo et al., 1994).

Phenolic compounds are a class of antioxidant compounds which act as free radical terminators (Shahidi and Wanasundara, 1992). 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 process (Cook and Samman, 1996). The compounds such as flavonoids, which contain hydroxyl functional groups, are responsible for antioxidant effect in plants (Cook and Samman, 1996). The correlation between total phenolic contents and antioxidant activity has been widely studied in different foodstuffs such as fruit and vegetables (Jayaprakasha and Girennavar, 2008; Kedage et al., 2007; Kiselova et al., 2006; Klimczak et al., 2007).

As reported, antioxidant activity of fruits and vegetables significantly increases with the presence of high concentration of total polyphenolic content. Also, a good correlation between iron chelatory activity and total phenol and flavonoid contents has been reported (Ebrahimzadeh et al., 2008, 2009c). In the present study, the relation between total phenolic and flavonoids contents and radical scavenging activity of 40 extracts from 26 medicinal plants were analyzed. The correlation graphs are depicted in Figures 1 and 2. Good correlations could be found between total phenolic contents and nitric oxide scavenging activity through linear regression analysis (R^2 = 0.63). There was better correlation between total flavonoids and nitric oxide scavenging activity, too (R^2 = 0.73). In addition to reactive oxygen species, nitric oxide is also implicated in inflammation and other pathological conditions (Sreejayan and Rao, 1997; Nabavi et al., 2008). The plant/plant products may have the property to counteract the effect of NO formation and in turn may be of considerable interest in preventing the ill effects of excessive NO generation in the human body. Moreover, the scavenging activity may also help to arrest the chain of reactions initiated by excess generation of NO that are detrimental to human health. Very powerful scavenging activity of *S. ebulus* fruit extract may explain the highest anti-inflammatory activity of *S. ebulus* fruit (Ebrahimzadeh et al., 2006, 2007). Further investigations on the individual compounds and their *in vivo* nitric oxide scavenging activities are needed for selected plants. These plants may also be beneficial in clinical states of excess nitric oxide.

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