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Antimicrobial and Preliminary Phytochemical Analysis of Solvent Extracts of *Hyptis suaveolens* from Banks of River Krishna

S.R.V. Prasanna[1], Sunil Babu Koppula[2]

[1]St Marys College of Pharmaceuticals, Guntur, A.P. India

[2]College of Pharmaceutical Sciences, Acharya Nagarjuna University, ANU, A.P. India

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Corresponding Author: S.R.V. Prasanna, Assistant Professor, St. Mary's College Of Pharmaceutical Sciences, Acharya Nagarjuna University [ANU], A.P. India.

ABSTRACT

The present work deals with the antimicrobial and preliminary phytochemical analysis on the different parts of *Hyptis suaveolens* (L. Poit) Lamiaceae. The plant material is 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* plants parts extracts were evaluated by two different solvent extracts were carried out by using three fungi like, *Canidida albicans, Rhizophus stoloniphera and Aspergillus Niger* and four bacteria viz. *Klebsiella pneumoneae, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*. The chloroform extract of plant material can not show any inhibition zone microbes like *C. albicanas, S. aureus* and *P. aeruginosa*. All the five microbes tested are susceptible to methanol extract with the inhibition zone range of 11-25mm. 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. The results indicate that the whole plant extracts of *Hyptis suaveolens* may recommend to use in preparation of herbal drugs.

Keywords: Antimicrobial, Phytochemical, Susceptible, *Hyptis suaveolens*, Agar Well Diffusion.

INTRODUCTION

In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world (Davis, 1994 and Robin *et. al.*, 1998). Infectious diseases are the world's leading cause of premature deaths, killing almost 50,000 people every day. The drug resistant bacteria and fungal pathogens have further complicated the treatment of infectious diseases. In the present scenario of emergence of multiple drug resistance to human pathogenic organisms, this has necessitated a search for new antimicrobial substances from other sources including plants. Recently, in world 80% of populations are using medicinal plants to cure various diseases caused by microbial pathogens. Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties (Chopra *et al.*, 1992). Increasing the failure of synthetic drugs, side effects and development of antibiotic resistance by pathogenic microorganisms leads to development of the identification and screening of several medicinal plants for their potential antimicrobial activity (Iwu *et al.*, 1999). Thousands of species are known to have medicinal value and several studies reported the antimicrobial activity of plants (Werner *et al.*, 1999; Samy and Ignacimuthu, 2000).

In recent years an antimicrobial properties of medicinal plants are being increasingly reported from different parts of world (Saxena, 1997). In India, from ancient times different parts of medicinal plants have been used to cure specific ailments. Today there is widespread interest in drugs derived from plants. In this regard value of traditional medicine has recognized by World Health Organization and involved in creating strategies, guidelines and standards for plant medicines (WHO, 2002). So, an extensive study is required to detect the medical properties of the plant. Several medicinal plants have been tried against pathogenic microorganisms (Haraguchi *et al.*, 1999; Sashikumar *et al.*, 2003). In ancient times parts of several medicinal plants are used to cure specific diseases (Hashim *et al.,* 2010). Several medicinal plants have been tried against pathogenic microorganisms, hence to detect the plant medicinal properties an extensive study is required (Haraguchi *et al.*, 1999; Sashikumar *et al.*, 2003).

Hyptis species are the most potent plants against pathogenic microorganisms. *Hyptis suaveolens* is one of the important traditional medicinal plant belongs to family lamiaceae. Antibacterial activity of *Hyptis suaveolens* was studied (Mandal *et al.*, 2007; Witayapan *et al.*, 2007;

Asekun *et al*., 1999) and presence of analysis of photochemical was also studied (Iwu *et al*., 1990). Analysis, presence of photochemical and potent antimicrobial activity of plant extracts were studied in *Hyptis suaveolens* (Pachkore *et al*., 2011). In view of this the present study was aimed to evaluate antimicrobial and phytochemical analysis of selected plant extracts of *Hyptis suaveolens* against to some bacterial pathogens.

MATERIALS AND METHODS

Collection of Plant Materials:

Fresh *Hyptis suaveolens* whole plant and plant parts like stem, roots were collected randomly from banks of river Krishna, Vijayawada. Botanical identification of plant material was done by Department of Botany, Acharya Nagarjuna University, Guntur. The plant materials were washed thoroughly for 3-4 times with running tap water to remove dust from the surface and twice with sterile distilled water, shade dried at room temperature on a sterile blotting paper, after complete drying the plant materials were powdered using the blender and stored in separate air tight bottles then used for the preparation of chloroform and methanol extracts.

Preparation of Chloroform and Methanol Extract:

Air-dried 10 g of each powdered plant material was mixed in a conical flask with chloroform, then shaken at 120 rpm for 30 minutes and kept for 24 h. After 24 h, each of the extracts was filtered rapidly through four layers of gauge and then by a more delicate filtration through Whatman no.1 filter paper. The resulting filtrates were then concentrated in a rotary evaporator for 24 hours to collect the extract. Similar method was used to prepare methanol extract.

Test Microorganism:

The four bacterial strains, *Klebsiella pneumoneae, Staphylococcus aureus, Escherichia coli*, and *Pseudomonas aeruginosa* and three fungi *Canidida albicans Rhizophus stoloniphera and Aspergillus Niger* were collected from IMTECH, Chandigarh as test pathogens for antimicrobial activity assay.

Antibacterial Activity Determination:

A direct suspension of the test organisms was prepared in broth and the optical density (OD) of the solution was adjusted to 0.5 at 456 nm which corresponded to 1x 107 CFU. The antimicrobial activity assay was performed by sensitivity tests by agar well diffusion method. Mueller Hinton Agar was used as media as per CLSI guidelines. The test pathogens were inoculated in to Muller Hinton agar medium. After that 6 mm diameter were punched in agar plate. Different concentrations (10ul, 50 ul, and 100 ul) of plant extracts were poured in the wells. Standard antibiotic Streptomycin (10mg/ml) and blank (solvent) were used as positive and negative control. The plates were then incubated at 37°C for 24 hr to allow maximum growth of the microorganisms the antibacterial activity of the test samples was determined by measuring the diameter of zone of inhibition expressed in millimetre. The assay was repeated thrice and the mean of the three experiments was recorded.

Phytochemical Analysis:

Different parts of plant material were used for phytochemical tests were performed according to the methods of Johansen (1940) and Guerin (1971). The positive tests were noted as present (+) and absent (-).

RESULTS AND DISSCUSION

The results of antimicrobial activities of the *Hyptis suaveolens* were shown in Table 1. The extracts showed varying degree of inhibitory effect. The inhibition zone of extracts was directly proportional to increasing concentration of field grown different extract of *Hyptis suaveolens*. Whole plant extracts showed highest antimicrobial activity in compared with stems and roots in both chloroform and methanol extracts (Table 1). The small zone of inhibition was observed in 10µg concentration of whole plant chloroform extract against to *Bacillus subtilis*. No zone of inhibition was observed against to *E coli, Klebsiella pneumonia* and *Pseudomonas aeruginosa*. But zone of inhibition was observed in 10µg concentration of methanolic extract of stem and root, against to all pathogens. Stem and root showed minimum zone of inhibition (3.0mm - 9.0mm) with 10µg concentration of chloroform extract against to tested microorganisms. Methanolic extracts of stem, root and whole plant were showed highest antibacterial activity than chloroform extracts. Maximum zone of inhibition was observed in 100µg concentration of methanolic extracts of stem, root and whole plant against to all selected strains. The methanolic extract of whole plant showed highest zone of inhibition against *E. coli* at concentration 100ug. All plant part extracts of both solvents showed inhibitory activity against the *Candida albicans* ranging from 3 mm to 20 mm except the chloroform extract of concentration 10ug/ml. In the same way the *Rhizophus stoloniphera* and *Aspergillus Niger* were also showed zone of inhibition against all the two plant part extracts (Table.1)

Similar antibacterial activity was showed by Hyptis species in different studies. Solvent extracts of Hyptis species against to pathogen *Xanthomonas campestris* was also showed antimicrobial activity (John and Herin, 2011).

Nwobu *et al.* (2010) evaluated the phytochemical and antibacterial of Hyptis suaveolens of chloroform and methanol extracts of Hyptis plant parts against gram positive and gram negative organism using agar well diffusion method.

In other studies, whole plant extract of *Camellia sinensis* (Yam et al., 1997), extracts of stem, root, leaf, flower and whole plant of *Tridax procumbens*, (Aniel Kumar and Mutyala Naidu 2010), *Andrographis paniculata* (Aniel Kumar *et al.*, 2010), *Andrographis serpyllifolia* (Revathi et al., 2012), *Sida spinosa* Linn. (Selvadurai *et al.*, 2011), also showed antibacterial activity, among all extracts whole plant extract showed highest activity against to bacteria. The standard drug streptomycin (10ìg/ml) showed high degree of inhibition against *E. coli* and *Klebsiella pneumoniae*.

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 1: Antimicrobial activity of chloroform and methanol extracts against selected pathogens

Zone of Inhibition (mm)								
		Chloroform extracts			Methanolic extracts			
Bacterial Strains	Concentration (µg)	Stem	Root	Whole plant	Stem	Root	Whole plant	Antibiotic
Bacillus subtilus	10	-	-	2..2 ± 0.25	6..5 ± 0.45	4..1 ± 0.41	13..2 ± 0.2	10
	50	3..2 ± 0.11	7..3 ± 0.57	8.5±0.3	15.5 ± 0.45	12.9 ± 0.60	22.8 ± 0.75	
	100	9.6 ± 0.15	8.0 ± 0.20	17.2±0.25	24.1± 0.95	18.4 ± 0.89	27.2 ± 0.87	
E. coli	10	-	4.0 ± 0.26	-	7..6 ± 0.55	11.0 ± 0.35	17.2 ± 0.25	16
	50	4.6 ± 0.15	6.4 ±0.15 7.	6±0.20	11.0 ± 0.20	16.0 ± 0.12	17.0 ± 0.15	
	100	8.2 ± 0.1	5.0 ± 0.25	15.1± 0..2	17.0 ± 0.20	27.1 ± 0.32	29.3 ± 0..9	
Klebsiella Pneumonia	10	-	-	-	10.0 ± 0.15	6..3 ± 0.30	12.2 ± 0..25	17
	50	4..5 ± 0.15	3.4 ± 0.10	6.1± 0.15	3.4 ± 0.45	4.1± 0.15	18.0 ±.0..25	
	100	6.0 ± 0..3	7.7 ± 0.20	13.0 ± 0.11	0.3 ± 0.30	23.2 ± 0.26	24.7 ± 0..32	
Pseudomonas aeruginosa	10	-	-	-	3.1±0.15	2.1±0.23	4.8 ± 0..20	10
	50	3..3 ± 0.1	3.0 ± 0.1 9	6 ± 0.15	8..3 ±0.32	5..9 ± 0.25	10.2 ± 0.25	
	100	6..2 ± 0.20	5.1± 0.20	19.1± 0.32	11.2 ± .20	12.1 ± 0.11	14.3 ± 0.30	
Candida albicans	10	-	-	-	8..6 ± 0.51	11.0 ± 0.35	15.2 ± 0.25	15
	50	3.6 ± 0.11	4.4 ± 0.15	6±0.20	10.0 ± 0.22	15.0 ± 0.10	17.0 ± 0.10	
	100	7.o ± 0.1	5.0 ± 0.25	10.1± 0..2	15.0 ± 0.20	17.1 ± 0.32	20.3 ± 0..9	
Rhizophus stoloniphera	10	-	-	2..2 ± 0.25	6..5 ± 0.45	4..1 ± 0.41	13..2 ± 0.2	10
	50	3..2 ± 0.11	7..3 ± 0.57	8.5±0.3	15.5 ± 0.45	12.9 ± 0.60	22.8 ± 0.75	
	100	9.6 ± 0.15	8.0 ± 0.20	17.2±0.25	24.1± 0.95	18.4 ± 0.89	27.2 ± 0.87	
Aspergillus niger	10	-	-	-	8..6 ± 0.51	11.0 ± 0.35	15.2 ± 0.25	20
	50	3.6 ± 0.11	4.4 ± 0.15	6±0.20	10.0 ± 0.22	15.0 ± 0.10	17.0 ± 0.10	
	100	7.o ± 0.1	5.0 ± 0.25	10.1± 0..2	15.0 ± 0.20	17.1 ± 0.32	20.3 ± 0..9	

Table 2: Phytochemical Test of *H. suaveolens*

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

CONCLUSIONS

By the present studies and above results it can be concluded that *Hyptis suaveolens* extracts have great potential as antimicrobial compounds against tested bacterial strains and that they can be used in the treatment of infectious diseases caused by resistant microorganisms. Antibacterial properties of *Hyptis suaveolens* plant extracts or whole plant extract can be effectively used for common infectious diseases causing bacterial species.

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