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Research Article

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A study on phytochemical analysis and antimicrobial activity of *Hyptis suaveolens (L.) poit*

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ABSTRACT

In the present work an attempt has been made to carryout screening phyto constituents and antimicrobial ability of various extract of Hyptis suaveolens (L.) poit .The aim of the study is to select an active plant extract useful in developing new lead compounds to control infectious disease. Antimicrobial activity was screened by disc diffusion method. Antibacterial and antifungal activities of aqueous, ethanol, methanol, chloroform extract of Hyptis suaveolens (L.) poit were tested against Escherichia coli, Staphylococcus aureus, pseudomonas aeruginosa, proteus vulgaris and Aspergillus Niger, Aspergillus flavus, Fusarium, Rhizopus. The antimicrobiall activity was observed by measuring the width of the inhibitory zones. The extent of antimicrobial activity is varied depending upon solvent that has been used. Chloroform extract showed good inhibitory activity against Escherichia coli, Staphylococcus aureus. Ethanolic extract showed highest inhibitory activity against pseudomonas aeruginosa, proteus vulgaris. For Aspergillus Niger , Methanolic extract showed maximum inhibitory activity where as chloroform extract showed maximum inhibitory zone against Aspergillus flavus. Methanolic extract showed best inhibitory ability against Fusarium where as chloroform extract showed highest inhibitory action against Rhizopus. Finally it can be concluded that the active chemical compounds present in Hyptis suaveolens (L.) poit showed certainly fine place in treatment of bacterial and fungal infectious disease.

Key words: *Hyptis suaveolens (L.) poit* phytochemical analysis Antimicrobial activity, Antifungal, Antibacterial.

INTRODUCTION

Plants have been used to treat or prevent illness since before recorded history. The sacred Vedas dating back between 3500 B.C and 800 B.C give many references of medicinal plants [1].Plants and plant based medicaments are the basis of many of the modern Pharmaceuticals we used today for our various ailments [2]. However, the knowledge of medicinal plant is rapidly dwindling due to the influence of Western lifestyle, reducing in number of generations to carry on the use of plant species in traditional medicine which has increased the interact throughout the world [3].

Chemical constituents in the plant are responsible for their medicinal as well as their toxic properties [4]. They are organic substances and could be obtained in both primary and secondary metabolic process; they also provide a source of medicine since the earliest time. The most important of these bioactive constituents of plants are steroids,

terpenoids, carotenoids, flavanoids, alkaloids, tannins, and glycosides. Plants in all facet of life have served a valuable starting material for drug development [5]

The traditional medicinal methods, especially the use of medicinal plants, still play a vital role to cover the basic health needs in the developing countries and moreover, the use of herbal remedies has risen in the developed countries in the last decade.

The present study was carried out to evaluate the antibacterial and antifungal activity of aqueous, ethanol, methanol, chloroform extract of Hyptis *Suaveolens (L.) poit* against *Escherichia coli, Staphylococcus aureus, pseudomonas aeruginosa, proteus vulgaris* and Aspergillus *Niger, Aspergillus flavus, Fusarium, Rhizopus.*

The plant, *Hyptis suaveolens* (L) Poit commonly known as *Wilayati tulsi* belongs to the family Lamiaceae and is an ethno botanically 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 [6]. Crude leaf extract is also used as a relief to colic and stomach-ache. Leaves and twigs are considered to be antispasmodic and used in antirheumatic and antisuporific baths [7], anti-inflammatory, antifertility agents [8], 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 [9].

Pictorial presentation of *Hyptis suaveolens* (L) Poit

EXPERIMENTAL SECTION

Collection of plant materials

Fresh leaves of *Hyptis suaveolens (L.) Poit* were collected from Kannukudi, Thanjavur (DT), Tamilnadu, India. They were identified by Dr.S.John britto, The Director, The Rapinet herbarium St.Joseph's college, Thiruchirapalli and a voucher specimens were deposited in the Rapinet herbarium of St.Joseph's College, Thiruchirapalli (Voucher number K G 001/ 2013).

Preparation of power

The leaves of plants were dried under shade. The dried materials were mechanically powdered sieved using 80 meshes and stored in an air tight container. The powdered material was used for further photochemical, and antimicrobial activity.

Extraction of plant material

Aqueous, Methanol, Chloroform and ethanol extracts were prepared according to the methodology of Indian pharmacopoeia. The coarse powder material was subjected to soxhlet extraction separately and successively with ethanol and distilled water, .methanol, chloroform, these extracts were concentrated to dryness in flash evaporator under reduced pressure and controlled temperature (40°c to 50°c) the aqueous and ethanol extracts put in air tight container stored in a refrigerator

Phytochemical Analysis

Qualitative phytochemical analyses were done by using the procedures of kokate et al. (1995). Alkaloids, Tannins, Phenols, Flavonoids, Steroids, Terpenoids, Glycosides Carbohydrates, Protein, Quinones, and Saponins were qualitatively analysed.

Antimicrobial activity

Microorganisms selected

Totally eight human pathogens were selected for the present investigation four microbial strains namely *E.coli, staphylococcus aureus, pseudomonas aerogens proteous valgari and* Fungi: *Aspargillus niger, Aspargillus flarus, fusarium, Rhizopus*, .The pure microbial cultures were collected from the microbiology laboratory of Sengamala Thayaar Educational Trust, Mannargudi.

Bacterial inoculam preparation

The young microbial inoculam was prepared and used in the entire research period. The nutrient broth were prepared and poured into tubes and sterilized. The culture was inoculated in the tube using inoculation needles or loops. The tubes were incubated at 37°c for 24 hours for bacteria.

Media preparation

Fungal inoculum preparation

The invitro method proposed by National committee for clinical Laboratory standard for testing molds [10] was followed for the present study. The fungal stock inoculam suspension was prepared from seven days old culture grown on PDA medium. The fungal colonies were covered with 10 ml of sterile distilled water and suspension well made by gently probing the surface with the tip of the Pasteur pipette and transferred to sterile tube. Heavy particles were allowed to settle for 5 – 20 minutes, and the homogenous suspension were collected and mixed with a vortex mixture. The density of this suspension was adjusted with a spectrophotometer at a wavelength of 530 nm for 80 – 85 % to obtain the standard inoculum.

Antimicrobial activity of plant materials

Disc diffusion method

The antimicrobial activities of the aqueous, ethanol, methanol, chloroform, extracts were tested using spread plate method. The disc was prepared by using whatmann no 1 filter paper, then the filter paper disc of 5 mm in diameter were sterilized and soaked in the different extract of plant material. The culture media is inoculated when the medium maintains a temperature of 45°c so that the cells can be distributed thoroughly , then the medium containing the culture is poured in the sterilized petriplates, allow to solidify and then incubated colonies appear on the agar surface. The filter paper disc soaked in aqueous, ethanol, methanol, and chloroform, extract of. Hyptis suaveolens

(L) poit. Were placed on the surface of the microbial seeded nutrient plate and then the plate was incubated at 37°c. The antimicrobial activities were recorded by measuring the width of the clear zone around each disc.

Preparation of Antibiotic disc

Commercially available ciprofloxacin antibiotic was used for the study and the antibiotic (500 mg) was prepared for disc by dissolving antibiotic powder in 100 ml of distilled water.

Antibiotic sensitivity method

The isolated microorganisms were tested with antibiotic for the susceptibility by disc diffusion method. Antibiotic disc were used to detect antibiotic sensitivity of the microbial suspension from nutrient broth. The antibiotic disc were placed on the inoculated plates and incubated at 38°c for 24 hours antibiotic sensitivity was assayed from the diameter of zones.

RESULTS AND DISCUSSION

Phytochemical analysis

Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, and flavonoids which have been found invitro to antimicrobial properties. The biologically active compounds are screened by dissolving the crude powder in various solvent. Phytochemical studies of various extract of *Hyptis suaveolens (L.) poit* were investigated. The alkaloids, flavonoids and phenolic compounds can serve as antimicrobial agent. The result showed that aqueous extract contains alkaloids, glycosides, carbohydrates, proteins, steroids, flavonoids, phenols, terpenoids and devoid of quinines, saponins. In case of ethanolic extract alkaloids, glycosides, carbohydrates, proteins, steroids, flavonoids, phenols, terpenoids, Quinones were present. Methanolic extract contains alkaloids, glycosides, carbohydrates, proteins, steroids, flavonoids, phenols. Chloroform extract gives positive result to the test for alkaloids, glycosides, carbohydrates, proteins, steroids, flavonoids, phenols.

Table 1: Qualitative Phytochemical Analysis of *Hyptis suaveolens (L.) Poit*

S.No	Phytochemical constituents	*Plant Extracts*			
		Aqueous	Ethanol	Methanol	Chloroform
1	Alkaloids	+	+	+	-
2	Carbohydrates	+	+	+	-
3	Glycosides	+	+	+	+
4	Terpenoids	+	+	-	-
5	Protein	+	+	+	+
6	Steroids	+	+	+	+
7	Flavonoids	+	+	+	-
8	Phenols	+	+	+	+
9	Tannins	+	+	+	+
10	Quinones	-	+	-	+
11	Saponins	-	-	-	-

(+) Indicates Presence, (-) Indicates Absence

Table2: Antimicrobial activity of *Hyptis suaveolens* Linn(poit)

Plant Extracts	Zone of inhibition in diameter(mm)							
	E.coli	*S.aureus*	*P.aeruginosa*	*P.vulgaris*	*A.niger*	*A.flavus*	*Fusarium*	*Rhizopus*
Aqueous	6	6	-	-	-	-	-	-
Ethanol	13	10	14	15	12	14	12	10
Methanol	14	11	13	12	14	13	14	12
Chloroform	15	1 3	12	13	10	15	11	14

Antibacterial activity

The antibacterial activity was observed by measuring the width of the inhibitory zones. The result obtained in this present study is summarized below

Aqueous extract of Hyptis *suaveolens (L.) Poit* showed 6mm in diameter of inhibitory zone against both *Escherichia coli* and *Staphylococcus aureus*. In case of ethanolic extract the inhibitory zone was found by 13mm, 10mm against Escherichia coli, *Staphylococcus aureus* respectively. Methanolic extract also showed significant inhibitory action (14mm) against *Escherichia coli* and 10mm against *staphylococcus aureus*. Chloroform extract

of *Hyptis suaveolens (L.) poit* Showed 15mm in diameter of inhibitory zone against *Escherichia coli* and the inhibitory zone against *staphylococcus aureus* was found to be 13mm in diameter.

Aqueous extract showed no inhibitory action against both *Pseudomonas aeruginosa*, *Proteus vulgaris.* Ethanolic extract showed 14mm in diameter of inhibitory zone against *Pseudomonas aeruginosa* and the inhibitory zone against *Proteus vulgaris* was found to be 15mm in diameter. The inhibitory zone of Methanolic extract against *Pseudomonas aeruginosa*, *Proteus vulgaris* were found to be 13mm, 12mm respectively. Chloroform extract exhibits 12mm of inhibitory zone against *Pseudomonas aeruginosa*, 13mm against *Proteus vulgaris*

Antifungal activity

Aqueous extract showed no inhibitory activity against both .*Aspergillus Niger*, *Aspergillus flavus*. Methanolic extract showed highest activity of 14mm inhibitory zone against *Aspergillus Niger followed* by 13mm against Aspergillus *flavus*. Ethanolic extract showed 12mm inhibitory zone against Aspergillus *Niger* where as chloroform extract showed 10mm inhibitory zones against *Aspergillus Niger*. Ethanolic extract showed 14mm of inhibitory zones against *Aspergillus flavus* where as chloroform extract showed 15mm against *Aspergillus flavus*.

Ethanolic extract showed 12mm inhibitory zones against *Fusarium* and 10mm against *Rhizopus,* inhibitory zone of 14, 12mm showed by Methanolic extract against Fusarium, *Rhizopus* respectively. Chloroform extract showed significant inhibitory activity (11mm, 14mm) against both *Fusarium*, and *Rhizopus.*

CONCLUSION

The extent of antimicrobial activity is varied depending upon solvent that has been used. Finally it can be concluded that the active chemical compounds present in *Hyptis suaveolens (L.) poit* showed certainly fine place in treatment of bacterial and fungal infections. The results from the present study is very encouraging and it indicates that the mechanism of activity of antimicrobial ability should be studied more extensively to explore its potential in the treatment of infectious disease

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