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Antioxidant Activity of *Hyptis suaveolens* Poit.

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Abstract: The antioxidant activity of the methanol extract of leaves of *Hyptis suaveolens* Poit. was evaluated *in vitro* by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity using gallic acid and butylated hydroxyanisole (BHA) as reference standards. They exhibited strong antioxidant radical scavenging activity with IC₅₀ value of 0.4, 1.15 and 14.04 µg mL⁻¹ for Gallic acid, BHA and *Hyptis suaveolens*, respectively. The antioxidant activity of methanol extract could be due to the presence of Flavonoids.

Key words: *Hyptis suaveolens* Poit., antioxidant activity, DPPH, methanol extract, gallic acid, butylated hydroxyanisole

INTRODUCTION

Antioxidants are the substances used by the body to protect itself from the damage caused by oxidation. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which starts chain reactions that damage cells. Free radical production is actually a normal part of life, part of the equation of simply breathing in oxygen. The body can cope with some free radicals and need them to function effectively. However, an overload of free radicals has been linked to many diseases, few of which include heart diseases (Aviram, 2000), liver diseases and cancers (Owen *et al.*, 2000). The reactive oxygen species and free radicals produced in the cells include hydrogen peroxide, hypochlorous acid, hydroxyl radical and superoxide anion (Valko *et al.*, 2007), which is known to cause cell damage by starting chemical chain reactions such as lipid peroxidation, or by oxidizing DNA or proteins (Sies, 1997). Hence the research has focused on use of antioxidants, with particular emphasis on naturally derived antioxidants, which may inhibit reactive oxygen species and may display protective effects. Plant phenolics, in particular phenolic acids, tannins and flavonoids are known to be potent antioxidants and occur in vegetables, fruits, nuts, seeds, roots and barks (Pratt and Hudson, 1990). In addition to their antioxidant properties, these compounds display a vast variety of pharmacological activities such as antiinflammatory, anticancer, anticarcinogenic and antibacterial activities.

Hyptis suaveolens Poit. (Lamiaceae), known as Ganga tulsi, is an aromatic strongly scented herb found in Deccan Peninsula, North East India, Andaman and Nicobar Island, Philippines and Tropical America. In the Traditional System of Medicine, the plant is used as a stimulant, carminative, for wounds, sudorific, galactagogue, catarrhal condition, infection of uterus, parasitic skin diseases (Anonymous, 2001). The phytoconstituents isolated from the plant are hyptadienic acid (Raja *et al.*, 1990), suaveolic acid, suaveolol, methyl suaveolate, β-sitosterol, oleanolic acid, ursolic acid, rosamarinic acid (Manchard *et al.*, 1974), dehydroabietinol (Ziegler *et al.*, 2002), 3β-hydroxy lup-12-en-28-oic acid (Misra *et al.*, 1983a), 3β-hydroxyl lup-20(29)-en-27-oic acid (Misra *et al.*, 1983b) and essential oil (Peerzada, 1997). It has been reported to possess antibacterial (Asekum, 1999), wound healing (Shirwaikar *et al.*, 2003), antiinflammatory activity (Grassi *et al.*, 2006), antiplasmodial activity (Chukwujekwu *et al.*, 2005), antimalarial (Ziegler *et al.*, 2002) and antinociceptive (Santos *et al.*, 2007) activities. Given the fact of traditional knowledge and the recent pharmacological studies, the aim in the present study was to evaluate its *in vitro* antioxidant DPPH free radical scavenging activity.

MATERIALS AND METHODS

Chemicals: 1, 1-diphenyl-2-picrylhydrazyl (DPPH), gallic acid and butylated hydroxyanisole was purchased from Loba Chemie Pvt Ltd., Mumbai. All the chemicals and reagents used were of analytical grade.

Plant material: Fresh leaves of *Hyptis suaveolens* Poit. were collected from Bangalore, Karnataka in July 2007, shade dried and authenticated by Prof. Gajendra Rao, Central Council for Research in Ayurveda and Siddha, Bangalore. Voucher specimen is deposited in the Department of Pharmacognosy, The Oxford College of Pharmacy, Bangalore.

Preparation of methanol extract: The shade dried powdered leaves (450 g) were exhaustively extracted with petroleum ether (60-80°C) followed by chloroform and methanol using soxhlet apparatus. The methanol extract (HSME) was concentrated in vacuum (yield: 4.86% w/w) and was only used for antioxidant activity.

Phytochemical screening: The coarse powder of leaves of *Hyptis suaveolens* (25 g) was subjected to successive extraction with different solvents in their increasing order of polarity from petroleum ether (60-80°C), chloroform, methanol and water. The extracts were concentrated and subjected to various chemical tests to detect the presence of different phytoconstituents (Khandelwal, 2003).

Evaluation of antioxidant activity: The free radical scavenging activity of the methanol extract of *Hyptis suaveolens* was measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH) using the method described by Shimoda *et al.* (1992). 0.1 mM solution of DPPH in ethanol was prepared. One milliliter of the solution was added to 3 mL of HSME solution in methanol at different concentration (100-1.56 µg mL⁻¹). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm by using a spectrophotometer (UV-VIS Shimadzu). Reference standard compound being used was gallic acid and butylated hydroxyanisole 0.125-1 and 1-5 µg mL⁻¹, respectively. The IC₅₀ value is the concentration of sample required to inhibit 50% of the DPPH free radical. The IC₅₀ value for the sample was calculated using log dose inhibition curve. Lower absorbance of the reaction mixture

indicated higher free radical activity. The percent DPPH scavenging effect was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = 100 \times A_1/A_0$$

where, A₀ was the absorbance of the control reaction and A₁ was the absorbance in presence of the standard sample or HSME.

RESULTS AND DISCUSSION

Preliminary phytochemical screening: Preliminary phytochemical screening revealed the presence of steroids, flavonoids, alkaloids, carbohydrates and tannins in the different extracts (Table 1). From the literature, it is clear that phytoconstituents viz., steroids only have been isolated from different extracts, still other phytoconstituents are yet to be isolated. Only methanol extract was chosen as it contains flavonoids which are generally potent inhibitors of free radicals (Havsteen, 1983).

DPPH radical scavenging activity: As shown in Table 2, HSME has potent DPPH radical scavenging activity with an IC₅₀ value of 14.04 µg mL⁻¹. IC₅₀ value of gallic acid and butylated hydroxyanisole were found to be 0.4 and 1.15 µg mL⁻¹, respectively.

DPPH is a stable free radical that can accept an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH radicals react with suitable reducing agents then losing colour stoichiometrically with the number of electrons consumed which is measured spectrometrically at 517 nm (Soares *et al.*, 1997; Lugasi *et al.*, 1998). Gallic acid is a potent free radical scavenging and butylated hydroxyanisole is known antioxidant and is used as preservative. So when compared to such pure components, IC₅₀ value of crude HSME is quite good proving that it is potent DPPH free radical scavenger. This can be attributed to flavonoids

Table 1: Phytochemical screening of different extracts

Extracts	Steroids	Alkaloids	Glycosides	Saponin	Flavonoid	Tannin	Carbohydrates
Petroleum ether	+	-	-	-	-	-	-
Chloroform	+	-	-	-	-	-	-
Methanol	+	+	-	-	+	+	-
Aqueous (water)	-	-	+	+	+	+	+

+: Presence; -: Absent

Table 2: DPPH free radical scavenging activity of methanol extract of *Hyptis suaveolens* [HSME]

Extract/standard	IC ₅₀ value (µg mL ⁻¹ ; mean±SEM)
Gallic acid	0.40±2.68
BHA	1.15±3.56
HSME	14.04±2.08

present in the extract. So the plant may be useful in the management of free radical mediated diseases. Further research is therefore needed for the isolation and identification of the antioxidant components.

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