Pharmacognosy Magazine
ISSN: 0973-1296

PHCOG MAG.
An official Publication of Phcog.Net

PHCOG MAG.: Research Article
In vitro antioxidant studies of the aerial parts of *Origanum majoram* Linn and *Artemesia sieversiana* Ehrh.

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ABSTRACT - The alcoholic extract of *Origanum majoram* (OM) and *Artemisia sieversiana* (AS) were studied for antioxidant activity on different *in vitro* models namely 1, 1-diphenyl, 2-picryl hydrazyl (DPPH) assay, nitric oxide assay and trichloroacetic acid based reducing power method. Ascorbic acid was also evaluated for comparison. The extracts showed dose dependent free radical scavenging property in the tested models. OM showed 96.07% inhibition of DPPH at 1000 µg and its activity at 500 µg was comparable to that of ascorbic acid at 20 µg. While the maximum percentage inhibition by OM and AS in the nitric oxide model was found to be only 41.14 and 33.63 respectively, the activity of 40 µg of OM and 10 µg of AS compares favorably with that of 20 µg ascorbic acid. AS showed marginal reductive ability. This study demonstrates the anti oxidant activity of the herbs.
KEY WORDS: Antioxidant, *Origanum majoram*, *Artemisia sieversiana*, free radicals, reductive ability.

INTRODUCTION

The role of oxygen derived free radicals in the pathogenesis of a number of degenerative disease is well known (1). Many plants contain substantial amounts of antioxidants including Vitamin C and E, carotenoids, flavonoids, tannins and thus can be utilized to scavenge the excess free radicals from the human body (2).Epidemiological studies have suggested the association between the consumption of antioxidant rich foods and beverages and the prevention of diseases (3).There is a lot of ongoing research on such plants for their potential usefulness as dietary supplements and as adjuvants for use in therapeutic management of stress related disorders.

Origanum majoram Linn. (Synonymn, *Majorana hortensis*, Lamiaceae) is a creeping aromatic, perennial herbaceous shrub, cultivated in several states in India for use in flower garlands, bouquets and wreaths. Native of South Europe it is commonly called 'sweet majoram'. Locally called 'marvam'(Tamil, Hindi: marwah) it is used in traditional medicine as an emmenagogue, galactogogue and carminative(4). The essential oil from the leaves is used in hot fomentations in acute diarrhoea and is also considered an excellent external application for sprains and bruises (5). The plant possesses antibacterial, antitumour, anti inflammatory activity (6) and it reportedly inhibits platelet aggregation (7).The plant essential oil is predominantly constituted of monoterpenes and novel flavonoids have been isolated from its aerial parts (8) Considering the reported anti-inflammatory activity and the flavonoid content, it is proposed to investigate its antioxidant potential.

Artemisia sieversiana Ehrh. (Synonymn: *Artemesia dracunculus*, *A.pallens*, Asteraceae) is a tall perennial aromatic herb with much divided lamina extensively cultivated in mountainous districts of India. Commonly called 'Indian wormwood' or 'davana'(Tamil, Hindi:'dauna') it is prized for its exquisite and delicate aroma with fruity fragrance. Leaf sprigs are commonly used in garlands, bouquets and religious offerings (9) The dried flowering tops are given for inflammations (10) and urinary problems (11) in folk medicine. The plant is reportedly antimutagenic and antiviral (12). It's essential oil is antibacterial and antifungal (13). Several aroma chemicals, flavonoids, acetylenic compounds and coumarin derivatives have been isolated from this plant (14-16). Antioxidant activity of AS is to be investigated to reason its proposed benefits in the modern context.

MATERIALS AND METHODS

All chemicals and solvents used were of analytical grade and obtained from Ranbaxy Fine Chemicals and SD Fine Chem. Ltd., Mumbai, India. Ascorbic acid was obtained from Merck Ltd., Mumbai and 1, 1-diphenyl,

2-picryl hydrazyl (DPPH) was obtained from Sigma chemicals, USA. The other chemicals used were N-(1-naphthyl) ethylene Diamine Dihydrochloride (NED), trichloro acetic acid, (TCA), Sodium nitoprusside, sulphanilamide, o- phosphoric acid, sodium chloride (NaCl), Ferrous sulphate ($FeSO_4$), Ferric Chloride ($FeCl_3$), disodium hydrogen orthophosphate, potassium dihydrogen phosphate and potassium ferricyanide.

Plant Material

The aerial parts of OM and AS were purchased from the local market in August 2005 and authenticated by a botanist by comparision with the preserved samples in the herbarium of our college.

Plant extracts

The plant parts were shade dried, powdered and extracted individually with ethanol by continuous percolation, using Soxhlet apparatus. The extracts were filtered and concentrated *in vacuo* and kept in a vacuum desicator for complete removal of the solvent. Alcohol extract of *Origanum majoram* (AOM) and *Artemesia sieversiana* (AAS) were obtained in the yield of 8.5% and 11.4% w/w respectively.

In vitro antioxidant study

AOM and AAS were tested for free radical scavenging property using DPPH and nitric oxide method. Reductive ability was tested by trichloroacetic acid method. Ascorbic acid was used as the standard antioxidant for comparision. All experiments were performed thrice and the results averaged.

DPPH radical scavenging activity

DPPH scavenging activity was measured by the spectrophotometric method (17). To 3 ml of an ethanolic solution of DPPH (200 µM), 0.05 ml of test extracts/ascorbic acid (20 µg) dissolved in ethanol were added. Test extracts were prepared in different concentrations (4-1000µg). The solutions were incubated at 37^0 for 30 min, absorbance measured at 517 nm using Systronics 118 model spectrophotometer. The percentage inhibition of DPPH radical was calculated by comparing the results of the test with those of the control (not treated with the extract) using the formula (18)

$$\text{Percentage inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance. of test}) \times 100}{\text{Absorbance of control}}$$

Nitric oxide scavenging activity (19) - Nitric oxide radicals were generated from sodium nitroprusside solution at physiological pH. 1 ml of sodium nitroprusside (10mm) was mixed with 1 ml of the test extracts / ascorbic acid (20 µg) in phosphate buffer (pH 7.4). The test extracts were prepared in different concentratiors (10-1000 µg). The mixture was incubated at 25^0 for 150 mins. To 1.5 ml of the incubated solution, 1 ml of Griess' reagent (1% sulphanilamide, 2% o-phosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride) was added. Absorbance was read at 546 nm and percentage inhibition calculated.

***Reductive* ability (20)**

Reducing power of the test extracts was determined based on the ability of antioxidants to form coloured complex with potassium ferricyanide, TCA and $FeCl_3$. 1 ml of the test extracts (100-800 µg) / ascorbic acid (20 µg) in ethanol were mixed with 2.5 ml potassium ferricyanide (1%) and 2.5 ml of phosphate buffer (pH 6.6). The mixture was incubated at 50^0 C for 20 min. 2.5 ml TCA (10%) were added to it and centrifuged at 3000 rpm for 10 min. 2.5 ml of the supernatant was mixed with 2.5 ml water and 0.5 ml $FeCl_3$ (0.1%). Absorbance was measured at 700 nm.

Statistical analysis

Linear regression analysis was used to calculate IC_{50} values wherever needed.

RESULTS

AOM and AAS in graded concentrations were tested for their antioxidant activity in three different *in vitro* models. It was observed that free radicals were scavenged by the test compounds in a concentration dependent manner up to the given concentration in the models studied. The maximum percentage inhibition of DPPH by AOM and AAS was 96.07 and 64.48% respectively at 1 mg concentration (Table 1). Standard drug ascorbic acid showed 93.58% inhibition of the DPPH radical at 20 µg. IC_{50} value of AOM and AAS was respectively 65 µg and 625 µg.

In the nitric oxide model, the maximum percentage of inhibition of nitric oxide radicals of AOM and AAS was 41.14% and 33.63% respectively (Table 2). However, ascorbic acid at 20 µg caused only 7.51% inhibition which is similar to the inhibition of 40 µg of AOM and 10 µg of AAS.

The reducing power of AOM and AAS was also dose dependent and shown in Table 3. The maximum absorbance of AOM at 800 µg, compares favourably with ascorbic acid. On a comparative basis AOM was better at quenching DPPH and nitric oxide radicals than AAS. Also its reductive ability is far greater than AAS.

DISCUSSION

DPPH is relatively stable nitrogen centered free radical that easily accepts an electron or hydrogen radical to become a stable diamagnetic molecule.

Table 1 - DPPH scavenging activity of AOM and AAS

Concentration (µg)	Percentage Inhibition	
	AOM	AAS
1000	96.07 ± 0.052	64.48 ± 0.037
500	93.45 ± 0.084	46.52 ± 0.056
250	89.52 ± 0.073	25.03 ± 0.072
125	84.14 ± 0.068	12.97 ± 0.084
62	47.84 ± 0.044	7.47 ± 0.044
32	15.72 ± 0.011	6.16 ± 0.011
16	3.67 ± 0.015	3.93 ± 0.015
10	3.01 ± 0.032	2.88 ± 0.032
7	0.65 ± 0.084	2.36 ± 0.064
5	0.39 ± 0.038	1.96 ± 0.036

% inhibition of DPPH due to ascorbic acid (20µg) is 93.58

Values are mean of triplicate determinations

Table 2 - Nitric oxide scavenging activity of AOM and AA

Concentration (µg)	Percentage	Inhibition
1000	41.14 ± 0.058	33.63 ± 0.024
800	33.02 ± 0.008	31.16 ± 0.056
600	30.04 ± 0.015	26.79 ± 0.045
400	28.36 ± 0.004	24.33 ± 0.032
200	23.54 ± 0.016	22.20 ± 0.042
100	10.76 ± 0.084	18.61 ± 0.066
40	6.95 ± 0.064	10.43 ± 0.078
20	5.83 ± 0.042	9.75 ± 0.082
10	3.92 ± 0.016	7.51 ± 0.032

% inhibition of NO due to ascorbic acid (20µg) is 7.51.

Values are mean of triplicate determinations

Table 3 - Reductive ability of AOM and AAS

Concentration (µg)	Absorbance at AOM	700 nm. AAS
800	0.588 ± 0.060	0.126 ± 0.032
600	0.414 ± 0.068	0.092 ± 0.054
400	0.412 ± 0.042	0.03 ± 0.044
200	0.0158 ± 0.021	0.025 ± 0.012
100	0.027 ± 0.007	0.003 ± 0.001

Absorbance due to ascorbic acid (20µg) is 0.605.

Values are mean ± SEM of triplicate determinations

DPPH radicals react with suitable reducing agents as a result of which the electrons become paired off forming the corresponding hydrazine. The solution therefore loses colour stoichometrically depending on the number of electrons taken up(21). From the results it may be postulated that both the plant extracts have hydrogen donors thus scavenging the free radical DPPH.

Nitric oxide is an important chemical mediator generated by endothelial cells, macrophages, neurons and involved in the regulation of various physiological processes. Excess concentration of nitric oxide is implicated in the cytotoxic effects observed in various disorders like AIDS, cancer, alzheimer's, and arthritis (22). Oxygen reacts with the excess NO to generate nitrite and peroxy nitrite anions, which act as free radicals. In the present study the nitrite produced by

the incubation of solutions of sodium nitoprusside in standard phosphate buffer at 25^0 c was reduced by AOM and AAS. This may be due to the antioxidant priciples in the extract, which compete with oxygen to react with nitric oxide thereby inhibiting the generation of nitrite. It is to be noted that AOM and AAS have caused a greater inhibition than ascorbic acid which has shown only 7.51% inhibition of NO. It is known that ascorbic acid acts as a pro oxidant *in vitro* in the presence of transition metal ions such as iron (23).This could explain its meagre antioxidant effect in this method. This effect is however unlikely to be important *in vivo* where metal ions are sequestered and other reductants are present.

An increase in absorbance in the reducing power method implies that extracts are capable of donating hydrogen atoms in a dose dependent manner.

It is evident from the results that AOM has a higher antioxidant potential than AAS.

Several flavonoids apart from chlorogenic acid have been reported from OM. These could be the antioxidant principles mediating the anti inflammatory activity reported earlier for this herb. The results thus support the folklore claim of the usefulness of the herb in inflammatory conditions.

The radical scavenging potential of AS is explained by the presence of flavonoids, acetylenic compounds and ascorbic acid in the herb. It also correlates with the anti mutagenic activity reported earlier, as chemicals that scavenge free radicals prevent DNA strand breaks (24).

Demonstration of the antioxidant potential of the herbs, especially in view of the presence of a rich spectrum of bio active molecules of therapeutic significance, makes them likely candidates for bio activity guided fractionation of useful phytomolecules.

REFERENCES

1. Cross C E. Oxygen radicals and human disease, *Annal Int Med.*, **107:** 526(1987).
2. Pratt D.E. Natural antioxidants from plant material, In: *Phenolic compounds in food and their effects on health II. Antioxidants and Cancer Prevention* (ACS symposium series 507) edited by M Hand, CHO and C Lee (American Chemical Society, Washington DC) 54 (1992).
3. Scalbert A, Williamson G. Dietary intake and bioavailability of polyphenols, J Nutr. **130:** 2073S-2085S, (2000).
4. Issar RK. Study of *Ageratum conyzoides* Linn. (Compositae) a weed being used in Unani Pharmacy as "Mirzanjosh" *J Res Indian Med Yoga Homeopathy*, **14:** 147 (1979).
5. Anonymous. *Wealth of India, Raw Materials* (Revised), Vol.VI, CSIR, New Delhi, India, 227(1985).
6. Okuyama T, Matsuda M, Masuda Y, Baba M, Masubuchi, H. Adachi Okada Y, Hashimoto T, Zou LB, Nshiuo H. Studies on cancer biochemoprevention of natural resources: Inhibitory effect of spices on TPA enhanced 3 H choline incorporation in phospholipid of (3HIOT1/2) cells on TPA induced ear edema. *Zhonghua Yaoxue Zazhi*, **47:** 421(1995).
7. Okayaki K, Nakayama S, Kawazoe K, Takaishi Y. Antiaggregant effects on human platelets of culinary herbs, *Phytother Res.*, **12:** 603 (1998).
8. Dolchi M, Tira S. Flavonoids of *Majorana hortensis*, Riv ital essenze profumi, plante office aromi saponi cosmet aer, 62,131 (1982).
9. Kak S N, Kaul BL. in *Supplement to cultivation and utilization of Aromatic Plants*, edited by Handa SS and Kaul M.K, Regional Research Laboratory, Jammu Tawi, India, 273 (1997).
10. Mascolo N, Autore G, Caposso F, Menghini A, Fasula MP. Biological screening of Italian medicinal plants for anti-inflammatory activity *Phytother. Res.*, **1:** 28 (1987).
11. Bocek BR. Ethnobotany of Costanoan Indians based on collections by John P. Harrington, *Econ Bot.*, **38:** 240 (1984).
12. Natake M. Kanayawa K, Miyuno M, Veno N, Kobayashi T, Danno GI, Minamotao S. Herb water extracts markedly suppress the mutagenicity of TRP – P2, *Agri Biol Chem.*, **53:**1423 (1989).
13. Mehrotra S, Rawat AKS, Shome U. Antimicrobial activity of the essential oils of some Indian *Artemesia* species, *Fitoterapia*, **64:** 65 (1993).
14. Thieme H Nguyen Thi TAM. Investigations on the accumulation and the composition of the essential oils of *Satureja hortensis*, *Satureja montana* and *Artemesia dracunculus* in the course of onto genesis: I Literature Review, Thin layer and Gas chromotographic investigations, *Pharmazie*, **27:** 255 (1972) .
15. Jakupovic J, Tan RX, Bohlmann K, Jia ZJ, Huneck S, Acetylenes and other constituents

from *Artemesia dracunculus*, *Planta Med.*, **57:** 450 (1991).

16. Steinegger E, Brantschen A. Coumarins of *Artemesia dracunculus*, *Sci Pharm.*, **27:** 184 (1959).
17. Cotelle A, Bernier JL, Catteau JP, Pommery J, Wallet JC, Gaydou EM. Antioxidant properties of hydroxyl flavones, *Free Rad. Biol Med.*, **20:** 35 (1996).
18. Shirwaikar A, Rajendran K, Dinesh Kumar C. *In vitro* antioxidant studies of *Annona Squamosa* Linn.Leaves, *Ind J Exp Biol.*, **42:** 804 (2004).
19. Sreejayan N, Rao MNA. Nitric Oxide scavenging by curcuminoids, *J Pharm Pharmacol.*, **49:**105 (1997).
20. Jay Prakash GK, Singh RP, Sakariah KK. Antioxidant activity of grape seed extracts on peroxidation models *in vitro, J Agri Food Chem.*, **55:**1018 (2001).
21. Blois MS. Antioxidant activity of grape seed extracts on peroxidation models in vitro, *J Agri Food Chem.*, **55:** 1018 (2001).
22. Sainani GS, Manika JS and Sainani RG. Oxidative stress- a key factor in pathogenesis of chronic diseases, Med Update, **1:**1 (1997).
23. Parthasarathy N, Santhanam N, Ramachandran P, Melhac O. Oxidants and antioxidants in atherogenesis : an appraisal, *J Lipid Res.* **40:** 2143 (1999).
24. Dharmendra KM, Thomas PAD, Cherupalli KKN. Some novel approaches for radio protection and the beneficial effect of natural products, *Ind J Exp Biol.*, **44:** 95 (2006).