Antimicrobial studies on the extracts of *Cocculus hirsutus* Linn. and *Hyptis suaveolens* Poit.

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Extracts of *Cocculus hirsutus* Linn. and *Hyptis suaveolens* Poit. were screened for their in vitro antimicrobial activity by agar disc diffusion method. The antimicrobial activity of petroleum ether, chloroform, methanol and aqueous extracts of the leaves of these plants were studied using *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, *Serratia marcescens* and *Aspergillus flavus* as test organisms. Petroleum ether extracts of *C. hirsutus* and *H. suaveolens* were found to be more effective against *Escherichia coli* and *Serratia marcescens* and *Aspergillus flavus* respectively when compared to other extracts of both the plants. Phytochemical screening of the petroleum ether extract of *C. hirsutus* and *H. suaveolens* revealed the presence of alkaloids and steroids, respectively which suggests that these phytoconstituents may be responsible for their antimicrobial activity.

**Keywords:** Antimicrobial activity, *Cocculus hirsutus*, *Hyptis suaveolens*, Disc diffusion method, Leaves, Phytochemical screening.

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**Introduction**

*Cocculus hirsutus* Linn. (Family — Menispermaceae), known as Broom creeper, is found throughout moderately cool and hot regions of India particularly West Bengal, Bihar, Punjab, Tamil Nadu and Himalayan regions (Fig. 1). In Traditional System of Medicine, the roots are used for the treatment of rheumatism, tuberculosis, leprosy, skin disease, dyspepsia, pruritis, flatulence, laxative, aphrodisiac, antipyretic and leaves are used in biliousness, eczema, gonorrhea, opthalmia, sexual debility, leucorrhoea and neuralgia. The phytoconstituents isolated from plant are alkaloids like cohirisine, cohirsinine, cohiristinine, hirustine, haiderine, syringasesinol, isotriboline, triboline, jamtinine; triterpenoid alcohol hirsudiol; essential oils; sitosterol and gineol.

*Hyptis suaveolens* Poit. (Family — Laminaceae), known as Ganga tulsi, is an aromatic strongly scented herb found in Deccan peninsula, North East India, Andaman and Nicobar Island, Philippines and Tropical America (Fig. 2). In the Traditional System of Medicine, the plant is used as a stimulant, carminative, for wounds, sudorific, galactogogue, catarrhal condition, infection of uterus and parasitic skin diseases. The phytoconstituents isolated from the plant are hyptadienic acid, suaveolic acid, suaveolol, methyl suaveolate, β-sitosterol, oleanolic acid, ursolic acid, rosamarinic acid, dehydroabietinol, 3β-hydroxy lup-12-en-28-oic acid, 3β-hydroxylup-20(29)-en-27-oic acid and essential oil.

Since there is no report on antimicrobial activity of *C. hirsutus* and *H. suaveolens*, an attempt was made...
to evaluate the antimicrobial activity of petroleum ether, chloroform, methanol and aqueous extracts of leaves of both the plants by agar diffusion method.

Materials and Methods

Plant Materials

*Cocculus hirsitus* and *Hyptis suaveolens* were collected from Hassan, Karnataka and authenticated by Central Council for Research in Ayurveda and Siddha, Bangalore. Voucher specimens (RRI/bng/smp/Drug Authentication/ 2007-08/247 and 248) have been preserved in our department for the future reference.

Shade dried leaves were coarsely powdered and subjected to successive solvent extraction by continuous hot extraction (soxhlet). The extraction was done with different solvents in their increasing order of polarity such as petroleum ether (60-80°C), chloroform, methanol and water. Each time, the marc was air dried and later extracted with other solvents. All the extracts were concentrated by distilling the solvent in a rotary flash evaporator. The dried extracts were dissolved in dimethyl sulphoxide (DMSO) and subjected to antimicrobial activity.

Preliminary Phytochemical Screening

The coarse powder of leaves of *C. hirsitus* and *H. suaveolens* (25g) was subjected to successive extraction with different solvents in their increasing order of polarity from petroleum ether (60-80°C), chloroform, methanol and water. The extracts were concentrated and subjected to various chemical tests to detect the presence of different phytoconstituents.

Microorganisms and Media

Gram positive bacteria: *Staphylococcus aureus* (ATCC 29213), *Listeria monocytogenes* (ATCC 19115); Gram negative bacteria: *Escherichia coli* (ATCC 25922), *Serratia marcescens* (ATCC 21074); and Fungus: *Aspergillus flavus* (ATCC 32612) were obtained from the Department of Microbiology, The Oxford College of Science, Bangalore for the study. The bacterial and fungal stock cultures were maintained on Muller Hinton agar and Sabourd-Dextrose agar slants, respectively, which were stored at 4°C.

Antimicrobial Screening

The extracts were screened for their antimicrobial activity in vitro by disc diffusion method using *S. aureus*, *L. monocytogenes*, *E. coli*, *S. marcescens* and *A. flavus* as test organism. Agar cultures of the test microorganisms were prepared. Three to five similar colonies were selected and transferred to 5ml broth with a loop and the broth cultures were incubated for 24 h at 37°C and suspension was checked to provide approximately 10^10 colony forming units per ml. 0.1 ml of organism’s suspension were spread evenly on the agar plates. For screening, sterile 3 mm diameter disc (Whatman filter paper No. 1) were impregnated with 0.2 ml of 1000 µg/ml of the various extracts of both the drugs, dried and then placed in inoculated plates of Muller Hinton agar and Sabourd-Dextrose agar medium. DMSO solvent was used as negative control. The plates were incubated at 37°C for 24h and room temperature for 48h for bacteria and fungi, respectively. After incubation for 24 and 48h, the results were recorded by measuring the zones of inhibition surrounding the disc. Pencillin (10 µg/disc) and Gentamycin (20 µg/disc) were used as reference standards for bacteria and fungi, respectively. Each experiment was done in triplicate.

Results and Discussion

Petroleum ether, methanol and aqueous extracts of *C. hirsitus* showed significant activity against *E. coli* and moderate activity against other organisms except *L. monocytogenes* and only the aqueous extract showed significant activity against *A. flavus* (Table 1).

Petroleum ether extract of *H. suaveolens* showed significant activity against *S. marcescens* and *A. flavus* and moderate activity by all the extracts against *S. aureus* (Table 2).

Preliminary phytochemical screening of different extracts of *C. hirsitus* and *H. suaveolens* showed the
presence of alkaloids, tannins, saponin, flavonoids, coumarins and sugars (Table 3).

Petroleum ether extracts of *C. hirsutus* and *H. suaveolens* were found to be more effective against *E. coli* and *S. marcescens* and *A. flavus*, respectively when compared to other extracts of both the plants. Phytochemical screening of the petroleum ether extract of *C. hirsutus* and *H. suaveolens* revealed the presence of alkaloids and steroids, respectively which suggests that these phytoconstituents may be responsible for their antimicrobial activity. Further studies are needed to isolate and characterize the bioactive principles to develop new natural drugs.

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