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Antimicrobial and phytochemical screening of *Hyptis suaveolens* (L.Poit) Lamiaceae

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Abstract: The present communication deals with the antimicrobial and preliminary phytochemical screening on the *Hyptis suaveolens* (L. Poit) Lamiaceae. The plant is stimulant, carminative, antispasmodic antirheumatic, antisuporitic bath. It is also used for parasitical cutaneous diseases, infection of uterus, and as sudorific in catarrhal condition, headache, stomach, snuff to stop bleeding of the nose. The antimicrobial effect of *H. suaveolens* leaves extract was evaluated aqueous and ethanol extracts was carried out by using fungus like, *Canidida albicans, Collectrotrichum capsici, Fusarium oxysporum F. sp. Lycopersici,* and four bacteria *viz. Klebsiella pneumoneae, Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa*. The aqueous extract of plant material can not show any inhibition zone microbes like *C. albicanas, S. aureus* and *P. aeruginosa*. All the seven microbes tested are susceptible to ethanol extract with the inhibition zone range of 12 – 29 mm. The *in vitro* antimicrobial evaluation was carried out by agar disc-diffusion method. Preliminary phytochemical screening shows the presence of volatile oil, starch, proteins, tannins, saponins, fats, alkaloids and glycosides etc.

Keywords: Antimicrobial, Phytochemical, Susceptible, *Hyptis suaveolens*

INTRODUCTION

The medicinal important plant *Hyptis suaveolens* is used by various tribal communities of Maharashtra, Marathwada region to cure various diseases like, parasitical cutanous, diseases, infection of uterus, and as a sudorofic in cutarrhal condition. The plant is stimulant carminative, antiplasmodic, antirhenmatic, antisuportic bath. It is also used for headache, stomachache and snuff to stop bleeding of the nose (Nadkarni 1976).

Silver and Bostian (1993) have documented the use of natural products as new antibacterial drugs. There is an urgent need to identify novel substances active towards highly resistant pathogens (Recio, 1989; Cragg *et al*., 1997). In an effort to discover new compounds, many research groups screen plant extracts to detect secondary metabolites with the relevant biological activities. In this regard, several simple bioassays have been developed for screening purposes (Hostettmann, 1991).

It is thought that herbal remedies have the advantage of combining their active components with many other substances which appear to be inactive but which give the plant as a whole a level of safety and efficiency superior to that of its isolated, pure active components; moreover, in developing countries, synthetic drugs are presently too expensive and also are often adulterated (Shariff, 2001).

The antimicrobial activity have been screened because of their great medicinal relevance with the recent years, infections have increased to a great extent and resistant against antibiotics, becomes an ever increasing therapeutic problem (Austin *et al*., 1999). Natural products of higher plants may give a new source of antimicrobial agents.

MATERIALS AND METHODS

Sample collection and Authentication: The fresh plant organs (Leaves, Stems, Roots, etc) of *H. Suaveolens* were collected from the Botanical garden of Government Institute of Science, Caves Road, Aurangabad. The voucher specimen is preserved in the Department of Botany, Government institute of science, Aurangabad. (M.S., India). The collected plants were washed repeatedly with tap and finally with distilled water. Then sliced in root, stem and leaves. They were dried and powdered with help of grindings and filtered through sieves and stored for extraction. (Daniel M. 1965, Sadashivam and Manikramal 1992, Khandelwal 1985, Kokate *et al* 2005).

Preparation of extract: The dried plant material was pulverized into fine powder using a grinder (mixer). About 50 g of powdered material was extracted in soxhlet extraction apparatus with 250 ml of the alcohol solvent and distilled water (Vogel, 1988). The extracts obtained were filtered through Whatman filter paper No. 1 and were evaporated (at 40ºC) with the help of heating mantle. The sticky greenish-brown substances were obtained and stored in refrigerator for

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prior to use (Beyer and Walter, 1997).

Preparation of microorganism

The bacterial cultures of *Klebsiella pneumoneae*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* were obtained from Department of Microbiology, Government institute of science, Aurangabad. (M.S., India). Whereas the fungus *Canidida albicans, Collectrotrichum capsici, Fusarium oxysporum F. sp. Lycopersici* obtained from home department. The bacterial cultures and fungal cultures were maintained in nutrient agar and sabouraud agar slants respectively and stored at 4˚C.

Preparation of Spore Suspension and Test Sample: Spore suspension of bacteria were prepared by inoculating loopful of target bacteria (25 hour old culture) in 5 ml nutrient broth and incubated at 27±2˚C for 5-8 hours till a moderate turbidity was developed. The turbidity was adjusted to 0.5 McFarland standard by adding distilled water which correspond to the cell density $1.5x10^8$ (CFU/ml). The spore suspension of fungi were prepared in sabouraud broth which grown overnight and OD was adjusted to 1.0 to 600 nm. Each sample of extracts residue were dissolve in 20 ml of 80% ethanol which corresponds to the $1/5^{th}$ of the original volume of the extracts.

Procedure for performing the Disc Diffusion test (Bayer *et al*., 1966): The required amount of Petri plates is prepared and autoclaved at 121˚C for 15 minutes. And they were allowed to cool under Laminar air flow. Aseptically transfer about 20 ml of media into each sterile Petri dishes and allowed to solidify. 1 ml inoculum suspension was spread uniformly over the agar medium using sterile glass rod to get uniform distribution of bacteria. The readily prepared sterile discs were loaded. The paper diffuse discs were placed on the medium suitably apart and the plate were incubated at 5˚C for 1 hour to permit good diffusion and then transferred to an incubator at 37˚C for 24 hours. The antimicrobial activity was recorded by measuring the width of the clear inhibition zone around the disc using zone reader (mm).

Histochemical Analysis:

Transections of leaf, stem and root were taken by free hand. Histochemical tests were performed on fresh plant materials according to the methods of Johansen (1940) and Guerin (1971). The positive tests were noted as present (+) and absent (-).

RESULTS AND DISCUSSION

Plants are known to have beneficial the therapeutic effect documented in traditional Indian system of medicine. Much work has been done on ethno medicinal plant in India. Interest in a large number of traditional natural product has increased.

The results obtained for the antimicrobial tests of *H. suaveolens* are presented in Table 1. Among the extracts tested, ethanolic extracts showed broader spectrum of activity, being active to both bacteria and fungi organisms compared to aqueous extracts. The ethanol extract for example, 29 mm was maximum diameter zone recorded as for *S. aureus* and 12 mm minimum diameter zone for *P. aeruginosa*. The microbes like *C. albicanas*, *S. aureus* and *P. aeruginosa* are not show any inhibition zone and other are showed inhibition spectrum from 05-14 mm to aqueous extract. All the seven microbes tested are susceptible to ethanol extract with the inhibition zone range of 12 – 29 mm. The antifungal activity of plant extracts against *Candiada albicans* is not common.

In the present antimicrobial activity of plant extract towards drug resistant or clinically significant microbes are reported and it was observed that active constituent of plant material seep out in organic solvent to display biological activity. The antifungal activity of plant extracts against *Candiada albicans* is not common. Further phytochemical studies for identification and elucidation of active constituent in plant material tested in expected to serve as lead in the development of novel bioactive antimicrobial compound.

Histological results indicate presence of volatile oil, starch, proteins, tannins, saponins, fats, alkaloids and glycosides in leaves while saponins are absent in stem and roots (Table 2).

Table No. 1: Antimicrobial Activity of *H. suaveolens*

Sr. No.	Name of the Microorganism	Diameter of the Inhibition Zone (mm)	
		Aqueous Extract	Ethanolic Extract
1.	*Candiada albicanas*	0	19
2.	*Collectotrichum capsici*	10	20
3.	*Fussarium oxysporum F.sp. Lycopersici.*	05	18
4.	*Klebsiella pnenmoneae*	14	22
5.	*Escherichia coli*	12	18
6.	*Staphylococcus aureus*	0	29
7.	*Psudomouas aeruginosa*	0	12

Table 2: Histochemical Test of *H. suaveolens*

Sr. No.	Test	Leaf	Stem	Root
1.	Volatile oil	+	+	+
2.	Starch	+	+	+
3.	Protein	+	+	+
4.	Tannin	+	+	+
5.	Saponin	+	-	-
6.	Fat	+	+	+
7.	Alkaloids	+	+	+
8.	Glycoside	+	+	+

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