

Original Research Article

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A Study on Antioxidant Activity of *Hyptis suaveolens* (L) poit

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ABSTRACT

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Hyptis suaveolens (L) *poit* commonly known as Vilayati tulsi, belongs to the family Lamiaceae. Production of reactive oxygen species (ROS) causes various diseases and cellular anomalies in human beings. Antioxidants inhibit generation of reactive species, or scavenge them, or raise the levels of endogenous antioxidant defenses. In this study the antioxidant activity of various extract of *Hyptis suaveolens* (L) *poit* leaves were evaluated using Total Antioxidant Activity Method, Nitric oxide radical scavenging activity, DPPH Scavenging method, H₂O₂ scavenging activity, Reducing power assay. The result obtained in the present study indicated that the ethanolic extract of *Hyptis suaveolens* (L) *poit* could be a potential source of natural antioxidant.

Introduction

The role of free radical reactions in disease pathology is well established and is known to be involved in many acute and chronic disorders in human beings, such as diabetes, atherosclerosis, aging, immuno suppression and neuro degeneration. An imbalance between ROS and the inherent antioxidant capacity of the body, directed the use of dietary and /or medicinal supplements particularly during the disease attack. The ingestion of natural antioxidants has been inversely associated with morbidity and mortality from degenerative disorders (Halliwell *et al.*, 1981). Antioxidant agents of natural origin have attracted special interest because of their free radical scavenging

abilities (Osawa *et al.*, 1990). The use of medicinal plants with high level of antioxidant constituents has been proposed as an effective therapeutic approach for free radical mediated diseases (Govind *et al.*, 2011).

Hyptis suaveolens is a very common plant in India. Indians used to call it "Chan/Wilaiti tulsi" and the morning soup made by mixing it with corn is called "Bate" meaning memory aid. Tea made from the roots of *H. suaveolens* is used to purify the blood, and it is also used as a remedy for the "diseases" of women (Fig. 1). It has been used as a medicinal tea in many places in Asia (Palmer, 1891) and as a food and source of essential oil in South America (Gentry *et al.*, 1990). The plant *Hyptis suaveolens* (L.) Poit; (Lamiaceae) is reported

to possess antifertility (Garg, 1976), Antimicrobial (Okonogi *et al.*, 2005), Anthelmintic (Oliver, 1960), Immunomodulatory activity (Bhagwat, 2003) and antiplasmodial properties (Ziegler *et al.*, 2002; Chukwujekwu *et al.*, 2005). *Hyptis suaveolens* is an important source of essential oils, alkaloids, flavonoids, phenols, saponins, terpenes, and sterol, tannins. The present study was carried out to evaluate the Antioxidant activity of Aqueous, Ethanol, Methanol, Diethyl ether, Chloroform extract of *Hyptis suaveolens* (L.) *poit* using various methods

Materials and Methods

Collection of plant materials

Fresh leaves of *Hyptis suaveolens* (L.) *Poit* were collected from Kannukudi, Thanjavur (DT), Tamilnadu, India. They were identified by Dr. S. John Britto, The Director, The Rapinet herbarium St. Joseph's college, Thiruchirapalli and voucher specimens were deposited in the Rapinet herbarium of St. Joseph's College, Thiruchirapalli (Voucher number K G 001/ 2013).

Extraction of plant material

The leaves of plants were dried under shade. The dried materials were mechanically powdered sieved using 80 meshes and stored in an air tight container. The powdered material was used for further photochemical, and antimicrobial activity. Aqueous, Methanol, Diethyl ether, Chloroform and ethanol extracts were prepared according to the methodology of Indian pharmacopoeia. The coarse powder material was subjected to Soxhlet extraction separately and successively with ethanol and distilled water, methanol, Diethyl ether, chloroform, these extracts were concentrated to dryness in flash evaporator under reduced pressure and controlled temperature (40°C to 50°C) the aqueous and

ethanol extracts put in air tight container stored in a refrigerator

Antioxidant activity

DPPH radical scavenging activity

For DPPH radical scavenging activity, Stock solutions of extracts was prepared to the concentration of 1mg/ml. 1 ml of each extract were added at an equal volume to methanol solution of DPPH (0.1mM). All the reaction mixture is incubated for 30min at room temperature. The absorbance was recorded at 517 nm. Ascorbic acid was used as standard (Blois, 1958).

Total antioxidant activity

Total antioxidant activity of plant extract was determined by dissolving 1ml of the all extracts of plant in water and mixed with 3ml of reagent solution (0.6M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate) in Eppendorf tube. The tubes were capped and incubated in a thermal block at 95°C for 90min. After 90min, the mixtures were cooled at room temperature; the absorbance was measured at 695nm against reagent blank. Ascorbic acid was used as the standard and the total antioxidant capacity is expressed as equivalents of ascorbic acid (Kumaran and Karunakaran, 2007).

Nitric oxide radical scavenging activity

Nitric oxide radical scavenging activity of plant drug was measured by mixing Sodium nitro prusside (1ml of 10Mm) with 1ml of plant drug of different extracts in phosphate buffer (pH 7.4). The mixtures were incubated at 25°C for 150min. To 1ml of the incubated solution, 1ml of Griess' reagent (1% sulphnilamide, 2% o-phosphoric acid and 0.1% naphthyl ethylene diamine hydrochloride) was added. Ascorbic acid was

used as the standard. Absorbance was read at 546nm and percentage inhibition was calculated (Streejayan *et al.*, 1997).

Hydrogen peroxide scavenging activity

In Hydrogen peroxide radical scavenging activity a solution of H₂O₂ was prepared in phosphate buffer (pH 7.4). H₂O₂ concentration was determined spectrophotometrically by measuring absorption with extinct coefficient for H₂O₂. Various extract of plant drug in distilled water was added in H₂O₂ solution (0.6ml, 40μM/). After 10min, absorbance of H₂O₂ was measured at 230 nm against blank solution containing the phosphate buffer without H₂O₂. The percentage of scavenging effect of plant and standard ascorbic acid were calculated (Ruch *et al.*, 1989).

Reducing power assay

Reducing ability of plant drug was measured by the method of Yildirim *et al.*, (2001). To 1.0ml extract) added 2.5ml of phosphate buffer (0.2m, pH6.6) and 2.5ml of 1% potassium ferricyanide and incubated at 50°C for 30 min. After that 2.5ml of trichloro acetic acid (10%) were added to the mixture and centrifuged for 10min at 3000xg. 2.5ml from the upper part were diluted with 2.5ml water and shaken with 0.5ml fresh 0.1% ferric chloride. Ascorbic acid was used as the standard. The absorbance was measured at 700nm using UV- spectrophotometer.

Results and Discussion

The study investigated that the antioxidant activity of aqueous, ethanol diethyl ether and ethanol extract of *Hyptis suaveolens* (*L*) *poit*. The aqueous, ethanolic, methanolic and diethyl ether extract of *Hyptis suaveolens* were tested for antioxidant activity ion different in vitro models. Experiments were carried out in triplet form to calculate mean and standard

deviation. The percentage of inhibition in various models viz. DPPH radical scavenging activity (%), Total antioxidant capacity, Nitric oxide radical scavenging activity (%), Hydrogen peroxide scavenging activity (%) Reducing power assay (%) was represented as mean± standard error in table 1.

DPPH radical scavenging activity

DPPH radical scavenging activity of aqueous, ethanol, methanol and diethyl ether extract of *Hyptis suaveolens* represented in table 1. The diethyl ether extract showed 55.2±0.56%, Chloroform extract showed 31.5±0.5%, whereas the methanolic extract showed 50.5±0.5% and aqueous extract having least total antioxidant potential of 30.3±0.5%. The ethanolic extract having greater antioxidant capacity (60.4±0.4) which was nearer to the value showed by vitamin C (65.5±0.5). DPPH assay is a good method for evaluating the antioxidant efficacy. This assay provided useful information on the reactivity of the test compounds with stable free radicals. The DPPH relatively stable free radicals and the test determine the capacity of the test material to reduce the DPPH radicals to the corresponding hydrazine. The inhibition of DPPH radical showed that *Hyptis suaveolens* cause reduction of DPPH radical in spectrometric manner.

DPPH is a stable free radical accepts an electron or hydrogen radical to become stable diamagnetic molecules. The reduction capability of the DPPH radical is determined by the decrease in its absorbance at 517 nm induced by antioxidants. The decrease in absorbance DPPH radical caused by antioxidant because of the reaction between antioxidant molecules and radical donation. Hence DPPH is usually used as a substrate to evaluate the antioxidant activity of antioxidant.

Fig.1 Pictorial representation of *Hyptis suaveolens* (L.) Poit



Table.1 Antioxidant activity of various extracts of leaf of *Hyptis suaveolens* Linn (poit)

S. No	Particulars	Plant Extracts					
		Aqueous	Ethanol	Methanol	Diethyl ether	chloroform	Vit C
1	DPPH radical scavenging activity (%)	30.3±0.5	60.4±0.4	50.5±0.5	55.2±0.56	31.5±0.5	65.5±0.5
2	Antioxidant activity (%)	33.5±0.4	65.4±0.5	55.2±0.5	40.2±0.2	35.5±0.4	70.5±0.5
3	Nitric oxide radical scavenging activity (%)	32.2±0.3	55.4±0.3	40.2±0.6	38.5±0.4	31.0±0.5	60.5±0.4
4	Hydrogen peroxide scavenging activity (%)	36.4±0.4	50.4±0.5	42.5±0.5	35.4±0.5	30.5±0.2	60.0±0.5
5	Reducing power assay (%)	28.5±0.2	60.5±0.5	45.3±0.4	40.5±0.5	25.8±0.5	65.0±0.5

Total antioxidant activity

The total antioxidant activity of various extract *Hyptis suaveolens* (L) poit. Was depicted in table 1. Scavenging activity of aqueous extract was found to be $33.5\pm 0.4\%$ whereas ethanolic extract showed the maximum scavenging activity such as $65.4\pm 0.5\%$. Scavenging activity of methanolic and diethyl ether extract were found as $55.2\pm 0.5\%$, $40.2\pm 0.2\%$ respectively. Chloroform extract showed $35.5\pm 0.4\%$ among five extract, ethanolic extract showed greater potentiality which was nearer to the value showed by vitamin C ($70.5\pm 0.5\%$).

The total antioxidant capacity of the extract was calculated based on the formation of phosphomolybdenum complex which was measured at spectrometrically at 695nm. The total antioxidant capacity of the extract of *Hyptis suaveolens*, was greater when compared to aqueous, Methanol, Diethyl ether, extracts. The total antioxidant capacity may be due to antioxidant principle in the extract.

Nitric oxide radical scavenging activity

Nitric oxide radical scavenging activity of, aqueous, ethanolic, methanolic, Diethyl ether, Chloroform extract of *Hyptis suaveolens* (L) poit represented in table 1. Nitric oxide radical scavenging activity of aqueous and ethanolic extract of were found to be $32.2\pm 0.3\%$, $55.4\pm 0.3\%$ respectively. Methanolic, diethyl ether, Chloroform extracts showed $40.2\pm 0.6\%$, $38.5\pm 0.4\%$, $31.0\pm 0.5\%$ respectively. In case of vitamin C it was found to be $60.5\pm 0.4\%$. Nitric Oxide (NO) is an important chemical mediator generated by endothelial cells, macrophages, neurons etc and involved in the regulation of various physiological processes (Lata *et al.*, 2003). Excess concentration of NO is associated with several diseases (Alenti, 1993

and Ross *et al.*, 1990). Oxygen reacts with the excess nitric oxide to generate nitrite and peroxy nitrate anions, which act as free radicals (Cotran *et al.*, 1999 and Sainani *et al.*, 1997). In the present study, the extract competes with oxygen to react with nitric oxide and thus inhibit the generation of the anions.

Hydrogen peroxide scavenging activity (%)

Table 1 revealed the Hydrogen peroxide scavenging activity of various extract of *Hyptis suaveolens* (L) poit, the aqueous extract showed $36.4\pm 0.4\%$ Free radical scavenging activity in ethanolic extract was found to be $50.4\pm 0.5\%$ and in methanolic extract it was found to be 42.5 ± 0.5 . Diethyl ether and Chloroform showed the potentiality of $35.4\pm 0.5\%$, $30.5\pm 0.2\%$ respectively. From this above, it was noted that ethanolic extract showed maximum capacity comparable with Ascorbic acid ($60.0\pm 0.5\%$).

Reducing power assay

Reducing power assay of aqueous, ethanolic, methanolic, Diethyl ether and Chloroform extracts showed $28.5\pm 0.2\%$, $60.5\pm 0.5\%$, $45.3\pm 0.4\%$, $40.5\pm 0.5\%$, $25.8\pm 0.5\%$ respectively. Among five extract, ethanolic extract showed greater potentiality which was nearer to the value showed by vitamin C ($60.5\pm 0.5\%$). The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The reducing ability is generally associated with the presence of reductones, which breaks the free radical chain by donating a hydrogen atom (Subhashini, 2011).

From the above, it was found to that ethanolic extract showed the highest antioxidant capacity and is a valuable source of antioxidant both for preparation of crude extracts and for further isolation and

purification of antioxidant components. *Hyptis suaveolens* (L) *poit* found to suppress hydroxyl radical formation, through different chemical mechanisms, including free radicals quenching, electron transfer, radical addition or radical recombination in-vitro. There is good scope in examining the herb for its antioxidant and free radicals scavenging activity in vivo.

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