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In-vitro evaluation of antioxidant activity of leaves of *Hyptis suaveolens* (L.) Poit

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Research Article

ABSTRACT

Hyptis Suaveolens (L.)Poit is a traditional pubescent annual herb found throughout India. On the basis of its traditional use and literature references, this plant was selected for the screening of antioxidant property. The leaves of *Hyptis Suaveolens* were exhaustively extracted by soxhlet apparatus with different solvents like methanolic, petroleum ether,and chloroform in ascending order of the polarity. All the three extracts were subjected to antioxidant screening by using the different method.. The extracts and the reference standard, butylated hydroxyl toluene (BHT) were evaluated for DPPH, nitric oxide, superoxide and hydroxyl radical scavenging activity. The ethanolic extract exhibited significant antioxidant activity but petroleum ether and chloroform extracts of *Hyptis Suaveolens* did not show any significant antioxidant activity in comparison with standard (BHT).

Key words: *DPPH, Nitric oxide, Butylated hydroxyl toluene, Superoxide and hydroxyl radical scavenging*

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INTRODUCTION

Natural products are a source of synthetic and traditional herbal medicine and are still the primary health care system [1]. The presence of various life sustaining constituents in plants made scientists to investigate these plants for their uses in treating certain infective diseases. Majority of the diseases/disorders are mainly linked to oxidative stress due to free radicals [2]. Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and metabolism [3].

The most common reactive oxygen species (ROS) include superoxide (O_2) anion, hydrogen peroxide (H_2O_2), peroxyl (ROO)

radicals and reactive hydroxyl (OH) radicals. The nitrogen derived free radicals are nitric oxide (NO) and peroxynitrite anion. ROS have been implicated in over a hundreds of diseases states which range from arthritis and connective tissue disorders to carcinogenesis, aging, physical injury, infection and acquired immunodeficiency syndrome [4].

In treatment of these diseases, antioxidant therapy has gained an immense importance. Current research is now directed towards finding naturally occurring antioxidants of plant origin. Antioxidants have been reported to prevent oxidative damage by free radical and ROS, and may prevent the occurrence of disease, cancer and aging. It can interfere with the oxidation process by reacting with free radicals, chelating, catalytic metals and also by acting as oxygen scavengers [5-6]. Plant and plant products are being used as a source of medicine since long. The medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent antioxidant activities, no side effects and economic viability [7].

Flavonoids and phenolic compounds widely distributed in plants which have been reported to exert multiple biological effect, including antioxidant, free radical scavenging abilities, anti- inflammatory, anti-carcinogenic etc. [8]. They were also suggested to be a potential iron chelator [9-10]. Novel natural antioxidants from some plants have been extensively studied in the past few years for their antioxidant and radical scavenging properties. In view of this and the present understanding about ROS-induced multiple diseases, we have selected one of such ayurvedic herb, *Hyptis Suaveolens* (L.)Poit. The plant, *Hyptis suaveolens* (L) Poit commonly known as *Wilayati tulsi* belongs to the family Lamiaceae and is an ethnobotanically important medicinal plant. The plant has been considered as an obnoxious weed, distributed throughout the tropics and subtropics. Almost all parts of this plant are being used in traditional medicine to treat various diseases. The leaves of *H. suaveolens* have been utilized as a stimulant, carminative, sudorific, galactogogue and as a cure for parasitic cutaneous diseases [11].

Crude leaf extract is also used as a relief to colic and stomachache. Leaves and twigs are considered to be antispasmodic and used in anti-rheumatic and anti- soporific baths, anti-inflammatory, antifertility agents [12], and also applied as an antiseptic in burns, wounds, and various skin complaints. The decoction of the roots is highly valued as appetizer and is reported to contain urosolic acid, a natural HIV- integrate inhibitor [13]. Fumes of the dried leaves are also used to repel mosquitoes and control insect pests of stored grains. The objective of this investigation was to ascertain the scientific basis for the use of this plant in the treatment of antioxidant, using different antioxidant models.

MATERIALS AND METHODS

Fresh leaves of *Hyptis suaveolens* (L) Poit. (Lamiaceae) were collected in flowering stage during late September from the natural population of Jhansi (U.P.) and authenticated by Dr. H.B. Singh, Head Raw material and museum, NISCAIR, New Delhi., shade dried and powdered then passed from 40# mesh size.

Preparation of various extracts of *Hyptis suaveolens* (L.) Poit

Around 1 kg fresh shade dried leaves were powdered and extracted by hot percolation method by soxhlet apparatus with five liters of each petroleum ether, methanol, chloroform. The percolation process was continued until the extraction process was completed (indicated by fade coloured menstrum). All the extracts finally reduced to dryness at 40^{o} C by Rotovapour. The traces of the solvents were removed by keeping the dried extracts in to a desiccator. The concentrated extracts were stored carefully for phytochemical investigation.

DPPH Radical Scavenging Activity

The free radical scavenging activity was measured in terms of hydrogen donating or radical scavenging ability, using the stable radical, DPPH [14]. A 0.1 mM solution of DPPH in methanol was prepared and 1 ml of this solution was added to 3 ml of control i.e. standard butylated hydroxyl toluene (BHT) at different concentration (25-100 µg/ml) and test solutions at different concentrations (5-100µg/ml) in different test tubes. Thirty minutes later, the absorbance were measured at 517 nm.

Nitric Oxide Scavenging Activity

Nitric oxide scavenging activity was measured by the spectrophotometric method [15]. Sodium nitroprusside (5 mM) in phosphate-buffer saline was mixed with a control without the test compound, but with an equivalent amount of methanol. Test solutions at different concentrations (5-100 µg/ml) were dissolved in methanol and incubated at 25°C for 30 min. After 30 min, to 1.5 ml of the incubated solution was diluted with 1.5 ml of Griess reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% naphthyl ethylenediamine dichloride). The absorbance of the chromophore formed during the diazotization of the nitrile with sulphanilamide and the subsequent coupling with naphthyethylene diamine dihydrochloride was measured at 546 nm.

Superoxide Scavenging

Superoxide scavenging was carried out using the alkaline dimethyl sulfoxide (DMSO) method [15]. Solid potassium superoxide was allowed to stand in contact with dry DMSO for at least 24 hrs and the solution was filtered immediately before use; the filtrate (200 µl) was added to 2.8 ml of an aquous solution containing nitroblue tetrazolium (56 µM), EDTA (10 µM) and potassium phosphate buffer (10 µM, pH 7.4). Test solutions at different concentrations (5-100 µg/ml) were added and absorbances were recorded at 560 nm against the control.

Hydroxyl Radical Scavenging Activity

The scavenging capacity for hydroxyl radical was determined according to the modified method [16]. The assay was performed by adding 0.1 ml of EDTA, 0.01 ml of ferric chloride, 0.1 ml of hydrogen peroxide, 0.36 ml of deoxyribose, 1.0 ml of test solutions (5-100 µg/ml) in distilled water, 0.33 ml of phosphate buffer (50 mM, pH 7.4) and 0.1 ml of ascorbic acid were dissolved in sequence. The mixture was then incubated at 37°C for 1 hr and 1.0 ml portion of the

incubated mixture was mixed with 10% TCA and 1.0 ml of 0.5% TBA to develop the pink chromogen and measured at 532 nm.

Statistical Analysis

The results are presented as mean ± SEM. All parameters were analysed using Student's *t*-test. P<0.05 was considered as significant.

RESULTS

Inhibition of DPPH Radical

The potential decrease in the concentration of DPPH radial due to scavenging property of MEIF and BHT showed significant free radical scavenging activity viz. 86.44 and 91.86%, respectively at 100 µg/ml. The IC of MEHS was found to be 24.56 µg/ml whereas PEEHS and CEIF did not show any significant activity (Table 1).

Nitric Oxide Scavenging Activity

The scavenging of nitric oxide by MEHS and BHT was concentration dependent. There was a moderate inhibition of nitric oxide formation with the maximum inhibition being 74.12 and 82.14% respectively at 100µg/ml MEHS and BHT. The IC of MEHS was found to be 24.45 µg/ml. similar results were not found in case of PEEHS and CEHS (Table 1).

Superoxide Radical Scavenging

The MEHS and BHT showed a moderate inhibition of the superoxide radical 75.50 and 81.87% respectively at 100 µg/ml. There was no significant inhibition of superoxide radical by PEEHS and CEHS (Table 2).

Hydroxyl Radical Activity

The effect of MEHS and BHT on hydroxyl radical and iron (II)- dependent deoxyribose damage was protected significantly at all concentrations; the percentage of inhibition of hydroxyl radical being and 73.56% respectively at 100 µg/ml. No significant inhibition of superoxide radical by PEEHS and CEHS (Table 2).

DISCUSSION

Oxidative stress, in which large quantities of reactive oxygen species (ROS) like hydrogen peroxide, superoxide, hydrogen radical, singlet oxygen and nitrogen species are generated, one of the earliest responses to stress. These ROS have a role in disease and aging in animals [17]. The antioxidative system protects the organism against ROS-induced oxidative damage. There are restrictions on the use of synthetic antioxidants such as BHT, as they are suspected to be carcinogenic [18].

Natural antioxidants therefore have gained importance. DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to form a stable diamagnetic molecule. The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm, which is induced by antioxidants. The significant decrease in the concentration of DPPH radical is due to the scavenging ability of MEHS. Nitric oxide was generated from sodium nitroprusside and measured by the Greiss reduction. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to

produce nitrate ions that can be estimated by use of Greiss reagent. Scavengers of nitric oxide complete with the oxygen, leading to reduced production of nitric oxide. [19]

The MEHS was shown significant scavenging activity. The potentially reactive hydroxyl radicals can cause oxidative damage to DNA, lipids and proteins, the effect of MEHS and BHT on the inhibition of free radicalmediated deoxyribose damage was assessed by means of iron (II)- dependent DNA damage assay, which showed significant results. [20]

The MEHS has potent antioxidant and free radical scavenging effects in different *in-vitro* systems, but PEEHS and CEHS showed no significant effects as compared to standard BHT. Further work is necessary to elucidate the mechanism involved in the antioxidant activity MEHS.

REFERENCES

1. Blanks T S, Brown B, Cosgrave J, Woody V, Bentley, Sullivan N. "The Body Shop Book of Wellbeing Mind, Body and Soul", Ebury Press London, 1998; 173-192.

2. Gutteridgde, J.M.C., Free radicals in disease processes: A complication of cause and consequence. Free Radical Research Communication, 1995, 19, 141-158.

3. Tiwari, A.,Imbalance in antioxidant defence and human diseases: Multiple approach of natural antioxidants therapy. Current Science 2001,81, 1179-1187.

4. Joyce, D.A., Oxygen radicals in disease. Adverse Drug Reaction Bulletin, 1987, 127, 476-79.

5. Buyukokuroglu, M.E., I. Gulcin, M. Oktay and O.I. Kufrevioglu, *In vitro* antioxidant properties of dantrolene sodium. Pharmacological Research, 2001, 44, 491-495.

6. Shahidi, F. and P.D. Wanasundara. Phenolic antioxidants. Critical Reviews in Food Science and Nutrition 1992,32, 67-103.

7. Auudy, B., F. Ferreira, L. Blasina, F. Lafon, F. Arredondo, R. Dajas and P.C. Tripathi, Screening of antioxidant activity of three Indian medicinal plants, traditionally used for the management of neurodegenerative diseases. Journal of Ethnopharmacology., 2003,84, 131-138

8. Miller, A.L., Antioxidant flavonoids: structure, function and clinical usage. Alternative Medicine Review 1996, 1, 103-111.

9. Boyer, R.F., H.M. Clark and A.P. Laroche, Reduction and release of ferritin iron by plant phenolics. Journal of Inorganic Biochemistry 1988, 32, 171-181.

10. Havsteen, B., Flavonoids, A class of natural products of high pharmacological potency. Biochemical Pharmacology 1983,30, 1141-1148.

11. The Wealth of India (Raw Materials), CSIR, New Delhi, Vol. V, 1964, 159.

12. Kirtikar K.R. and Basu B.D. "Indian medicinal plants", Singh B & Singh, M.P. Publishers, India, Vol. 3, 1991: 2032.

13. Chatterjee A. and Pakrashi S.C. "The Treatise on Indian Medicinal Plants", PID, New Delhi, Vol. 5, 1997:15.

14. Sreejayan, N. and M.N. Rao, 1996. Free radical scavenging activity of curcuiminoids. Drug Research 1996,46, 169-171.

15. Madan, M.P., G. Raghavan, A.K. Singh and Palpu P., Free radical scavenging potential of *Saussarea costus*. Acta Pharmaceutica 2005, 55, 297-304.

16. Rajeshwar, Y., Senthil G.P., Malay A.G. and Mazumder U.K., Studies on *in vitro* antioxidant activities of methanol extract of Mucuna pruriens (Fabaceae) seeds. European Bulletin of Drug

Research 2005, 13, 131-138.

17. Melov, S., Animal models of oxidative stress, aging and therapeutic antioxidant interventions. International Journal of Biochemistry and Cell Biology 2002, 34, 1395-1400.

18. Singh, R.P., K.N. Chidamara Murthy and G.K. Jayaprakasha, Studies on the antioxidant activity of Pomegranate (*Punica granatum*) peel and seed extracts using *in-vitro* models. Journal of Agriculture and Food Chemistry 2002, 50, 81-86.

19. Govindarajan, R., S. Rastogi, M. Vijayakumar, A. Shirwaikar, A.K.S. Rawat and S. Mehrotra, Studies on antioxidant activities of *Desmodium gangeticum*. Biopharm Bulletin 2003, 26, 1424-1427.

20. Jornot, L., Petersen H. and Junod A.F., Hydrogen peroxide induced DNA damage is independent of nuclear calcium but dependent on redox active ions. Biochemical Journal 1998, 335, 854-855.

Table 1: Free radical scavenging activity of various extracts of *Hyptis suaveolens* (L.) Poit

Drug	Concentration (µg/ml)	DPPH radical inhibition (%)	Nitric oxide
Methanolic extract of *Hyptis Suaveolens* (MEHS)	5	11.24±0.517	10.59±0.112
	10	22.36±0.453	22.72±0.451
	25	51.44±0.004*	51.14±0.009*
	50	72.64±0.005**	72.92±0.001**
	100	86.44±0.002***	74.12±0.001***
Chloroform extract of *Hyptis Suaveolens* (CEHS)	5	05.14±0.101	02.54±0.103
	10	11.41±0.121	05.68±0.138
	25	21.26±0.006	13.99±0.004
	50	23.26±0.009	25.29±0.007
	100	33.59±0.004	43.81±0.021
Petroleum ether extract of *Hyptis Suaveolens* (PEEHS)	5	03.65±0.121	04.99±0.244
	10	05.24±0.124	12.74±0.411
	25	10.09±0.014	32.10±0.011
	50	15.45±0.007	38.14±0.008
	100	48.65±0.005	48.25±0.005
Butylated hydroxyl toluene (BHT)	25	83.12±0.141	76.69±0.054
	50	86.94±0.125	80.12±1.215
	100	91.86±0.156	82.14±0.512

Values are mean± SEM, 6 independent analysis, P<0.05*, P<0.01**, P<0.001*** as compared to standard (Student's *t*-test)

Table 2: Free radical scavenging activity of various extracts of *Hyptis suaveolens* (L.)Poit

Drug	Concentration (µg/ml)	Superoxide inhibition (%)	Hydroxyl radical inhibition (%)
Methanolic extract of *Hyptis Suaveolens* (MEHS)	5	11.87±0.016	11.04±0.135
	10	26.69±0.592	24.58±0.876
	25	68.46±0.158*	54.63±0.745*
	50	70.43±0.364**	61.93±0.984**
	100	75.50±0.654***	68.84±0.647***
Chloroform extract of *Hyptis Suaveolens* (CEHS)	5	04.12±0.846	04.11±0.526
	10	09.55±0.532	06.76±0.548
	25	22.12±0.641	18.96±0.643
	50	23.46±0.684	29.35±0.351
	100	36.46±0.214	44.56±0.386
Petroleum ether extract of *Hyptis Suaveolens* (PEEHS)	5	02.45±0.184	06.82±0.125
	10	04.46±0.514	15.34±0.279
	25	13.43±0.128	35.33±0.357
	50	15.94±0.052	37.99±0.549
	100	48.56±0.119	48.87±0.217
Butylated hydroxyl toluene (BHT)	25	75.48±0.784	68.65±0.386
	50	76.02±0.887	71.88±0.423
	100	81.87±1.246	73.56±0.368

Values are mean± SEM, 6 independent analysis, P<0.05*, P<0.01**, P<0.001*** as compared to standard (Student's *t*-test)