AkiNik

International Journal of Herbal Medicine
Available online at www.florajournal.com

IJHM
International Journal of Herbal Medicine

ISSN 2321-2187
IJHM 2013; 1 (3): 73-75

Received: 1-9-2013
Accepted: 8-9-2013

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In vitro Anti-bacterial Activity of Methanolic Extract of *Cardiospermum canescens*- A Wild Medicinal Plant

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ABSTRACT

The methanolic extract of *Cardiospermum canescens* was evaluated for its antibacterial activities against six bacterial strains, *Streptococcus pyogenes, Pseudomonas aeruginosa, Bacillus subtilis, Streptococcous faecalis, Salmonella typhi* and *Escherichia coli*. The extracts have inhibited all the tested bacterial species with different manner at various conshations. However, *E.coli* was found to be mostly inhibited by the plant extract among the tested microbial strains with its MIC value was 7.86 mg/ml. It could be a potential source for the preparation of anti-microbial drugs from natural habitats.

Keywords: *Cardiospermum canescens,* Methanol Extract, Disc Diffusion and Minimum Inhibitory Concentration.

1. Introduction

Medicinal plants are the important sources of phytochemical substances with potential therapeutic effects. Generally application for the treatment of many diseases is conserved with folk medicine from different parts of the world. Naturally occurring compounds from various sours like plants, fungi and microbes are still used in pharmaceutical preparations in different forms. In worldwide, infectious disease is the number one cause and accounting for approximately one-half of all deaths in tropical countries. The third generation approach is treating the infectious disease with herbal drugs with minimal side effects [1].

Cardiospermum canescens is a climber belongs to the family Sapindaceae. The plant is a twinner, pubescent or nearly glabrous annual or perennial with slender branches, liming by means of tendrillar hooks. The roots are diuretic, diaphoretic, emetic, mucilaginous, laxative and emmenagogue. They are useful in fever, arthritis, amenorrhea, lumbago, neuropathy, rheumatism, stiffness of limbs, snake bite, nervous disorders and piles. The leaves are rubefacient and are good for arthritis. The plant is normally used as a leafy vegetable in the interior parts of Tamilnadu[2]. This study will render new frontiers for microbial diseases and provides a scientific basis for the traditional claims of this ethnic medicinal plant.

2. Materials and Methods

2.1 Preparation of plant sample

Cardiospermum canescens (whole plant) was collected freshly from the rural part of Aanaikatti, Western Ghats, Coimbatore and they were identified by taxonomist. The plant material was washed with tap water to remove the impurities and shade dried at room temperature. The dried plant materials were ground into fine powder in an electric blender and subsequently sieved for obtaining fine powder. The plant powder first soaked with petroleum ether for removing the fatty acids, followed by methanol used to prepare crude and filtered extract. The filtrate was stored in refrigerator at 4 ˚C and used for antimicrobial studies.

2.2 Bacterial Strains Used

Streptococcus pyogenes, Pseudomonas aeruginosa, Bacillus subtilis, Streptococcous faecalis, Salmonella typhi and *Escherichia coli* were purchased from department of Microbiology, Bharathidasan University, Tirchirappalli, Tamilnadu.

2.3 Disc Diffusion Assay

The antibacterial activity of various concentrations of methanolic extract of *C. canescens* was evaluated by disk diffusion method [3].Petri dishes containing 20 ml of Nutrient agar medium were seeded with a 24 h culture of the bacterial strains in Nutrient broth. The inoculum size was adjusted to approximately 10^8 colony forming units (CFU)/ml. After the residual solvent was evaporated, dried and sterilized filter paper discs (6mm diameter) impregnated with known amount of the test substances 25-100 mg/ml using micropipette was employed for the experiment evaporated. Discs containing the test materials were placed on nutrient agar medium uniformly seeded with the test microorganisms. For this study, standard disc of Tetracycline (10µg/disc) used as positive control. These plates were then kept at room temperature for 24 hours to allow maximum diffusion of test samples. The plates were then incubated at 37ºC for 24 hours to allow maximum growth of the organisms. The test materials showing symbol of antimicrobial activity inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the disc. The antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition in millimeter. The experiment was carried out in triplicate and the average zone of inhibition was calculated.

2.4 Minimum Inhibitory Concentration for Bacterial Strains

MIC was determined by the standard method [4]. Nutrient broth was prepared and sterilized using autoclave. One ml of the prepared broth was dispensed into the test tubes numbered 1-8 using sterile syringe and needle. A stock solution containing 500µl/ml of the extract also was prepared. Then 1 ml of the solution was dispensed into the tubes numbered 1. Subsequently, from tube 1, serial dilution was carried out and 1 ml from tube 1 was transferred up to tube number 7 and 1 ml from the tube 7 was discarded. Tube 8 was kept as control for sterility of the medium. An overnight culture (inoculum) of each of the test isolates was prepared in sterile nutrient broth. 10µl of the inoculum was transferred into each tube from 1 to tube 8. The final concentration of the plant extract in each of the test tubes numbered 1-7 after dilutions of 500µg, 250µg, 125µg, 62.5µg, 31.25µg, 15.62µg and 7.81µg/ml were incubated at 37°C for 24 h and examined for growth. The last tube in which growth failed to occur was the MIC tube. The tubes with the extract and broth were inoculated with a microorganism suspension at a density of 10^5 CFU per ml. The tubes were incubated at 37°C for 24 h and then observed for the Minimum Inhibitory Concentration (MIC). The growth of organisms was observed as turbidity determined by a spectrophotometer (Elico SL177) at 620 nm. Control tubes without the tested extracts were assayed simultaneously. MIC of each extract was taken as the lowest concentration that showed no growth.

3. Results and Discussion

In vitro antibacterial activity of various concentrations of *Cardiospermum canescens* against three gram positive and three gram negative bacterial strains were studied. The results showed that the extracts were more active towards the gram positive bacteria. The activity increased in increasing concentrations of the sample. The 100mg/ml concentration of *C. canescens* extract exhibited a good inhibition against all the bacterial strains when compared to control. The 100mg/ml concentration of *C. canescens* extract exhibited a good inhibition against all the bacterial strains when compared to control. The strains *B. subtilis* and *S. faecalis* are effectively inhibited in lower concentrations. The gram negative bacteria *E. coli* is significantly inhibited at 75mg/ml concentrations itself (Table 1 and Fig 1).

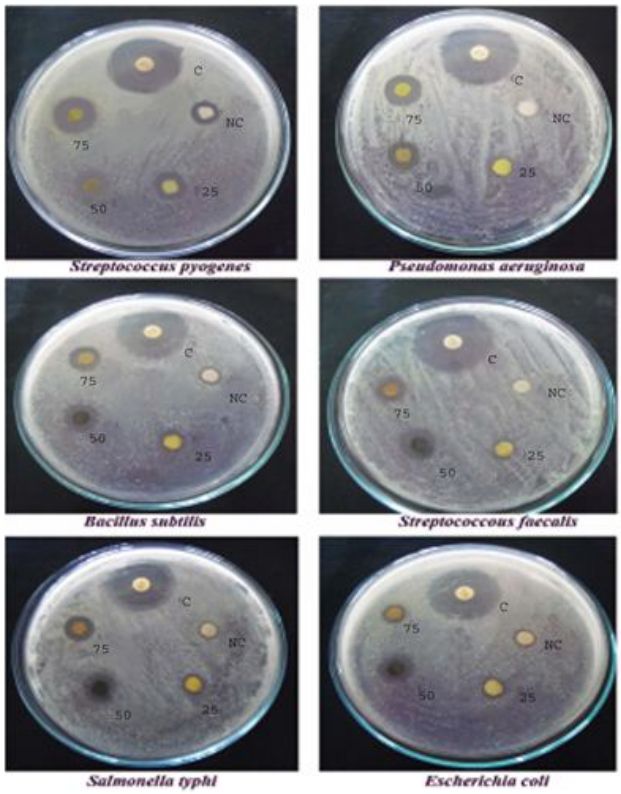
Table 1: Antibacterial activity of methanolic extracts of *Cardiospermum canescens*

S. No	Microorganisms	Zone of Inhibition (mm)				
		Various concentrations used (mg/ml)				Control Tetracycline
		25	50	75	100	
1	*Streptococcus pyogenes*+	8	11	12	16	18
2	*Pseudomonas aeruginosa*-	7	10	11	15	17
3	*Bacillus subtilis*+	10	12	12	16	21
4	*Streptococcous faecalis*+	9	9	13	13	18
5	*Salmonella typhi*	8	11	10	12	14
6	*Escherichia coli*-	9	12	14	14	22

Table 2: MIC values of methanolic extract of *Cardiospermum canescens* for bacterial strains

S. No.	Microorganisms	Minimum Inhibitory concentration (µg/ml)
1	*Streptococcus pyogenes*	125
2	*Pseudomonas aeruginosa*	31.25
3	*Bacillus subtilis*	62.25
4	*Streptococcous faecalis*	125
5	*Salmonella typhi*	250
6	*Escherichia coli*	7.86

Fig 1: Antibacterial Activity of Methanolic Extracts of *Cardiospermum canescens*



The various concentrations of plant extracts showing considerably good antibacterial activity for each test organisms were selected to determine MIC. Values for MICs were dependent on the bacterial species. Generally the MIC values were low concentrations of plant extracts showing potent activity against the strains. *E. coli* and *P. aeruginosa* were the most potent extracts against all the bacteria. The inhibition against all the bacterial strains showed 250-7.86 µg/ml. *S. pyogenes* and *S. faecalis* are having the MIC value of 125µg/ml concentration (Table 2).

The differences in the sensitivity of the bacteria may be attributed to the fact that the cell wall. Gram positive bacteria consist of a single layer whereas the gram negative bacteria cell wall is multilayered. The walls act as a diffusional barrier making gram negative bacteria less susceptible to the antimicrobial agents than the gram positive bacteria [5].Generally, the plant extracts more effective in positive bacteria than the negative bacteria. Various workers have already reported similar results [6]. Microbial resistance to antibiotics in use nowadays, provides the need for the search of new compounds with potential effects against pathogenic bacteria [7]. In addition, high cost and adverse side effects are commonly associated with popular synthetic antibiotics (such as hypersensitivity, allergic reactions, immune suppression etc.) and are major burning global issues in treating infectious diseases [8].

Sapindaceae members are showed significant antibacterial activity. In *Cardiospermum helicacabum* leaf and stem extracts in different solvent were subjected to antimicrobial screening against selected Gram positive and Gram negative bacteria. Acetone and chloroform extracts of leaf had higher inhibitory action against *Salmonella typhi* and *Streptococcus subtilis*. Acetone extracts of stem showed maximum inhibitory action against *S. typhi* and benzene extracts of stem had moderate inhibitory action against *Escherichia coli*. Ethanolic extracts of *Sapindus saponaria* has been used as antimicrobial agent against various organisms [9].The antimicrobial properties exhibited by the extract may be associated with the presence of saponins, tannins, glycosides found in the plant extract. The family members are also ideal for saponin content. Several studies have been shown that saponins possess antimicrobial activity [10]. *Saponaria officinalis* which has a rich source of saponins mainly used for cosmetic industry and have antimicrobial activity reported [11].

Plants contain thousands of constituents and are valuable sources of new and biologically active molecules possessing antimicrobial property. The present study which deals with *C. canescens* has significant antimicrobial activity. Further, the potential of this plant must be explored more and more, in order to develop an alternate therapy for the treatment of infections caused by antibiotic-resistant bacteria.

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