

In Vitro Antioxidant and Antimicrobial Activity of Methanolic root Extracts of *Hyptis suaveolens*

Javed Ahmad¹, Iffat khan², Ashfaq Ahmad² and kaushar Imam¹

¹Department of biochemical Engineering and Food Technology, Harcourt butler technological Institute, Kanpur, INDIA

²Department of Bioscience, Integral University Lucknow, INDIA

Available online at: www.isca.in

Received 29th November 2012, revised 27th December 2012, accepted 24th January 2013

Abstract

The plant *Hyptis* is a potent medicinal herb and a well known medicinal plant in herbal world. Crude methanolic extract of *Hyptis suaveolens* were screened for their in vitro antimicrobial activity against pathogenic microorganisms; *S.epidermidis*; *K.pneumoniae* *B.subtilis*; *E.aerogens*; *B.cereus*. In-vitro antioxidant and antimicrobial activity determined using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and agar well diffusion method respectively. In addition, extract of *Hyptis suaveolens* prepared by soxlet apparatus and were partially purified by preparatory thin layer chromatography (TLC). Results indicated a potent antioxidant and antimicrobial activity of methanolic root extract of *Hyptis suaveolens*.

Keywords: *Hyptis suaveolens*, methanolic extract, antioxidant, antimicrobial, DPPH, agar well diffusion assay.

Introduction

The plant, *Hyptis suaveolens* commonly known as “Wilayati tulsi” belongs to the family Lamiaceae and is an ethnobotanically important medicinal plant. Almost all parts of this plant are being used in traditional medicine to treat various diseases¹. It is approximately 2 meters high, having branches and long, white piliferous stems. Its flowers are purple or white, its leaves oval, wrinkled and pointed.

An antioxidant is a molecule that slows or prevents the oxidation of the molecules. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often considered as reducing agents such as thiols, ascorbic acid, polyphenols². Antioxidants are widely used as ingredients in the dietary supplements in order to maintain health and to prevent diseases such as cancer and coronary heart disease³. These compounds may be synthesized in the body or obtained from the diet⁴. Many aromatic, medicinal and spice plants contain compounds that possess confirmed strong antioxidative components. One of the documented health promoting activities of many fruits and vegetables is their ability to scavenge naturally produced free radicals and hence acting as antioxidants⁵.

The leaves of *H. suaveolens* have been utilized as a stimulant, carminative, galactagogue and as a cure for parasitic cutaneous diseases. Crude leaf extract is also used as a relief to colic and stomach ache^{6,7}.

Hyptis suaveolens has been reported to as tonics, emmenagogues, diaphoretics, antispasmodics, burns and

wounds, antimicrobial, antibacterial, antispasmodic, analgesic, anti-inflammatory, headaches, anticatarrhal, anticutaneous, Insecticidal Effect, Acaricidal Effect, 12 Larvicidal Effect, Toxicity Concerns Hepatotoxicity, Chronic Toxicity Study^{1,6-11}. The efficacy of *Hyptis suaveolens* oil as a preservative of the cereals, pulses, nuts and spices against fungal spoilage *Hyptis suaveolens* (L.) and has been widely used as a stimulant, carminative, lactagogue and to treat colic disorders^{1,6}.



Figure-1
Plant of *Hyptis suaveolens*

A wide range of chemical compounds including terpenoids, alkaloids, acidic polysaccharide and 33 constituents were identified in the oil of *Hyptis suaveolens* isolated from its leaves. Extracts and metabolites from this plant have been found to possess pharmacological and insecticidal activities¹. *Hyptis suaveolens* was targeted on the basis of folkloric uses which suggest its toxicity to microbes, coupled with its importance as

food to humans¹¹. Soluble solvent extracts of the plant were tested for phytochemicals which revealed the existence of alkaloids, flavonols, flavones, flavonones, terpenoids, tannins, aldehydes and ketones and the absence of steroids, saponins and anthraquinones⁷. Wound Healing Activity of *Hyptis suaveolens* is a traditional pubescent annual herb found throughout India. On the basis of traditional use and literature references, this plant was selected for the screening of wound healing property¹¹.

Toxic secondary metabolites from plants were extracted, tested and proved to affect insect nerve functions and behaviors. A considerable number of studies have emphasized the research and development of herbal substances for controlling mosquitoes^{12,13,14}. These botanical extracts could also be used along with other insecticides under integrated vector control. Studies carried out so far have shown that some photochemicals acts as general toxicant (insecticide/ Larvicide) both against adult as well as larval stage of mosquito while others interfere with reproduction¹⁴.

In this communication bioactivity of essential oil extracted from *Hyptis suaveolens* was tested against *Aspergillus flavus* Link, *Aspergillus niger*¹⁵. Antimicrobial Study of the volatile oil distilled from the overground parts of *H. suaveolens* showed activity against bacteria and fungi⁶.

Material and Methods

Chemicals: 2, 2-diphenyl-1-picrylhydrazyl (DPPH), dimethyl sulfoxide (DMSO), Methanol (MeOH), Thin layer chromatography (TLC) plates, silica gel. All other chemicals were of AR grade.

Test microorganism which were used in this experiment are: *Klebsiella pneumoniae*, *B.cereus*, *E.aerogens*, *B.subtilis*, *S.epidermidis*, at different concentration (20, 30, 40, 50 µg/ml) concentration was used as a standard.

Sample Preparation: Plant Material: Plant material *Hyptis suaveolens* was collected in flowering stage from local area of Lucknow near Integral University. The shade dried plant material was crushed into fine powder.

Preparation of Methanolic Extract of *Hyptis suaveolens*: Around 15 gm fresh shade dried plant material was powdered and wrapped in muslin cloth. It was extracted by Soxhlet apparatus with 150 ml of methanol. The percolation process was continued until the extraction process was completed (indicated by transparent colour). The extract was allowed to cool and then poured in to a petri plate, left for drying. The dried extract was scratched and was collected in eppendorf tube and weighed, used for further phytochemical screening.



Figure
shows the soxhlet apparatus

In vitro antimicrobial assay: Agar well diffusion method: Cell suspensions to a final concentration of 10 to 10⁸ cfu/ml were prepared from different subcultures. 0.1 ml of inoculums of test plant extract was spread on Muller Hinton agar plates. 4 millimetres – diameter wells were punched into MHA. A 30 µl aliquot of each suspension was dispensed into the wells. The plates were incubated for 18hr at 37°C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test plant extract. Each experiment was carried out in triplicates.

Determination of Minimum Inhibitory Concentration (MIC): MIC may be defined as the lowest concentration of the extract that prevent visible bacterial growth. Broth micro dilution assay was performed for determination of MIC, bacterial strain were cultured at 37°C and suspended in nutrient broth to obtain final inoculums density of 10⁸ cfu/ml. The 0.5 ml of this suspension was added to 0.5 ml of susceptibility test broth containing serial two fold dilution of plant extract. All the test tubes were incubated at 37°C for 20 hours.

Antioxidant assay: Antioxidant assays: Each sample was dissolved in 95% methanol at a concentration 1 µg/ml and then diluted to prepare the series concentrations for antioxidant assays. Reference chemicals were used for comparison in all assays. DPPH radical scavenging activity assay. The DPPH assay was done according to the method of Brand-Williams, Cuvelier, and Berset with some modifications. The stock solution was prepared by dissolving 24 mg DPPH with 100 ml methanol and then stored at 20°C until needed. The working solution was obtained by diluting DPPH solution with methanol to obtain an absorbance of about 0.980 (±0.02) at 517 nm using the spectrophotometer. A 3 ml aliquot of this solution was mixed with 100 µl of the fractions at varying concentrations (25– 250 µg/ml). The solution in the test tubes were shaken well and incubated in the dark for 15 min at room temperature. Then the absorbance was taken at 517 nm. The scavenging activity was estimated based on the percentage of DPPH radical scavenged using the following equation: Scavenging effect (OD of control absorbance-OD of sample

absorbance)/OD of control absorbance×100: EC50 value is the effective concentration that could scavenge 50% of the DPPH radicals. Ascorbic acid was used as standard.

TLC Study of Flavonoids: 1 gm dried mass of plant was extracted with 10 ml methanol on rotary shaker for 24hrs. The flavonoid spots were separated using chloroform and methanol (4:1) solvent mixture. The colours of these spots were recorded under Ultraviolet light (254nm).

Results and Discussion

Crude methanolic extract of *Hyptis suaveolens* were screened for their *in vitro* antioxidant and antimicrobial properties. DPPH method was used to determine total antioxidant activity. Antimicrobial activity was determined by using agar well diffusion assay. Our results indicated a potent antioxidant and antimicrobial activity of methanolic root extract of *Hyptis suaveolens*. As shown in Table1 a significant decrease was

observed in absorbance with increasing concentration, which showed better antioxidant power. In addition, our data also showed (table 2 and figure 1) an increase in percent inhibition of free radicals by increasing concentration of plant extracts. The DPPH antioxidant assay was based on the ability of DPPH a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance. The antioxidant capacity observed was not solely from the phenolic contents, but could possibly be due to the presence of some other phytochemicals such as ascorbic acid, tocopherol and pigments as well as the synergistic effects among them, which also contribute to the total antioxidant capacity.

Table-1
 Show the absorbance of *Hyptis suaveolens* methanolic root extract at various sample concentration

Sample concentration(µg/ml)	Ascorbic acid(OD)	HsMe(root)(OD)
25 µg/ml	1.279±.001	1.234±.002
50 µg/ml	1.212±.005	1.219±.012
100 µg/ml	1.048±.008	1.208±.005
150 µg/ml	0.884±.009	1.169±0.004
250 µg/ml	0.571±.015	1.134±0.001

Table-2
 Percent Inhibition of DPPH mediated free radical generation by *Hyptis suaveolens* methanolic root extract at various concentrations

Sample concentration(µg/ml)	Ascorbic Acid (%scavenging inhibition)	HsME(root) (% scavenging inhibition)
25 µg/ml	5.1	0.1615
50 µg/ml	10.3	1.373
100 µg/ml	22.3	2.323
150 µg/ml	34.4	5.411
250 µg/ml	57.4	8.481

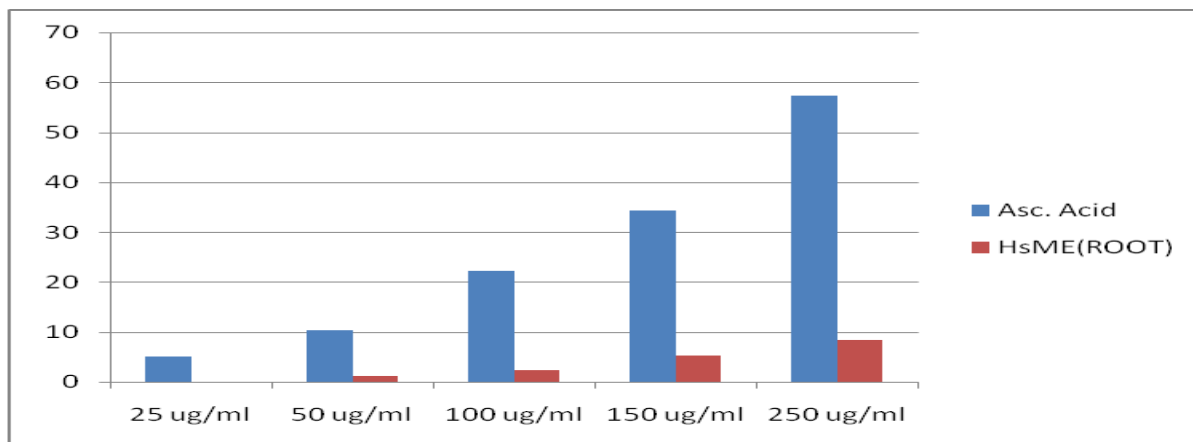


Figure-1
 Graph representing Percentage inhibition of *Hyptis* (root) against Ascorbic acid

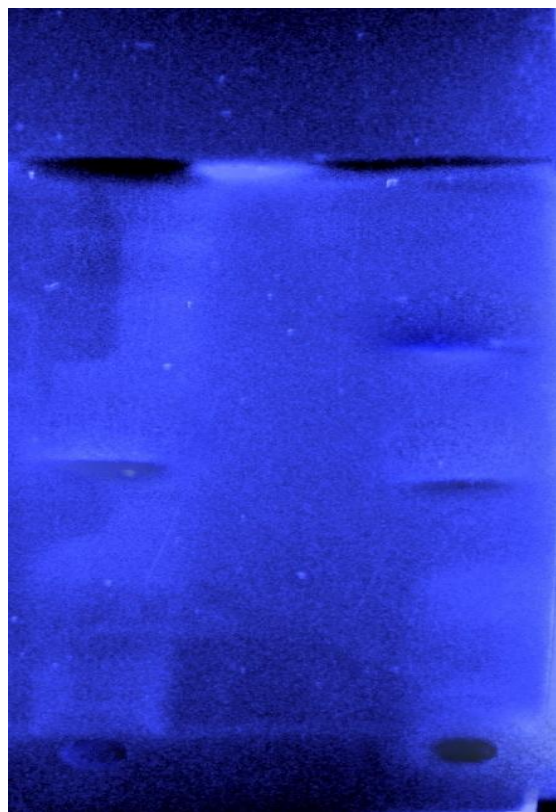


Figure-2(a)
 The TLC plate under ultraviolet light

Rf values of bands

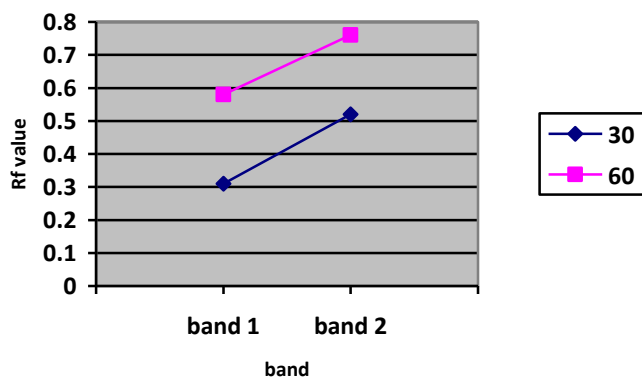


Figure-2(b)
 Rf values of bands

Emergence of multi-drug resistance in human and animal pathogenic bacteria as well as undesirable side effects of certain antibiotics has triggered immense interest in the search for new antimicrobial drugs from the plant source. In the present study methanolic extracts of plant have been tested against resistant bacteria. The antimicrobial activity of the extracts and their potency was quantitatively assessed by the presence or absence

of inhibition zone and zone diameter. The results of antimicrobial activity of *Hyptis suaveolens* was encouraging and that the plant extract showed significant antimicrobial activity against different bacterial strains. In the figure 3-6, the antimicrobial activity of methanolic extract of *Hyptis suaveolens* (root) against five bacterial strain *B.cereus*, *E.aerogens*, *K.pneumoniae*, *B.subtilis*, *S.epidermidis* at different concentration (20,30,40,50 µg/ml) was found in the following decreasing order *B.cereus* > *S.epidermidis* > *E.aerogens* > *K.pneumoniae* > *B.subtilis*



Figure-3
 Zone of inhibition of *Hyptis* methanolic root extract against *S.epidermidis*

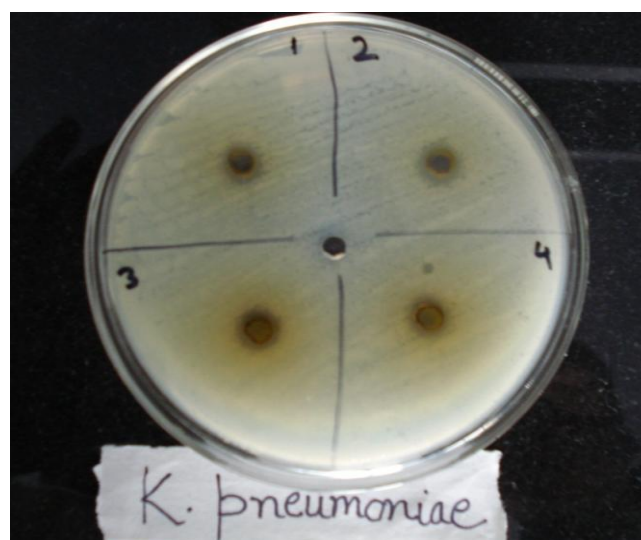


Figure-4
 Zone of inhibition of *Hyptis* methanolic root extract against *K.pneumonia*

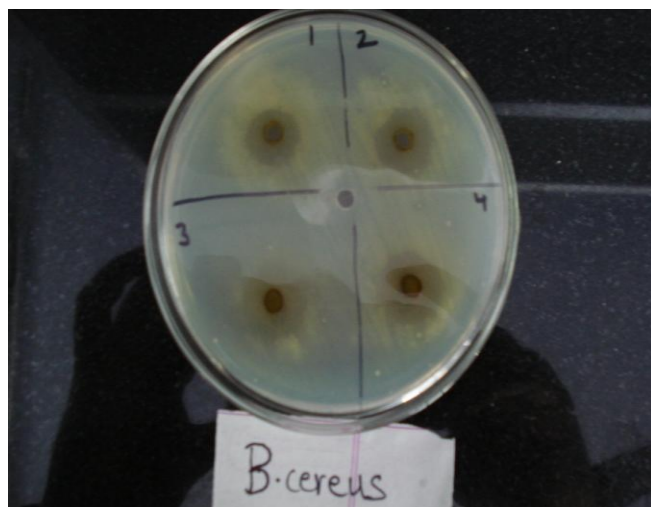


Figure-5
Zone of inhibition of *Hyptis* methanolic root extract against *B.cereus*



Figure-6
Zone of inhibition of *Hyptis* methanolic root extract against *B.subtilis*

Determination of antibacterial activity by agar well diffusion assay showed that methanolic extract of *Hyptis* root exhibited the antibacterial effect against pathogenic as well as non-pathogenic test bacteria. Significant effect on growth inhibition of gram positive and gram negative bacteria was also noticed. It was noted that among all tested organisms: the gram positive bacterial strain *S.epidermidus* and *B.cereus* registered maximum susceptibility to the methanolic extract at 50 µg/ml of the entire *Hyptis* root used, with the maximum inhibitory zone of 12 mm and 17 mm respectively. These differences may be attributed to the fact that while the cell wall in Gram-positive bacteria consist of a single layer that of Gram-negative is a multi-layered and quite complex structure. The results provided evidence that the studied plant might indeed be potential sources of natural antioxidant and antimicrobial agents. Based on these results, it

is possible to conclude that methanolic extracts of *Hyptis suaveolens* had different levels of antioxidant and antimicrobial activity. The obtained results might be considered sufficient to further studies for the isolation and identification of the active principles and to evaluate of possible synergism among extract components for their antioxidant and antimicrobial activity.

Conclusion

Crude methanolic extract of *Hyptis suaveolens* were screened for their in vitro antioxidant and antimicrobial properties. DPPH method was used to determine total antioxidant activity. Antimicrobial activity was determined by using agar well diffusion assay. Results indicated a potent antioxidant and antimicrobial activity of methanolic root extract of *Hyptis suaveolens*. Based on these results, it is possible to conclude that methanolic extracts of *Hyptis suaveolens* possesses potent antioxidant and antimicrobial activity. The obtained results might be considered sufficient to further studies for the isolation and identification of the active compounds, to evaluate possible synergism among extract components for their antioxidant and antimicrobial activity.

References

1. Asprey G.F., Medicinal Plants of Jamaica, *West Indian Medical Journal*, **2(4)**
2. Nayak Praveen S., Nayak Shweta, Shety Ranjan and Das P., *Hyptis suaveolens* Poit: A Review on Its Phytochemical and Pharmacological Profile, *research journal of pharmacognosy and phytochemistry review*, **2(1)**, (2010)
3. Sies. H., Oxidative stress: Oxidants and antioxidants, *Exp-Physiol*, **82(2)**, 291-295 (1997)
4. Vertuani S., Angust A. and Manfredini S., The antioxidants and proantioxidants network: an overview, *CurrPharm Des*, **10(4)**, 1677-1694 (2004)
5. Bauer, The Complete Idiot's Guide to Total Nutrition, *Alpha Books*, 76-78 (2002)
6. Mandal S.M., Mondal K.C., Dey S. and Patil B.R., Antimicrobial activity of the leaf extracts of *Hyptis suaveolens* (L.) poit, *Indian Journal of Pharmaceutical Sciences*, **69(4)**, 568-569 (2007)
7. Vermab Amita, Mukerjeea Alok, Vijayakumarc M. and Mishraa Shanti Bhushan, Anti-hyperglycemic activity of leaves extract of *Hyptis suaveolens* L. Poit in streptozotocin induced diabetic rats, *Asian Pacific Journal of Tropical Medicine*, **4(9)**, 689-693(2011)
8. Sharma N., Verma U.K. and Tripathi Abhishek, Bioactivity Of Essential Oil From *Hyptis Suaveolens* Against Storage Mycoflora, *Proc. Int. Conf. Israel*, 99-116 (2007)
9. Barbara Conti, Giovanni Benelli, Guido Flamini, Pier Luigi Cioni, Raffaele Profeti, Lucia Ceccarini, Mario

- Macchia, Angelo Canale, Larvicidal and repellent activity of Hyptis suaveolens (Lamiaceae) essential oil against the mosquito Aedes albopictus Skuse (Diptera: Culicidae), *Parasitol Res.*, **110**, 2013–2021, DOI 10.1007/s00436-011-2730-8 (2012)
10. Renisheya Joy Jeba Malar T., Sushna S.L., Johnson M., Janakiraman N. and Renola Joy Jeba Ethal T., Bio-Efficacy Of The Leaves Extracts Of Hyptis Suaveolens(L.) Poit Against The Fish Pathogens, *Life Science Microbiology*, **2(1)**, (2012)
 11. Shenoy Chitra, Patil M.B. and Kumar Ravi, Wound Healing Activity of Hyptis suaveolens (L.) Poit (Lamiaceae), *International Journal of Pharm Tech Research*, **1(3)**, 737-744 (2009)
 12. Bielakovic G., Nikolova D., Glud L.L. and Simonetti. R.G., Glud C., Mortality in randomised trials of antioxidant supplements for primary and secondary prevention: systematic review and meta analysis, *JAMA* , **297(8)**, 842-857 (2007)
 13. Fabricant D.S. and Farnsworth N.R., The value of plants used in traditional medicine for drug discovery, *Environ. Health Perspect.*, **109(1)**, 69–75 (2001)
 14. Okigbo R.N., Okeke J.J. and Madu N.C., Larvicidal effects of Azadirachta indica, Ocimum gratissimum and Hyptis suaveolens against mosquito larvae, *Journal of Agricultural Technology*, **6(4)**, 703-719 (2010)
 15. Parsons W. and Cuthbertson E., Weeds of Natural Ecosystems: a field guide to environmental weeds of the Northern Territory., 490–492 (1992)