Antioxidant Activity of some Medicinal Species using FRAP Assay

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Abstract

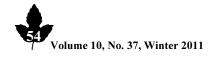
Background: Medicinal plants (especially belong to Lamiaceae family) are potential sources of new drugs to improve the treatment of diseases whose treatment is associated to anti-oxidative agents. In this paper, the Ferric Reducing Ability of Plasma (FRAP) activity of some Lamiaceae and Apiaceae species,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

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 tornefortii (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 mostly polyphenols antioxidants, 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 (Ocvmum 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 investigated by 1,1-diphenyl-2also picrylhydrazyl (DPPH) radical-scavenging, βcarotene/linoleic acid assay, scavenging of hydrogen peroxide (HRPO test), 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, S. aethiopis, S. candidissima, namely 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 ($IC_{50}=18.3 \mu g/ml$) [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 μg) showed no antioxidant activity (compared to α -tocopherol, IC₅₀= 0.60 µg) [9]. The antioxidant activity of four Lamiaceae plants, S. viridis, S. multicaulis, S. byzantina and E. laciniata have been determined using 1,1diphenyl-2-picrylhydrazyl (DPPH). The results followed descending order as: S. viridis > S. byzantina > S. multicaulis > E. laciniata. 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 antioxidative 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

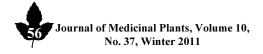
FRAP solution (3.6 mL) add to distilled water (0.4 mL) and incubated at 37°C for 5 min. Then this solution mixed with certain concentration of the plant extract (80 mL) and incubated at 37°C for 10 min. The absorbance of the reaction mixture was measured at 593 nm. For construction of the calibration 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 EDAP assay.

| Plant samples | Origin | Mean* | SD |
|----------------------------|--------|----------|----------|
| Salvia limbata (MeOH) | Semnan | 43.88782 | 1.97171 |
| Salvia limbata (AcOEt) | | 72.67498 | 1.750472 |
| Salvia macrosiphon(MeOH) | Tehran | 404.1227 | 27.81543 |
| Salvia macrosiphon (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 SD Plant samples Origin Mean* Scutellaria tornefortii (MeOH) Mazandaran 22.86673 0.658854 16.35666 0.850376 Scutellaria tornefortii (AcOEt) Stachys byzanthina (MeOH) Mazandaran 39.52061 0.850376 Stachys byzanthina (AcOEt) 36.93193 0.495108 Salvia hypoleuca (MeOH) Tehran 65.48418 1.23777 Salvia hypoleuca (AcOEt) 28.33174 1.206429 Lomatopodium staurophyllum (MeOH) 2.12594 Semnan 27.85235 Lomatopodium staurophyllum (AcOEt) 23.53787 1.206429 Pimpinella tragioides (MeOH) 4.951081 Qazvin 153.0201 Pimpinella tragioides (AcOEt) 18.9837 1.750472

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

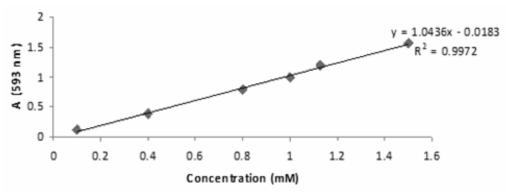


Figure 1- Linearity of FRAP (dose-response line) for standard solutions

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 Salvia macrosiphon and Salvia limbata were carried out, resulting the isolation of some flavonoids (ladanein, salvigenin, luteolin 7-methyl ether, cirsiliol, luteolin 7-O-glucoside) eupatorin, 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, antimutagenic, 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

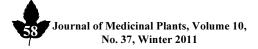
water extract exhibited greater antioxidant capacity than that of ethanol [15]. Here we report a high antioxidant activity of *Pimpinella* tragioides, an endemic species in Iran, for the first time. Chemical constituents of this species have not been investigated yet. Lomatopodium staurophyllum is member of Apiaceae samples in this study. essential oil (extracted by steam distillation) of the aerial parts 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 t-butyl myrcene, against 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].

Acknowledgements

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