

Antioxidant Activity of some Medicinal Species using FRAP Assay

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Abstract

Background: Medicinal plants (especially belong to Lamiaceae family) are potential sources of new drugs to improve the treatment of diseases whose treatment is associated to anti-oxidative agents. In this paper, the Ferric Reducing Ability of Plasma (FRAP) activity of some Lamiaceae and Apiaceae species, has been evaluated.

Methods: Aerial parts of the plants were dried, cut into small pieces and extracted with ethyl acetate and methanol, respectively, by percolation at room temperature. Different concentrations of the plant extracts were investigated for antioxidant power using FRAP assay.

Results: The extracts showed a considerable antioxidant effect from 16.36 mmol of $\text{FeSO}_4/100$ g dry plant equivalents in *Scutellaria tenebrifolia* (AcOEt extract) to 404.12 mmol of $\text{FeSO}_4/100$ g dried plant in *Salvia macrosiphon* (MeOH extract).

Conclusions: All the plant samples possessed antioxidant activity, while *Salvia macrosiphon* (MeOH extract), *Pimpinella tragioides* (MeOH extract) and *Salvia limbata* (AcOEt extract) showed significantly the highest results and *Scutellaria tenebrifolia* (AcOEt extract) showed the lowest power. Antioxidant activity of these species might be due to the presence of flavonoids, rosmarinic acid, coumarins even monoterpenes (like myrcene) in the plant extracts.

Keywords: Antioxidant power, FRAP, Lamiaceae, Apiaceae, *Salvia macrosiphon*, *Pimpinella tragioides*

Introduction

Medicinal plants contain various types of antioxidants, mostly polyphenols and flavonoids which exhibit high antioxidant activity. The FRAP assay (ferric reducing ability of plasma) evaluates total antioxidant power and is chosen to assess the presumable effects of medicinal plants [1]. FRAP assay depends upon the ferric tripyridyltriazine (Fe(III)-TPTZ) complex to the ferrous tripyridyltriazine (Fe(II)-TPTZ) by a reductant at low pH. Fe(II)-TPTZ has an intensive blue color and can be monitored at 593 nm [2]. The literature show that FRAP method is sensitive in the measurement of total antioxidant power of the fresh biological fluids, such as plant homogenates and pharmacological plant products [1, 3].

Antioxidant agents are closely associated to the prevention of degenerative diseases, such as cardiovascular and neurological illnesses, oxidative stress malfunctions and cancer [4].

Literature review show that the methanol extracts of oregano (*Origanum vulgare*), dittany (*Origanum dictamnus*), thyme (*Thymus vulgaris*), marjoram (*Origanum majorana*), spearmint (*Mentha viridis*), lavender (*Levandula vera*) and basil (*Ocymum basilicum*), belong to Lamiaceae family, have been tested in lard (pig fat) stored at 75° C in order to antioxidant evaluation. Oregano (followed by thyme and dittany) extract was found to be the most effective [5]. The antioxidant activity of the methanol extract of three endemic species of *Salvia* from Iran (*S. lachnocalyx*, *S. reuterana* and *S. sahendica*) has been reported using ferric reducing antioxidant power. The results showed that *S. reuterana* extract was the highest antioxidant effective and total phenolic contents among the three species [6]. The essential oil from

fruits and roots of *Ferulago campestris* (Apiaceae) growing in central Italy was analysed and the antioxidant activities were also investigated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging, β -carotene/linoleic acid assay, scavenging of hydrogen peroxide (HRPO test), and hypochlorous acid scavenging (taurine test). The IC₅₀ to Trolox or BHT was less than 2 ml and the oils had an activity equivalent to 1mg Trolox in the DPPH radical-scavenging [7].

Methanolic extracts of eight *Salvia* species, namely *S. aethiopis*, *S. candidissima*, *S. limbata*, *S. microstegia*, *S. nemorosa*, *S. pachystachys*, *S. verticillata*, and *S. virgata*, sampled (from Turkey), were screened for their antioxidant activities by DPPH free radical scavenging and beta-carotene/linoleic acid. A wide variation has been observed among species in terms of antioxidant activity and total phenolic content. The most potent plant was *S. verticillata* (IC₅₀=18.3 μ g/ml) [8]. The antioxidant activity of 60 Iranian plants of Iran was reported by linoleic acid peroxidation. Among them, *S. macrosiphon* (IC₅₀= 2.96 μ g) and *S. hypoleuca* (IC₅₀= 5.27 μ g) showed no antioxidant activity (compared to α -tocopherol, IC₅₀= 0.60 μ g) [9]. The antioxidant activity of four Lamiaceae plants, *S. viridis*, *S. multicaulis*, *S. byzantina* and *E. laciiniata* have been determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH). The results followed descending order as: *S. viridis* > *S. byzantina* > *S. multicaulis* > *E. laciiniata*. The most potent extract on H₂O₂-induced peak chemiluminescence was that of *S. viridis* and on HOCl-induced peak chemiluminescence was that of *S. byzantine* [10].

In this paper, we aim to report the anti-oxidative activity, using FRAP assay, of some Iranian medicinal plants belonging to

Lamiaceae and Apiaceae family which has not been previously reported.

Experimental

Plant material

All the plants were collected from wild growing arias of Iran (Tehran, Mazandaran, Qazvin and semnan provinces) in June and July (2005-2008) (during full flowering stage) and identified by Dr. A. R. Naghinejad and Mr. Y. Ajani. The voucher herbarium specimens were deposited at the herbarium of Medicinal Plants Research Center housed in Faculty of Pharmacy, Tehran University of Medical Sciences.

Extraction

Aerial parts of the plants were dried and reduced to small pieces then extracted with ethyl acetate and methanol, respectively, employing percolation (72 h) at room temperature. The solvents evaporated under reduced pressure to concentrate the extracts and fully dried by freeze dryer.

Reagents for FRAP assay

a) Acetate buffer 300 mM pH 3.6: Weigh 3.1g sodium acetate trihydrate and add 16 ml of glacial acetic acid and make the volume to 1 L with distilled water.

b) TPTZ (2, 4, 6-tripyridyl-s- triazine): (M.W. 312.34), 10 mM in 40 mM HCl (M.W. 36.46).

c) FeCl₃. 6 H₂O: (M.W. 270.30), 20 mM.

The working FRAP reagent was prepared by mixing a, b and c in the ratio of 10:1:1 just before testing. Standard was FeSO₄. 7 H₂O: 0.1 - 1.5 mM in methanol. All the regents were prepared from Merck (Germany) company.

Procedure

FRAP solution (3.6 mL) add to distilled water (0.4 mL) and incubated at 37°C for 5 min. Then this solution mixed with certain concentration of the plant extract (80 mL) and incubated at 37°C for 10 min. The absorbance of the reaction mixture was measured at 593 nm. For construction of the calibration curve, five concentrations of FeSO₄, 7H₂O (0.1, 0.4, 0.8, 1, 1.12, 1.5 mM) were used and the absorbance values were measured as for sample solutions [8].

Results

Linearity of FRAP (dose-response line) for standard solutions is shown in Figure 1. The results of the FRAP assay are reported in Table 1. The antioxidant activities were expressed as the concentrations of antioxidant having a ferric reducing ability equivalent to that of 1 mM of FeSO₄.

Table 1- Antioxidant activity of the ethyl acetate and methanol extracts from aerial parts of 14 species, belonging to Lamiaceae and Apiaceae families, using FRAP assay

Plant samples	Origin	Mean*	SD
<i>Salvia limbata</i> (MeOH)	Semnan	43.88782	1.97171
<i>Salvia limbata</i> (AcOEt)		72.67498	1.750472
<i>Salvia macrosiphon</i> (MeOH)	Tehran	404.1227	27.81543
<i>Salvia macrosiphon</i> (AcOEt)		24.47267	1.198466

Continue Table 1- Antioxidant activity of the ethyl acetate and methanol extracts from aerial parts of 14 species, belonging to Lamiaceae and Apiaceae families, using FRAP assay

Plant samples	Origin	Mean*	SD
<i>Scutellaria tornefortii</i> (MeOH)	Mazandaran	22.86673	0.658854
<i>Scutellaria tornefortii</i> (AcOEt)		16.35666	0.850376
<i>Stachys byzantina</i> (MeOH)	Mazandaran	39.52061	0.850376
<i>Stachys byzantina</i> (AcOEt)		36.93193	0.495108
<i>Salvia hypoleuca</i> (MeOH)	Tehran	65.48418	1.23777
<i>Salvia hypoleuca</i> (AcOEt)		28.33174	1.206429
<i>Lomatopodium staurophyllum</i> (MeOH)	Semnan	27.85235	2.12594
<i>Lomatopodium staurophyllum</i> (AcOEt)		23.53787	1.206429
<i>Pimpinella tragioides</i> (MeOH)	Qazvin	153.0201	4.951081
<i>Pimpinella tragioides</i> (AcOEt)		18.9837	1.750472

* FRAP values are indicated as weight (g) of FeSO₄ in 100 g of the plant extracts

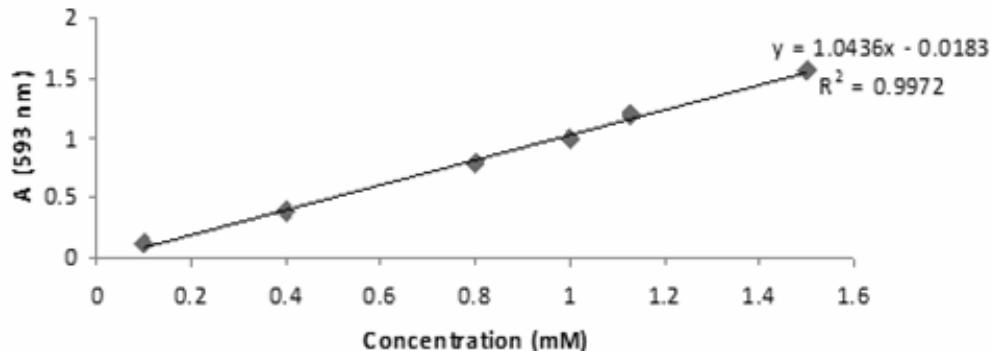


Figure 1- Linearity of FRAP (dose-response line) for standard solutions

Discussion

Most of the extracts showed a considerable antioxidant effect from 16.36 mmol of FeSO₄/100 g dry plant equivalents in *Scutellaria tornefortii* (AcOEt extract) to 404.12 mmol of FeSO₄/100 g dried plant in *Salvia macrosiphon* (MeOH extract). As it was shown (Table 1), all the plant samples possessed antioxidant activity, while *Salvia macrosiphon* (MeOH extract), *Pimpinella tragioides* (MeOH extract) and *Salvia limbata*

(AcOEt extract) showed significantly the highest results and *Scutellaria tornefortii* (AcOEt extract) showed the lowest power. Since, previous investigations on the Iranian medicinal plants showed no considerable activity for *S. limbata* and *S. macrosiphon* [9]. This difference might be due to the evaluating methods which was reported linoleic acid peroxidation. Anyhow, the methanolic extract of Turkish species of *S. limbata* was not active even by DPPH method [8]. It seems that,

composition of the extracts should be different because of the various growing regions, geographical habitat and climate.

In the literature, the antioxidant effects of the methanol extracts of the aerial parts of several *Stachys* species including *S. inflata*, *S. subaphylla*, *S. setifera* and *S. laxa* have been reported [9, 10] but different methods were used for the studies so the results are not comparable with our results. In our previous studies, the phytochemical investigation of the species, *Salvia macrosiphon* and *Salvia limbata* were carried out, resulting the isolation of some flavonoids (ladanein, salvigenin, luteolin 7-methyl ether, cirsiliol, eupatorin, luteolin 7-O-glucoside) and rosmarinic acid as the main components [11, 12].

Rosmarinic acid is a natural phenolic ester of caffeic acid and 3,4-dihydroxyphenyllactic acid, being found to have several biological activities such as anti-inflammatory, anti-mutagenic, and antioxidant, which is mainly due to its redox properties [13]. Most of these *Salvia* species enriched rosmarinic acid content possess strong radical scavenger activity [11, 12, 14].

The antioxidant properties of water and ethanol extracts of *Pimpinella anisum*, (Apiaceae) seed were previously evaluated using various antioxidant tests. Results showed that water and ethanol extracts exhibited 99.1 and 77.5% inhibition of peroxidation in linoleic acid system, which was greater than the same concentration of α -tocopherol. The

water extract exhibited greater antioxidant capacity than that of ethanol [15]. Here we report a high antioxidant activity of *Pimpinella tragoides*, an endemic species in Iran, for the first time. Chemical constituents of this species have not been investigated yet. *Lomatopodium staurophyllum* is another member of Apiaceae samples in this study. The essential oil (extracted by steam distillation) of the aerial parts of *Lomatopodium staurophyllum* was previously analyzed by capillary GC and GC/MS. Among the 15 compounds identified, the major components were (*E*)-beta-ocimene (26.8%), myrcene (26.3%), (*Z*)-beta-ocimene (17.7%), beta-caryophyllene (4.6%) and limonene (4.6%) [16]. There are a few reports about the anti-oxidative activity of the monoterpene myrcene, against t-butyl hydroperoxide (t-BOOH which induced genotoxicity). Results indicated that myrcene had substantial protective effect against oxidant induced genotoxicity, which is predominately mediated by its radical scavenging activity [17]. In conclusion, the Apiaceae plants are enriched of coumarins (such as furocoumarins in *Pimpinella* genus) [18]. The antioxidant activity of the natural coumarins could be estimated from the values of the rate constants of the cleavage of the lactone ring [19].

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