



Research Article

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**Analysis of phytochemical, antimicrobial and disinfectant properties of leaf extracts of *H. suaveolens* (L.) Poit**

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**ABSTRACT**

*Hyptis suaveolens* L. Poit., is an aromatic herb which is reported to have high medicinal value. This plant is widely seen in Kerala, India. In the present study the fluorescence analysis was performed and the plant powder was found pure. The phytochemical analysis revealed the presence of phenols, tannins, flavanoids etc. in chloroform, ethyl acetate, methanol and aqueous extracts. Disc diffusion method was performed to evaluate the antimicrobial activity of chloroform, ethyl acetate, methanol and aqueous extracts of *H. suaveolens* leaf against six bacterial strains (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Bacillus cereus* and *Staphylococcus aureus*) and five fungal strains (*Trichophyton rubrum*, *Aspergillus fumigatus*, *Penicillium expansum*, *Fusarium oxysporum* and *Aspergillus niger*). Methanolic extract showed the highest and consistent inhibition with an inhibition zone of 30 mm against *K. pneumonia*, 25 mm against *B. cereus* and 12 mm against *P. aeruginosa*. The aqueous extract showed the highest antifungal activity with an inhibition zone of 31 mm against *F. oxysporum*. Disinfectant property (as a floor cleaning agent) of fresh aqueous leaf extract of *H. suaveolens* was tested and an initial bacterial count of 435 colony forming units (CFU) was observed from the floor before the application of extract. Five minutes after the extract application, the bacterial count was decreased from 435 to 3 colony forming units.

**Keywords:** phytochemical, antimicrobial, disinfectant, floor cleaning, *Fusarium oxysporum*

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**INTRODUCTION**

Plants are a rich source of secondary metabolites and a potent source of antimicrobial compounds. Researchers around the globe are screening the antimicrobial agents present in medicinal plants, as they are being used in traditional/alternative healthcare systems. Screening of medicinal plants for therapeutically active bio-molecules including those with antimicrobial properties has gained an unprecedented importance in the recent years and World Health Organization has recently shown genuine interest in promoting the development and utilization of indigenous medicinal plant resources in the developing countries so as to extend safe and effective healthcare to maximum number of population in those countries [1]. Now a days many pharmaceutical companies are showing great interest in plant derived drugs mainly due to the current widespread belief that 'Green Medicine' is effective, safer and more reliable than synthetic drugs [2].

*Hyptis suaveolens* L. Poit., a potent medicinal herb, is commonly known as wilayati tulsi and it belongs to the family Lamiaceae. The extracts and oils of *H. suaveolens* were reported to have antimicrobial activities against some

bacteria, fungi [3, 4] and insecticidal effects on stored grain coleopteran pests and larvae of Yellow fever mosquito [5, 6]. The leaves of this plant have been used as a stimulant, carminative, sudorific and as a cure for parasitic cutaneous diseases whereas the crude leaf extract is used as a relief to colic and stomach ache. Leaves and twigs are considered to be antispasmodic and used in anti rheumatic and antisudorific baths, an anti-inflammatory agent and also applied as an antiseptic in burns, wounds and other skin related problems [7]. The decoction of the roots is highly valued as an appetizer and is reported to contain urosolic acid, a natural HIV – integrase inhibitor [8]. *H. suaveolens* is also used in the treatment of respiratory and gastrointestinal infections, indigestion, colds, pain, fever, cramps and skin diseases [9, 10]. The leaves are used as an anticancer and antifertility (in females) agents, while their aqueous extract has showed an antinociceptive effect and acute toxicity [11]. The results of different properties and uses of *H. suaveolens* reported by investigators prompted us to study further.

The present investigation was carried out to screen the phytochemical, antibacterial, antifungal activities and disinfectant property (as a floor cleaning agent) of different solvent extracts of *H. suaveolens* leaf samples.

## EXPERIMENTAL SECTION

### Collection and identification of plant

The plant *Hyptis suaveolens* was collected from S.D.V. College of Arts and Applied Science, college campus, Alappuzha, Kerala, India. The plant material was identified by Dr. Shaji P.K., Scientist, Environmental Resources Research Centre (ERRC), P.B. No. 1230, P.O. Peroorkada, Thiruvananthapuram, Kerala state, India. The plant leaves were washed several times with water, shade dried and then pulverized to coarse powder in an electric grinder. The powder was then stored in airtight bottles for further studies.

### Fluorescence analysis

Fluorescence analysis was performed as per the method described by Kakoshi *et al.* [12]. Many herbs fluoresce when the cut surface or powder is exposed to UV light and this can help in their identification method. A small quantity of leaf powder was taken and two to three drops of different organic solvents like 5% H<sub>2</sub>SO<sub>4</sub>, 1N HCl, 5% FeCl<sub>3</sub>, 1N NaOH, 5% KOH, 1N NaOH in water, methanol, acetic acid, chloroform, acetone, water and 50% HNO<sub>3</sub> were added separately and mixed well. The fluorescence character of the plant powders (40 mesh) was studied under short UV (280 to 100 nm), long UV (400 to 315 nm), and visible (390 to 700 nm) light.

### Analysis of phytochemical constituents

Analysis of the plant for various phytochemical constituents present was carried out using standard methods [13, 14, 15].

### Test for Carbohydrates

Molisch's test was performed to detect carbohydrates. Added a few drops alcoholic solution of alpha naphthol to the extracts. Then added 1 ml of concentrated sulphuric acid along the sides of the test tube. Formation of violet ring at the junction of the liquids indicated the presence of carbohydrates.

### Alkaloids

Crude extract was mixed with 2 ml of Wagner's reagent. Reddish brown colored precipitate indicated the presence of alkaloids.

### Cardiac Glycoside

Keller-Kelliani test was performed to detect cardiac glycoside. 5 ml of extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayered with 1 ml of concentrated H<sub>2</sub>SO<sub>4</sub>. A brown ring of the interface indicated a deoxy sugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout the thin layer.

### Coumarin glycoside

10 % NaOH was added to the extract and chloroform was added for observation of yellow colour, which showed the presence of coumarin.

**Saponins**

Foam test was performed to test the presence of saponins. To 2 ml of the extract, added 6 ml of water in a test tube and was shaken vigorously, then observed for the formation of persistent foam that confirms the presence of saponins.

**Flavonoids**

Alkaline reagent test was performed to test the presence of flavonoids. Crude extract was mixed with 2 ml of 2% solution of NaOH. An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid which indicated the presence of flavonoids.

**Phytosterols**

Salkowski test was used to detect phytosterols. To 2 ml of aqueous extract, 2 ml of chloroform and 2 ml of concentrated H<sub>2</sub>SO<sub>4</sub> was added. The solution was shaken well. As a result the chloroform layer turned red and acid layer showed greenish yellow fluorescence.

**Fats and oil**

Spot test was performed for fats and oils. This was done by preparing spot on the filter paper with the test solution and oil staining on the filter paper indicated the presence of fixed oil and fats.

**Phenols and tannins**

Crude extract was mixed with 2 ml of 2 % solution of FeCl<sub>3</sub>. A blue green or black coloration indicated the presence of phenols and tannins.

**Proteins**

Ninhydrin test was performed to detect the presence of proteins. Crude extract when boiled with 2 ml of 0.2 % solution of ninhydrin, violet color appeared indicating the presence of amino acids and proteins.

**Antimicrobial activity: Bacterial and fungal strains**

Six bacterial strains and five fungal strains were used in the present study. Out of the six bacterial cultures investigated, two are gram positive, *Staphylococcus aureus* (MTCC 902), *Bacillus cereus* (MTCC 430) and the remaining four are gram negative. They are *Escherichia coli* (MTCC 729), *Salmonella typhi* (MTCC 3216), *Klebsiella pneumoniae* (MTCC 432) and *Pseudomonas aeruginosa* (MTCC 4676). *Trichophyton rubrum* (MTCC 296), *Aspergillus fumigatus* (MTCC 343), *Penicillium expansum* (MTCC 2006), *Fusarium oxysporum* (MTCC 284) and *Aspergillus niger* (MTCC 282) are the fungal strains used in the study. All the microbial strains were procured from the Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India. These bacterial strains were sub cultured frequently in suitable agar slants and stored at 4°C for further studies.

**Preparation of inoculum**

The bacterial strains were inoculated in nutrient broth and incubated at 37°C in shaking incubator, while the fungal strains were inoculated in potato dextrose broth and incubated at 25°C in shaking incubator. The 24 h old microbial cultures were used for further studies.

**Disc diffusion assay**

Antibacterial assay was carried out by disc diffusion method and it was evaluated by measuring the diameter of the inhibition zone [16]. Different solvent (chloroform, ethyl acetate, methanol and aqueous) extracts of *H. suaveolens* were tested against the six bacterial and five fungal strains. The test bacterial and fungal cultures were evenly spread over nutrient agar and potato dextrose agar plates respectively using a sterile cotton swab. The sterile discs (6 mm in diameter) were impregnated with the respective extract solution was placed over the inoculated agar plates. Nutrient agar plates were then incubated at 37°C for 24 hours and potato dextrose agar plates at 25°C [17]. The zone of inhibition was subsequently measured in millimeter (mm). This experiment was done in triplicates and mean of the three experiments was recorded.

**Disinfectant property of *H. suaveolens* fresh leaf extract**

For analyzing the disinfectant property (as a floor cleaning agent) of *H. suaveolens* fresh aqueous leaf extract, 25 g fresh plant leaf was collected and grind using mortar & pestle in 25 ml sterile distilled water. 10 ml of aqueous extract was applied on the floor and the swabs were taken after an interval of 5, 10, 30, 50, 70, 90 and 120 minutes

and spread on nutrient agar plates for the enumeration of bacteria. The initial microbial load (denoted as zero) before the application of plant extract was also enumerated. The nutrient agar plates were incubated at 37°C in an incubator and potato dextrose agar plates were incubated at 25°C. All the experiments were done in duplicates and the average values were presented.

## RESULTS AND DISCUSSION

### Fluorescence analysis

The different colour characteristics observed for *H. suaveolens* leaf powder when exposed to different chemicals. The samples showed unique colours characteristics. It varied from light green to dark green and in certain cases it was yellowish green. The chemicals such as methanol, 1N NaOH, chloroform, 1N NaOH in water and in acetone, when exposed to short and long UV, it showed black colour. The presence of unique colours indicated the absence of any adulteration. The results of other fluorescence analysis performed when exposed to other chemicals are given in table 1.

Table 1: Fluorescence analysis of dried powder of *H. suaveolens*

Sl. No.	Chemical/solvent	Visible light	Short UV	Long UV
1	Powder as such	Light green	Dark green	Dark green
2	Methanol	Dark green	Dark green	Black
3	Acetic acid	Dark green	Yellowish green	Dark green
4	Water	Light green	Light green	Dark green
5	1N NaOH	Dark green	Black	Black
6	50% HNO <sub>3</sub>	Light green	Light green	Dark green
7	1N HCl	Light green	Dark green	Light green
8	5% FeCl <sub>3</sub>	Light green	Yellowish green	Dark green
9	1N NaOH in water	Dark green	Black	Black
10	Chloroform	Dark green	Black	Black
11	Acetone	Dark green	Dark green	Black
12	5% H <sub>2</sub> SO <sub>4</sub>	Light green	Dark green	Light green
13	5% KOH	Dark green	Dark green	Light green

The ultra violet light produces fluorescence in many natural products which do not visibly fluoresce in day light. If substance themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by applying different reagents. Hence crude drugs are often assessed qualitatively in this manner and it is an important parameter for pharmacognostic evaluation of crude drugs [12, 18]. Fluorescence studies help in the identification and authentication of the plant material and this information can act as reference information for exact identification of a particular plant and will also be useful in making a monograph of the plant. In addition to this, it will act as a tool to detect adulterants and substituent and will help in maintaining the quality, reproducibility and efficacy of natural drugs [19, 20].

Table 2: Phytochemical analysis of leaf extracts of *H. suaveolens*

Sl. No.	Tests	Method	Presence (+) or absence (-) phytochemicals			
			Chloroform	Ethyl Acetate	Methanol	Aqueous
1	Carbohydrates	Molisch's test	+	-	-	-
2	Alkaloids	Wagners test	-	+	-	-
3	Cardiac Glycoside	Keller killiani test	+	+	+	-
4	Coumarin glycoside	Made alkaline	-	+	+	+
5	Saponins	Foam test	+	-	+	+
6	Flavonoids	Alkaline reagent test	+	+	+	+
7	Phytosterols	Salkowski test	+	+	-	-
8	Fats & oil	Spot test	+	+	+	-
9	Phenols	Ferric Chloride test	+	+	+	+
10	Tannins	Ferric Chloride test	+	+	+	+
11	Proteins	Ninhydrin test	-	-	-	-

“+” indicate presence and “-” indicate absence

### Phytochemical analysis

Qualitative phytochemical analysis of *H. suaveolens* leaf was performed for chloroform, ethyl acetate, methanol and aqueous extracts. The results indicated the presence of phenol, tannin and flavanoid in ethyl acetate, methanolic,

chloroform and aqueous extracts and also indicated the absence of proteins in the respective extracts. The results of other phytochemical tests performed were given in the table 2.

Phytochemical studies proved that *H. suaveolens* is a good source of phenols, flavonoids, tannins and other phytochemicals. Prince *et al.* [21] also reported that *H. suaveolens* is an important source of essential oils, alkaloids, flavonoids, phenols, saponins, terpenes, and sterols. It has been reported that phytochemicals present in plants are vital sources of antiviral, antitumor and antimicrobial agents so they are used as constituents in allopathic medicine [22, 23]. Phenolic compounds are used in several industrial processes to manufacture chemicals such as pesticides, explosives, drugs and dyes. They are also used in the bleaching process of paper manufacturing. Apart from these functions, phenolic compounds have substantial allelopathic applications in agriculture and forestry as herbicides, insecticides, and fungicides [24].

### Antibacterial activity

Table 3: Antibacterial activity of leaf extracts of *H. suaveolens*

Extracts	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i>	<i>S. typhi</i>	<i>B. cereus</i>	<i>S. aureus</i>
Chloroform	2 ±0.25	25±0.18	9±0.2	Nil	14±0.25	1±0.13
Ethyl acetate	1±0.08	Nil	1±0.08	Nil	4±0.08	1±0.14
Methanol	9±0.18	30±0.27	12±0.22	2±0.15	25±0.17	10±0.13
Aqueous	Nil	Nil	23±0.17	Nil	2±0.15	1±0.13
Ciprofloxacin (5 µg/ml)	32±1.15	28±1.52	30±0	30±1.52	27±1	31±0.13

Effect of chloroform, ethyl acetate, methanol and aqueous leaf extracts were tested against *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. typhi*, *B. cereus* and *S. aureus*. Methanolic extract showed the highest inhibition with an inhibitory zone of 9, 30, 12, 2, 25 and 10 mm respectively. It is also noted that the zone of inhibition obtained in methanolic extract was higher (30 mm) than that of antibiotic ciprofloxacin (28 mm) against *K. pneumoniae*. The aqueous extract showed an inhibition zone of 23 mm against *P. aeruginosa*, where as the control drug ciprofloxacin exhibited a zone of inhibition of 30 mm. The zone of inhibition for other strains against other solvent extracts is given in table 3.

The present study showed promising antibacterial activity. This has been mainly attributed to the presence of phenols, flavanoids and other phytochemical compounds present in *H. suaveolens*. The presence of antimicrobial activity in a particular part of a particular species may be due to the presence of one or more bioactive compounds such as alkaloids, glycosides, flavonoids, steroids, saponins etc. [25].

### Antifungal activity

Table 4: Antifungal activity of leaf extracts of *H. suaveolens*

Extracts	<i>T. rubrum</i>	<i>A. fumigatus</i>	<i>P. expansum</i>	<i>F. oxysporum</i>	<i>A. niger</i>
Chloroform	2±0.25	Nil	Nil	5±0.08	2±0.13
Ethyl acetate	1±0.25	Nil	Nil	1±0.14	1±0.13
Methanol	Nil	Nil	3±0.08	3±0.18	Nil
Aqueous	Nil	3±0.18	5±0.17	31±0.7	Nil
Amphotericin B (10 µg/ml)	27±1	31±0.8	24±0.10	26±0.13	23±0.08

The chloroform, ethyl acetate, methanol and aqueous leaf extract on fungal strains *T. rubrum*, *A. fumigatus*, *P. expansum*, *F. oxysporum* and *A. niger* showed low inhibition when compared to antibiotic Amphotericin B. The aqueous extract showed an inhibition zone of 31 mm against *F. oxysporum*, where as the control drug Amphotericin B exhibited a zone of inhibition of 26 mm. The zone of inhibition for other strains and different solvent extracts are given in table 4.

Mandal *et al.* [3] reported that the steam distilled extract of *H. suaveolens* exhibited broad spectrum antibacterial against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Micrococcus luteus* and antifungal activity against *Fusarium oxysporum*, *Aspergillus niger*, *Helminthosporium oryzae*. The same extract also showed highest antifungal and antibacterial activity against *Aspergillus niger* and *Micrococcus luteus*, respectively.

**Disinfectant property (as a floor cleaning agent)**

Disinfectant property (as a floor cleaning agent) of *H. suaveolens* fresh leaf extract was evaluated. 25 g of fresh leaf was weighed and crushed with 25 ml of sterile distilled water. 100 % leaf extract was used for the study. Initial bacterial count of the floor was determined using swab culture technique. Swabs were taken from the marked area of the floor and spread over the nutrient agar plates and the plates were incubated at 37°C in an incubator for 24 hours. After incubation it was found that 435 colony forming units were present. Similarly, this method was repeated after applying 10 ml of the aqueous leaf extract of *H. suaveolens* on the same place from where the initial swabs were taken and bacterial swabs were taken for the following time schedule given in materials and methods. After 5 minutes of application of aqueous leaf extract, swabs were taken from the floor and spread on nutrient agar plates and incubated at 37°C in an incubator for 24 hours. Only 3 bacterial colonies were observed after incubation. Similarly, swabs were taken from the test site up to 120 minutes. An increasing trend in bacterial population was observed when the time increases. The highest bacterial population observed was 253 colony forming units after 120 minutes. This test was performed in triplicates and the average value was represented in figure 1.

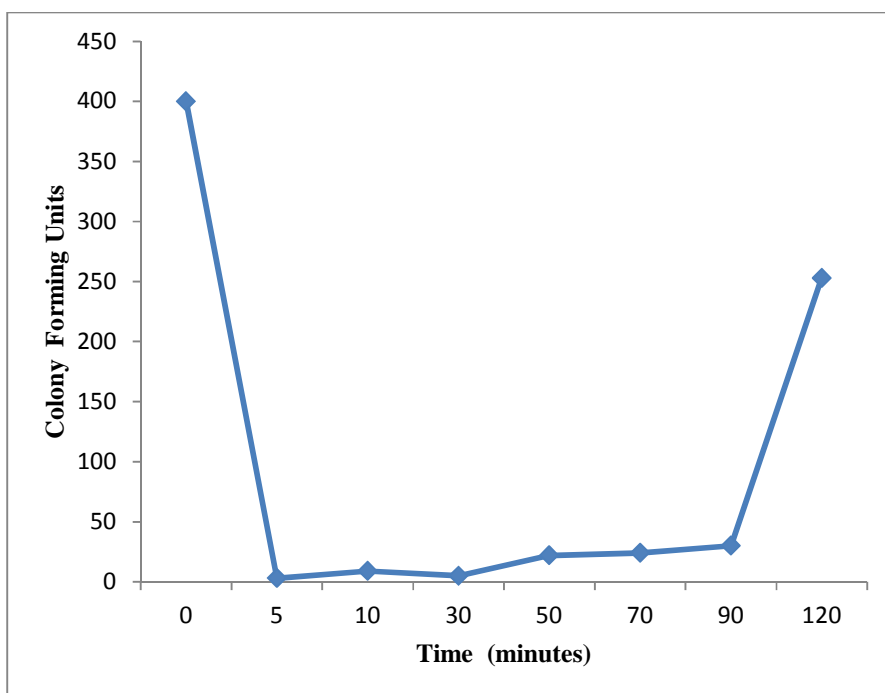


Figure 1: Reduction of bacterial population after application of *H. suaveolens* leaf extract

The above values showed a considerable decrease in bacterial population after the application of extract on the floor. The number of bacterial colonies decreased from 435 to 3 CFU immediately after 5 minutes of extract application. But as the time increased, without any further application of extract, the number of colonies increased. After 120 min, 253 CFUs were observed. Thus, it is concluded that repeated application of this extract can keep in control the microbial population on the floor and aqueous extract of fresh leaf of *H. suaveolens* can be used as a floor cleaning agent. The presence of very strong aromatic mint/thyme-like smell leads to the use of the plant as an insectifuge [21]. The presence of antimicrobial compounds and good aroma prompted us to perform disinfectant property as a floor cleaning agent. Prince *et al.* [21] reported the presence of essential oils as major components mainly in leaves; shoots and seeds of *H. suaveolens* and Asekun *et al.* [9] reported that the essential oil of *H. suaveolens* leaves showed antibacterial activity against gram positive and gram negative bacteria.

**CONCLUSION**

The phytochemical analysis revealed the presence of wide array of phytochemicals in *H. suaveolens* in four different solvent extracts. The results clearly indicated that the medicinal plant *H. suaveolens* possess strong antibacterial activity against the pathogenic bacterial strains such as *K. pneumoniae*, *P. aeruginosa*, *S. typhi*, *B. cereus* and *S.*

*aureus*. *H. saueolens* also showed antifungal property against *F. oxysporum*. The aqueous extract of fresh leaf of *H. saueolens* was found to have good disinfectant property when used as a floor cleaning agent.

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