# Antimicrobial Activity of the Leaf Extracts of *Hyptis suaveolens* (L.) Poit

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Steam distillation, petroleum ether, and ethanol extracts from *Hyptis suaveolens* leaves were evaluated for their antimicrobial activity *in vitro*. Steam distillation extract exhibited broad-spectrum antibacterial and antifungal activity against the tested organisms. It showed highest antifungal and antibacterial activity against *Aspergillus niger* and *Micrococcus luteus*, respectively. Activity indices of *A. niger* against miconazole (25 µg/ml) and *M. luteus* against chloramphenicol (10 µg/ml) were 0.89 and 0.67, respectively.

Key words: Antibacterial, antifungal, *Hyptis suaveolens*, leaf extracts



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[1]. Crude leaf extract is also used as a relief to colic and stomachache. Leaves and twigs are considered to be antispasmodic and used in antirheumatic and antisuporific baths[2], an antiinflammatory, antifertility agents[3], 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-integrase inhibitor[4]. Fumes of the dried leaves are also used to repel mosquitoes and control insect pests of stored grains. In the present work an attempt was made to study the antimicrobial activity of different leaf extracts of *Hyptis suaveolens* (L.) Poit.

Fresh leaves of *Hyptis suaveolens* (L) Poit. (Lamiaceae) were collected in flowering stage during late September from the natural population of the IIT-Kharagpur campus, India and identified in the Department of Botany, University of Burdwan, India.

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Bacteria (listed in Table 1) were obtained from the pure stock culture of Microbiology Department, Vidyasagar University, Midnapur (W), India. Fungi (listed in Table 2) were obtained from the Mycology and Plant Pathology Laboratory, Department of Botany, University of Burdwan, Burdwan, India.

Steam distillated (yield: 0.24%), petroleum ether extract (yield: 1.6%) and ethanol extract (yield: 2.64%) were prepared separately from fresh leaves following Souza *et al*[5]. The ethanol and petroleum ether extracts were reduced to dryness *in vaccuo* and solutions of each as well as of the steam distillation fraction (v/v) were dissolved in 10% DMSO (dimethylsulfoxide). Chloramphenicol (10 µg/ml) and

**TABLE 1: ANTIBACTERIAL ACTIVITY OF THE LEAF EXTRACTS OF *H. SUAVEOLENS***

| Extracts | Concentration | Bacteria | | | | |
|---|---|---|---|---|---|---|
| | (mg/ml) | B. s. | S. a. | E. c. | P. a. | M.l. |
| E.E. | 1.0 | - | - | 0.21 | | 0.25 |
| | 2.0 | 0.26 | - | 0.28 | 0.33 | 0.33 |
| | 3.0 | 0.35 | 0.27 | 0.39 | 0.47 | 0.41 |
| P.E. | 1.0 | 0.30 | - | 0.28 | 0.29 | 0.33 |
| | 2.0 | 0.43 | - | 0.36 | 0.42 | 0.41 |
| | 3.0 | 0.56 | 0.36 | 0.50 | 0.61 | 0.62 |
| S.E. | 0.25 | 0.35 | - | 0.32 | 0.38 | 0.37 |
| | 0.50 | 0.52 | 0.31 | 0.46 | 0.42 | 0.50 |
| | 1.0 | 0.61 | 0.41 | 0.56 | 0.63 | 0.67 |
| C | | 23 | 22 | 28 | 22 | 24 |

Values are; AI (Activity Index) = inhibition zone of test sample divided by inhibition zone of a standard [C= chloramphenicol (10 µg/ml)] drug, zone inhibition (mm, including cork borer diameter 5 mm), values of 'C' in mm. Abbreviations are E.E. = ethanol extract, P.E. = petroleum ether extract, S.E. = Steam distillation extract % (v/v), B. s.= *Bacillus subtilis*, S. a. = *Staphylococcus aureus*, E. c. = *Escherichia coli*, P. a. = *Pseudomonas aeruginosa*, M. l. = *Micrococcus luteus* and - No inhibition.

**TABLE 2: ANTIFUNGAL ACTIVITY OF THE LEAF EXTRACTS OF *H. SUAVEOLENS***

| Extracts | Concentration (mg/ml) | Fungi | | |
|---|---|---|---|---|
| | | A. n. | H. o. | F. o. |
| E.E. | 1.0 | 0.39 | - | 0.29 |
| | 2.0 | 0.44 | 0.35 | 0.38 |
| | 3.0 | 0.61 | 0.47 | 0.48 |
| P.E. | 1.0 | 0.44 | 0.35 | 0.43 |
| | 2.0 | 0.61 | 0.47 | 0.62 |
| | 3.0 | 0.78 | 0.64 | 0.71 |
| S.E. | 0.25 | 0.50 | 0.47 | 0.48 |
| | 0.50 | 0.72 | 0.64 | 0.71 |
| | 1.0 | 0.89 | 0.76 | 0.81 |
| M | | 18 | 17 | 21 |

Abbreviations are; M = miconazole (25 µg/ml), F. o = *Fusarium oxysporum*, A. n = *Aspergillus niger*, H. o. = *Helminthosporium oryzae* and other abbreviations/ conditions are same as in Table 1.

miconazole (25 µg/ml) were used as positive control. Well-diffusion assay method was used to screen the extracts for antimicrobial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Micrococcus luteus*. Nutrient agar plates were swabbed with the respective broth culture of the organisms and kept for 15 min in laminar chamber for absorption to take place. Wells were made in agar plates using a sterile cork borer (5 mm diameter) and 10 µl of extract was added to each well. The plates were incubated at 37º for 24 h and the diameters of the inhibition zone were measured in millimeter. The antifungal activity of the extracts against *Fusarium oxysporum*, *Aspergillus niger*, *Helminthosporium oryzae* was evaluated in molten potato dextrose agar medium and inoculated similar to antibacterial assay. The plates were incubated at 27º for 3 d.

The steam distillation and petroleum ether extracts displayed antifungal activity and broad spectrum of antibacterial activity against all the tested strains except *Staphylococcus aureus* (Tables 1 and 2). The ethanol extract showed less activity than that of steam distillation and petroleum ether extract at the tested concentration against both bacterial and fungal strains. Activity indices of tested bacteria were different in value against chloramphenicol (10 µg/ml). The microorganisms studied here showed obvious differences in their susceptibility to *H. suaveolens* leaf extracts. Steam distillation extract showed highest antifungal and antibacterial activity against *Aspergillus niger* and *Micrococcus luteus,* respectively.

Essential oils are the major constituents of steam distillation extract. Volatile oils of *H. suaveolens* with major component of β-caryophyllene, 1,8-cineole and sabinene, and β-pinene[6], sesquiterpenes and monoterpenes, terpenoids and sterols[7]. A wide variety of essential oils are known to possess antimicrobial properties and in many cases this activity is due to the presence of monoterpene constituents which exert membrane-damaging effects to microbial strains[8] and also stimulates leakage of cellular potassium ions which provides evidence of a lethal action related to cytoplasmic membrane damage[9].

In conclusion, our observations confirm that the steam distillation extracts are better than that of ethanol or petroleum ether extracts of *H. suaveolens* leaf in respect to their antimicrobial activity and the broad spectrum of activity makes it a promising indigenous drug.



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