

ESSENTIAL OIL EXTRACTION AND PRELIMINARY PHYTOCHEMICAL SCREENING OF LEAVES EXTRACT OF *Hyptis suaveolens* AND ITS ANTIBACTERIAL ACTIVITY

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ABSTRACT

Hyptis suaveolens (L.) Poit (Lamiaceae) is an aromatic plant found throughout India. Essential oil produced from plant reported to possess antibacterial. Antifungal, antiplasmodial properties. The extraction of essential oil from *Hyptis suaveolens* using water distillation method by Clevenger apparatus, The antibacterial activity of essential oil of *Hyptis suaveolens* is screened by disc diffusion method. The antibacterial potential of essential oil of *Hyptis suaveolens* were tested against *Escherichia coli*, *Staphylococcus aureus*. The antibacterial activity was observed by measuring the width of inhibitory zones. The antibacterial potential of Essential oil varied depending upon the strains of bacteria. Traditionally, the plant extracts were used to cure swellings and various disorders. the leaves extract of plant in ethanol was screened by phytochemically. Phytochemical screening of the plants showed the presence of Flavonoids, Alkaloids, Saponins, Phenols, Proteins, Tannins etc. Phytochemicals like alkaloids, flavonoids, phenols, saponins and tannins isolated from the plant has great medicinal and pharmaceutical importance.

KEYWORDS: *Hyptis suaveolens*, Phytochemistry, Essential Oil, Secondary Metabolites, Antibacterial Activity

The Lamiaceae is a relatively common botanical family; many of the species are used as the medicinal or aromatic herbs in the industries such as the Cosmetics, foods, hygienic products and perfumery (Rajesh, *et al.*, 2011).

Hyptis suaveolens (L.) Poit is a medicinal plant used in traditional medicines. Medicinal constituents include the strong essential oils, tannin, Flavanoids etc. Essential oils are the odoriferous oils obtained from plant. Essential oil shows antibacterial activity, phytochemical screening of leaf extracts lead to the presence of secondary metabolites. Essential oils and secondary metabolites are the major reason for medicinal properties (Gamble, 1921).

Chemical Composition and Physico-chemical characteristics of *H. suaveolens*, A work by Oliveria. In essential oil composition of *H. suaveolens*, edaphic factors and grown phase (vegetative, flowering and fruiting stage) have been shown to present a significant correlation with the essential oil composition.

In other report, the essential of Tanzania showed strong antifungal activity against *Mucor sp* and *Fusarium moniliforme* (Malele, *et al.*, 2003). From *H. suaveolens* collected in India, (Sharma, *et al.*, 2008) obtained an essential oil composed of 1,8-cineole(44.4%), α -pinene (11.7%), (E)-caryophyllene (10.0%), camphene (5.7%) and β -myrcene (5.3%).this oil was active against the pathogen *Fusarium oxysporum*, *F. gladioli* (Sharma, *et al.*, 2008)and the oil treatment caused a decrease of conidiation and anomalies in the hyphae such as decrease in the diameter of hyphae and granulation of cytoplasm (Tripathi, *et al.*,2009).

The preliminary phytochemical analysis of leaf extract of *Hyptis suaveolens* revealed the presence of sterols, alkaloids, flavanoids and tannins, flavonoids are known to reduce lipid peroxidation not only by preventing or slowing the onset of cell necrosis but also by improving vascularity. Hence, any drug that inhibits lipid peroxidation is believed to increase the viability of collagen fibrils by increasing the strength of collagen fibres, increasing the circulation, preventing the cell damage and by promoting the DNA synthesis (Getie, *et al.*, 2002).

MATERIAL AND METHODS

Hyptis suaveolens is a member of Lamiaceae very common weed, strongly aromatic.it is an important source of essential oil.



Extraction of Essential Oil

Extraction of essential oil through Hydrodistillation, the plant parts are packed with 700 ml of water in a round flask of water is added and brought to a boil about 75-100°C. due to the influence of hot water and steam, the oil is freed from oil glands. Hydrodistillation carried out by Clevenger apparatus.

Antibacterial Activity

The antibacterial activity of essential oil from the plant parts of *H. suaveolens* were screened through Disc diffusion method. *S. aureus* (Gram positive) and *E. coli* (Gram negative) are used in antibacterial assay. Muller Hinton agar medium was used for the disc diffusion

method. 1.0 ml bacterial broth of 10⁵ cells were swabbed with sterile cotton swab on the sterile medium in the petriplates, already and kept, under the aseptic conditions of the inoculation chamber. Blank sterile discs was impregnated with 25µl of the essential oil containing about 100-500 µg of essential oil. For each set of experiments controls were maintained pure solvents were used instead of the extract.

Preliminary Phytochemical Analysis

Qualitative phytochemical analysis done by *H. suaveolens* ethanolic leaf extract. Secondary metabolites like Alkaloids, Flavonoids, Phenols, Steroids, Saponins, Tannins and Proteins were detected by test.

Test	Reagents	Result
Alkaloids	1 mL extract + Dragendroff's reagent	Orange colour
Flavonoids	2 mL filtrate(0.1mg extract + 10mL ethanol) + conc.Hcl + magnesium ribbon	Pink/red colour
Phenols	2 mL extract + 10% aqueous ferric chloride	Blue/Green colour
Steroids	Extract + Chloroform + Conc. H ₂ SO ₄	Bluish red/Violet red/Reddish brown colour
Saponins	1 mL extract + 1 mL water	Foam produced & persistent for 10 minute
Tannins	1 mL extract + 1% ferric chloride	Blue green/Brownish green colour
Proteins	2 mL extract + 2 drop of Millions reagent (heat)	White ppt turns brick red after boiling

RESULT AND DISCUSSION

Essential Oil Extraction

100gm of plant parts are weighed out and packed with 700 ml water in a bottom flask and heated in 75-100°C; due to the influence of hot water and steam, the essential oil freed from oil glands. The essential oils are isolated by hydrostillation using Clevenger apparatus.

The extraction is fast at beginning but gets slow gradually. When the maximum amount of extractable oil is obtained, the oil yield level remains constant even by extending the extraction time. Approximately 0.3-0.5ml essential oil is extracted using Clevenger apparatus.

Antibacterial Activity of Essential Oil

The antibacterial activity of essential oil from the plant parts of *H. suaveolens* were screened against 2 bacterial strains, 1 gram positive (*S. aureus*) and 1 gram

negative (*E. coli*). The antibacterial potential of essential oil were measured in terms of zone of inhibition of bacterial growth. The results of the present study are shown in (Fig.)

The antibacterial effect of essential oil of *H. suaveolens* is compared with the antibacterial potential of a standard antibiotic (Streptomycin) and the essential oil is dissolved in control (Dimethyl sulphoxide). In present study according to the data the essential oil of the *H. suaveolens* shows more killing effect towards gram positive bacteria (*S. aureus*) than gram negative bacteria (*E. coli*). The growth inhibition zone measured in centimeters. The zone of inhibition of *E. coli* is negligible and zone of inhibition of *S. aureus* is 0.4 cm. The standard drug streptomycin have high zone of inhibition ranging from 2-2.3 cm. The control is DMSO –Dimethyl Sulphoxide doesn't have any antibacterial potential.

Cultures	Essential Oil (Sample)	Streptomycin (Antibiotic)	Dimethyl Sulphoxide (Control)
<i>E.coli</i>	0	1.7	0
	0	1.6	0
	0	1.7	0
	0	1.6	0
	0	1.6	0
	0	1.6	0
<i>S.aureus</i>	0.3	2.3	0
	0.4	2.3	0
	0.4	2.2	0
	0.3	2.2	0
	0.4	2.3	0
	0.3	2.3	0

Phytochemical Screening

The members of Lamiaceae are rich in a wide variety of secondary metabolites such as alkaloids, flavonoids, steroids, tannins etc. Therefore the present study involves a preliminary screening of the phytochemicals in leaf extracts of *Hyptis suaveolens*. Ethanol is used to make the extract.

Sl. No.	Phyto-constituents	intensity
1	Alkaloids	+++
2	Flavanoids	+++
3	Phenol	++
4	Steroid	+
5	Saponins	-
6	Tannins	+++
7	Proteins	+

Key:+=present;+=present in moderately high level;+++present in high level;-=absent

DISCUSSION

The essential was extracted using Clevenger apparatus, the extraction of oil increased with time.it is also can be observed that the rate of extraction is fast at the beginning of the extraction but gets slow gradually (Sayyar, 2009).

Appearance of orange colour indicates the presence of Alkaloids in the leaf extract. The pharmacological significances of alkaloids are reported by various workers in a variety of plants.

Red colour indicates the presence of Flavonoids. It forms complex with cell wall, binds to adhesions and showed antimicrobial activity(Cowan,1999).

The foam produced does not persists so the ethanolic extract of leaf is devoid of saponins

Blue green or brown green colour in ferric chloride test indicates the presence of tannins. Tannins are well known antimicrobial agents that could inhibit the growth of microorganisms and can be used against diarrhea (Trease and Evans,2002).

Proteins are present in the ethanolic extract of plants. The proteins are found to be bioactive against certain ailments (Kreis, 1989).

Antimicrobial screening of plant extracts and phytochemicals then, represents a starting point for antibacterial drug discovery. The present study utilises disc diffusion technique to evaluate the antibacterial potential of essential oil of *Hyptis suaveolens*.

The bacteria assayed include both gram positive (*Staphylococcus aureus*) and gram negative (*Escherichia coli*) strains. The essential oil of produced good results against Gram positive bacteria (*S. aureus*) and does not shows any antibacterial activity against Gram negative bacteria *E. coli*.

Plants are shown to be important source of antibacterial agents capable of inhibiting *S. aureus* including drug resistant strains. A number of studies have been undertaken which report the efficacy of plant extracts and their purified components to inhibit *S. aureus* (Mandal, *et al.*, 2011).

Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids and flavanoids, which have been found *in vitro* to have antimicrobial properties (Cowan, 1999).

The ethanolic leaf extracts of *H. suaveolens* expressed positive results towards secondary metabolites. The presence of Alkaloids, Flavonoids, Phenols, steroids, Tannins and Proteins were moderately high in ethanolic leaf extract of *Hyptis suaveolens*.

CONCLUSION

The present study revealed that about 0.2-0.3ml essential oil can be extracted from 100gm of fresh material of *Hyptis suaveolens* through water distillation by Clevenger apparatus. In this work the Antibacterial activity of essential oil is studied and it is revealed that that the antibacterial potential of essential oil is varied for gram negative and gram positive bacteria. The essential oil shows more activity against gram positive bacteria. In conclusion the present study demonstrates that the ethanolic extract of *Hyptis suaveolens* contains pharmacologically active substances, The preliminary phytochemical screening revealed that the presence of secondary metabolites in the ethanolic extract. This could be crucial step in further studies on the phytochemical, biological. Structure-function relationship of *Hyptis suaveolens* which is already reported to be of therapeutic importance.

The present data provided a support for the traditional use of the plant as a medicine for many diseases. However further studies will be necessary to isolate and characterize the active principles which are responsible for antibacterial activity and detect the role of phytochemical constituents in the plant for understand the exact medicinal value of *Hyptis suaveolens*.

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