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Research Article

Antimicrobial activity of a medicinal plant *Hybanthus enneaspermus* (Linn.) F. Muell.

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Abstract

Present investigation deals with the antimicrobial activity of a medicinal plant ***Hybanthus enneaspermus* (Linn.) F. Muell.**, against two Gram positive bacteria, viz. *Staphylococcus aureus* and *Bacillus subtilis* and five Gram negative bacteria, viz. *Escherichia coli*, *Enterobacter aerogens*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Citrobacter freundii*. Two active fractions were isolated from the benzene extract of the plant. The antimicrobial activity justifies its use in traditional medicine.

Keywords: *Hybanthus enneaspermus*, Antimicrobial activity, Antibacterial activity, Gram positive bacteria, Gram negative bacteria, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Enterobacter aerogens*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Citrobacter freundi*.

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Introduction

Microorganisms are the causative agents of almost all kinds of acute and chronic diseases. Many kinds of diseases have been treated with herbal medications throughout the history of mankind. The therapeutic value of a medicinal plant depends on the presence of one or more constituents possessing certain physiological and pharmacological activity. Plants based antimicrobials have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. Many commercially proven drugs used in modern medicine were initially used in crude form in traditional or folk healing practices or for other purposes that suggested potentially useful biological activity[1].

***Hybanthus enneaspermus* (Linn.) F. Muell.**, a traditional medicinal herb belonging to the family Violaceae is found throughout the warmer parts of India. The whole plant is used medicinally in Siddha, Ayurveda and other traditional systems of medicine for curing various ailments. In Ayurveda, it is known as *Sthalakamala*. The plant is reported to possess tonic, diuretic and demulcent properties. The root is diuretic and administered as an infusion in gonorrhoea and urinary affections. The *Sandals* employ the root in bowel complaints of children. The leaves and tender stalks are demulcent and used as a decoction or electuary; in conjunction with oil, they are employed in preparing a cooling liniment for the head[2]. An infusion of the plant extract is given in case of cholera[3].

Approximately 119 pure chemical substances extracted from higher plants are used in medicine throughout the world[4] and about 10% of our leading drugs now contain phytochemicals still extracted directly from higher plants[5]. Higher plants have been described as chemical factories that are capable of synthesizing unlimited numbers of highly complex and unusual chemical substances. Antimicrobial agents are chemicals that either kill the microorganisms or inhibit their growth. The purpose of present work is to analyse the antibacterial activity of separated fractions in a medicinal plant, ***H. enneaspermus***.

Materials and Methods

H. enneaspermus was collected from Tirunelveli hills, air dried, powdered and extracted in Soxhlet apparatus successively with petroleum ether, benzene, chloroform and methanol. The solvent was removed under vacuum at 40°C and the extracts were tested for antimicrobial activity against two Gram positive bacteria and five Gram negative bacteria. Further separation of the active extract (5g) was done by column chromatography on silica gel (230-400 mesh) built in petroleum ether (60- 80°C) and eluted with different solvent combinations, viz. petroleum ether, petroleum ether with increasing amount of benzene, benzene with increasing

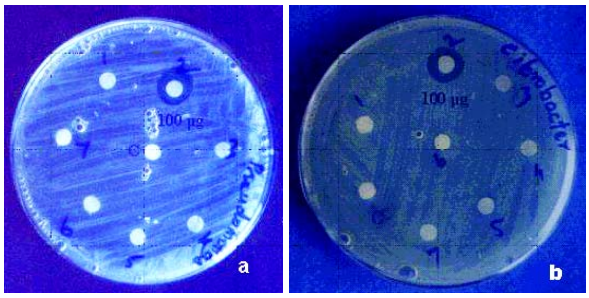

Fig. 1 (a-b) : Fraction No. 2 isolated from *Hybanthus enneaspermus* showing antimicrobial activity against: (a) *Pseudomonas aeruginosa,* and (b) *Citrobacter freundii*

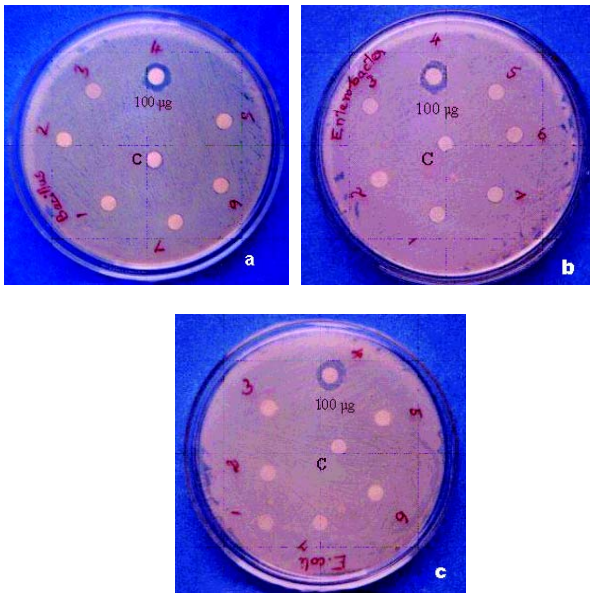

Fig. 2 (a-c) : Fraction No. 4 isolated from *Hybanthus enneaspermus* showing antimicrobial activity against: (a) *Bacillus subtilis,* (b) *Enterobacter aerogens,* and (c) *Escherichia coli*

amount of chloroform and chloroform with increasing amount of methanol. Combination of similar fractions was made by thin layer chromatography comparison. The fractions were dissolved in dichloromethane and the solvents were removed under vacuum distillation to give a residue that contained the neutral fraction.

Antimicrobial susceptibility testing was performed on Diagnostic Sensitivity Testing agar plates method[6] against two Gram positive bacteria, viz. *Staphylococcus aureus* (MTCC-96) and *Bacillus subtilis* (MTCC-1789) and five Gram negative bacteria, viz. *Escherichia coli* (ATCC-1650), *Enterobacter aerogens* (MTCC-111), *Pseudomonas aeruginosa* (MTCC-1688), *Salmonella typhi* (MTCC-733), and *Citrobacter freundii* (MTCC-1658). Inoculums were prepared in the same medium at a density adjusted to the 0.5 McFarland turbidity standards[7]. All bacterial strains were obtained from the Department of Microbiology, Vivek Institute of Laboratory Medicine, Nagercoil. Kieselgel GF_{254} plates were used for thin layer chromatography. Sterilized antibiotic discs (6mm in diam.) were prepared by using Whatmann No. 1 paper. To find out its antimicrobial activity 100µg of the isolated compounds were transferred to each disc with the help of a micropipette. For preparing the 100µg disc, 1mg of the isolated compound was dissolved in 1ml of dichloromethane (CH_2Cl_2) which was used as stock solution. From this stock solution 100µl were transferred per disc.

The diameter of the clear zone (zone of inhibition) around the disc was measured and the results were reported as the diameter of the zone of inhibition around each disc (in mm). For each experiment pure solvent was used as blind control disc. The minimum inhibitory concentrations (MICs) of the active fractions were determined by micro-dilution techniques in Mueller-Hinton broth (Merck) for bacteria[8]. On the chromatographic paper 50µl of the fractions were applied and the chromatogram was developed using Butanol: Acetic acid: Water (4:1:5) as solvent. Spots and bands were visualized by UV irradiation (366nm).

Results and Discussion

Column chromatographic separation of the active benzene extract of ***H. enneaspermus*** and comparison of similar fractions through thin layer chromatography lead to their combination into seven larger fractions (Table 1). Among seven fractions isolated only two fractions (No. 2 and 4) were found active. Fraction No. 2 was active against two Gram negative bacteria, *P. aeruginosa* and *C. freundii*. The zone of inhibition reported around 100µg disc was 17 mm for *P. aeruginosa* and 15mm for *C. freundii* (Fig. 1a-b). Minimum Inhibition Concentration (MIC) of this fraction was 30µg/ml against *P. aeruginosa* and 35µg/ml against *C. freundii* (Table 1). This fraction was active against Gram negative bacteria only.

Fraction No. 4 was active against a Gram positive bacteria *B.subtilis* and two Gram negative bacteria *E. aerogens* and *E. coli*. The zone of inhibition reported around 100µg disc was 14 mm for *B. subtilis,* 15mm for *E. aerogens,* and 16mm for *E. coli* (Fig. 2a-c). This

Table 1: Nature and MIC of various fractions isolated from *Hybanthus enneaspermus*

Fraction No.	Nature & Consistency	Colour	Rf Value	Under UV 366 nm	MIC in µg/ml						
					Sa	Bs	Ec	Ea	Pa	St	Cf
1	Powder	Golden yellow	0.36	Red	-	-	-	-	-	-	-
2	Powder	Orange*	0.25	Yellow	-	-	-	-	30	-	35
3	Sticky	Bluish green	0.44	Red	-	-	-		-	-	-
4	Powder	Orange*	0.52	Yellow	-	35	25	30	-	-	-
5	Sticky	Green	0.20	Blue	-	-	-	-		-	-
6	Sticky	Dark brown	0.45	Orange	-	-	-	-	-	-	-
7	Powder	Light brown	0.72	Red	-	-	-	-	-	-	-

*Active fractions, Bs - *Bacillus subtilis,* Cf - *Citrobacter freundii,* Ea - *Enterobacter aerogens,* Ec - *Escherichia coli,* Pa - *Pseudomonas aeruginosa,* Sa - *Staphylococcus aureus*, St - *Salmonella typhi.*

fraction was active against both Gram positive bacteria and Gram negative bacteria. MIC are given in Table 1. The differences in the antibacterial effect of both the isolated fraction against Gram positive and Gram negative bacteria may be due to differences in permeability barriers. In Gram negative species, an outer membrane is a fairly effective barrier for amphipathic compounds.

Conclusion

In the present work, the antibacterial activity of ***H. enneaspermus*** suggests its potential usefulness in traditional medicine for the treatment of urinary infections and bowel movement complaints. Therefore, this plant can be used to treat wound and burn infections, pneumonia, food poisoning and dermatitis due to Gram positive and Gram negative bacteria.

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