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**Phytochemical analysis and antibacterial potential of
Cardiospermum halicacabum Linn. (Sapindaceae)**

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Abstract

Methanolic stem extract of *Cardiospermum halicacabum* was examined for their phytochemical and antibacterial properties. The preliminary phytochemical screening of methanolic extract of *Cardiospermum halicacabum* showed the presence of bioactive components like Alkaloids, Flavonoids, Saponins, Tannins and Glycosides was absent in the plant extract. The methanolic stem extracts of *Cardiospermum halicacabum* was evaluated for antibacterial activity against medically important bacteria viz. *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus vulgaris*. The in vitro antibacterial activity was performed by agar disc diffusion method. Among this *Staphylococcus aureus* and *Bacillus subtilis* showed the high zone of inhibition activity (18 ± 0.057 and 17 ± 1.5) and also showed lesser activity against the control (Ampicilin). It is concluded that the plant studied might be a good antibacterial source.

Key-Words: *Cardiospermum halicacabum*, *Klebsiella pneumoniae*, Phytochemical, *Staphylococcus aureus*

Introduction

Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. India is rich in all the three levels of biodiversity, namely species diversity, genetic diversity and habitat diversity. In India thousands of species are known to have medicinal value and the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient times. Herbal medicine is still the mainstay of about 75-80% of the whole population, mainly in developing countries. These plants possess various phytochemicals and active biomolecules, which play a major role in the treatment of cancer. Many plants have been examined to identify new and effective anticancer compounds, as well as to elucidate the mechanism of cancer prevention and apoptosis (Swamy, and Tan, 2000). Microbial infections are an important health problem throughout the world and plants are possible sources of antimicrobial agents (Burapadaja, and Bunchoo, 1995). The interest to evaluate plants possessing antibacterial activity for various diseases is growing Clark and Hufford 1993.

It has been suggested that the aqueous and ethanolic extracts from plants used in allopathic medicine are potential source of antiviral, anti-tumoral and antimicrobial agents Chung, et al., 1995. Interest in large number of traditional natural products has increased Taylor et al., 1996.

The study plant *Cardiospermum halicacabum* Linn. belongs to the family *Sapindaceae*, commonly known as Balloon vine or Love in a puff. *Cardiospermum* is the combination of the Latin words cardio, meaning heart, and sperma, meaning seed and refers to the white heart-shaped pattern on the seed. *Halicacabum* is derived from the Latin word halicacabus, a plant with inflated fruits Brown, 1954. It is an annual or sometimes perennial climber, widely distributed in tropical and subtropical Africa and Asia. It has been examined for antidiarrhoeal as well as homoeopathic medicinal properties. *C. halicacabum* has been used in the treatment of rheumatism, nervous diseases, stiffness of the limbs and snakebite. Young leaves can be cooked as vegetables Pieroni, et al., 2002. Considering the above features of *Cardiospermum halicacabum* was investigated for their phytochemical and antibacterial activity.

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Material and Methods

Collection of Plant Material

Fresh plant was collected from nearby places, and the stem were separated and washed under running tap water. Thoroughly washed stem were allowed for shade drying under room temperature in the laboratory. The dried leaves were ground to fine powder using a blender. The powder was preserved in an air tight bottle for further studies.

Preparation of Solvent Extracts

10 grams of thoroughly grounded powder were then soaked in 100ml of organic solvent (methanol) in a conical flask, plugged with cotton and kept on a rotary shaker at 190- 220 rpm for 24 hours at 40°C. After 24 hours, it was filtered through a Whatmann filter paper No 1 and the supernatant was collected and the solvent was evaporated to make the final volume one fourth of the original volume.

Preliminary Phytochemical analysis

Cardiospermum halicacabum methanolic methanolic stem extract was preliminary qualitatively screened for phytochemicals as per standard biochemical procedure (Thenmozhi et al., 2010 and Santhi, et al., 2011). The crude extract was diluted with methanol to the concentration of 1mg/ml. The qualitative phytochemical analysis of crude methanolic extract was performed to determine the presence of flavonoids, saponins, tannins, terpenoids and cardio glycosides.

Antimicrobial Assay

Micro-organisms used

Five species of bacteria, two gram - positive (*Staphylococcus aureus* & *Bacillus subtilis*) and three gram negative (*Escherichia coli*, *Klebsiella pneumoniae* & *Proteus vulgaris*) were obtained from KMCH, Coimbatore.

Preparation of Inoculum

A loopful of strain was inoculated in 30ml of nutrient broth in a conical flask and incubated on a rotary shaker at 37° C for 24 hours to activate the strain.

Bioassay

The bioassay used was the standard Agar Disc Diffusion assay adapted from Nair, et al., 2005. Mueller Hinton Agar was prepared for the study. Mueller Hinton agar plates were swabbed with a suspension of each bacterial species, using a sterile cotton swab. Subsequently, the sterilized filter paper discs were completely saturated with the test compound. The impregnated dried discs were placed on the surface of each inoculated plate. The plates were incubated overnight at 37° C. Each organism was tested against each organism in triplicate. Methanol was used as negative control. Standard discs of Ampicillin served as positive antibacterial control. The test

materials having antimicrobial activity inhibited the growth of the micro organisms 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 mm.

Results and Discussion

Preliminary Phytochemical Analysis

The phytochemical analysis of methanolic stem extract of *Cardiospermum halicacabum* was analysed for the compounds such as Alkaloids, Cardiac Glycosides, Flavonoids, Glycosides, Saponins, Steroids and Tannins. The preliminary phytochemical analysis revealed the presence of six compounds i.e. Alkaloids, Cardiac Glycosides, Flavonoids, Saponins, Steroids and Tannins and absence of glycosides (Table-1). Various tests has been performed to find out the phytochemical constituents.

Antibacterial Activity

The antibacterial property of methanolic stem extract of *Cardiospermum halicacabum* was analysed against bacterial pathogens using ampicillin (commercial antibiotic) as control. Out of these five bacterial pathogens three were found to be negative (*Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*) and two were positive (*Bacillus subtilis*, *Staphylococcus*). Disc diffusion method was used to evaluate the antibacterial activity of taken samples. After twenty-four hours the minimum inhibitory zone of methanolic stem extract of *Cardiospermum halicacabum* and control were measured. The maximum activity (18 ± 0.57) in extract was observed against *Staphylococcus aureus* gram positive bacteria (Table2, Figure-2). The control ampicillin showed 19 ± 0.13 . The least activity (10 ± 0.03) was observed against *Proteus vulgaris* a gram negative bacteria. The control against *Proteus vulgaris* showed 12 ± 0.89 with tested 10 ± 0.0 . The treated disc shown zone of 17 ± 1.15 against *Bacillus subtilis*, against *E.coli* and *Klebsiella pneumoniae* showed a moderate activity. similar results were also reported by venkatesan et al 2006. In general, the plant sample has maximum activity against gram positive bacterial pathogens than that of gram negative bacterial pathogens. Plant-based antibacterial have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antibacterial (Iwu et al., 1999). Natural products, either as pure compounds or as standardized plant extract, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal

plants for their potential antimicrobial activity. The potential for developing antimicrobials from higher plants appears rewarding, as it will lead to the development of a phytomedicine to act against microbes. Plant based antimicrobial represents the vast untapped source for medicine. Plant based antimicrobials have enormous therapeutic potential as they can survive the purpose without any side effects that are often associated with synthetic antimicrobials, continued further research and exploration of plant derived antimicrobials is needed today. Medicinal plants are important source for the development of potential, new chemotherapeutic drugs and the in vitro antibacterial test form the basis (Toona, et al., 1998 and Srivastava et al, 1996).

Conclusion

The phytochemical analysis, antibacterial activity, antioxidant potential and anticancer studies of *Cardiospermum halicacabum* showed positive in all aspects. This may be due to the presence of various compounds present in *Cardiospermum halicacabum* and this may be the source for the wide activity of the plant against such activities. The present study reveals the potentiality of the plant taken for the study and extensive study with a particular area of focus in future may form a basis for a new drug discovery.

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Table 1: Phytochemical & biochemical analysis of methanolic stem extract of *Cardiospermum halicacabum*

Compound	Observation	Result
Alkaloids	Presence of precipitation	Present
Flavonoids	Formation of yellow colour	Present
Glycosides	No colour change	Absent
Saponins	Frothing present	Present
Steroids	Presence of pink colour	Present
Tannins	Extract change into blue black	Present

Table 2: Antibacterial activity of methanolic stem extract of *Cardiospermum halicacabum*

Bacteria	Control ampicillin (mm)	Zone of inhibition (mm)
<i>Bacillus subtilis</i> (+ve)	18 ± 0.57	17 ± 1.5
<i>Escherichia coli</i> (-ve)	14 ± 0.03	12 ± 0.03
<i>Klebsiella pneumoniae</i> (-ve)	12 ± 0.01	10 ± 0.57
<i>Proteus vulgaris</i> (-ve)	12 ± 0.89	10 ± 0.03
<i>Staphylococcus aureus</i> (+ve)	19 ± 0.13	18 ± 0.057