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Preliminary phytochemical screening and antibacterial of *Cardiospermum halicacabum* L.

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ABSTRACT

The present investigation of crude extracts from leaf, seed coat and stem of cardiospermum halicacabum L. in different solvent, were subjected to phytochemical and antibacterial screening against the selected Gram positive and Gram negative bacteria. The phytochemical solvent extracts were used for screening and antibacterial activity, were analysed. The Phytochemical studies indicate that the leaf, seed coat and stem contain a broad spectrum of secondary metabolites. Flavonoid, Terpenoids and cardiac glylosides were predominantly found in all the three tested solvent extracts of leaf followed by Tannin, Flavonoid, Terpenoids and cardiac glylosides (Acetone, Chloroform and Diethyl ether). Likewise, Tannins, Flavonoid, Terpenoids and cardiac glylosides and anthrquinonus were predominantly found in all the tested solvent extracts of the stem. Then seed coat followed by Tannins, Flavonoids and Terpenioids were predominantly found in all the tested solvent in plant extracts. Saponins were not found in any of the solvent extract of stem, leaf and seed coat. All the extracts showed varying degree of inhibitory potential against all the tested bacteria. (Acetone, Chloroform and Diethyl ether) extracts of seed coat had higher inhibitory action against Pseudomonas, Escherichia coli, Staphylococcus aureus and Salmonella respectively. Chloroform extract of stem, leaf and seed coat had moderate inhibitory action against Escherichia coli and Staphylococcus.

Key words: *Cardiospermum halicacabum* L. Phytochemical screening, Antibacterial activity

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INTRODUCTION

Herbal medicines play an important role in health care programs in the developing countries. Ancient Indian literature incorporates a remarkably broad definition of medicinal plants to the potential sources of medicinal substances. Diseases that remain most challenging for today's health care system tend to be more complex than could be treated by current combination therapies. 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 occupy of about 75-80% of the whole population, mainly in developing countries. **Cardiospermum halicacabum* possesses 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 [1]. Microbial infections are an important health problem throughout the world and plants are possible sources of antimicrobial agents [2]. The interest to evaluate plants possessing antibacterial activity for various diseases is growing [3]. 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 [4]. Interest in large number of traditional natural products has increased [5]. **Cardiospermum halicacabum* L. belongs to the 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 [6]. 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. *Cardiospermum halicacabum* L has been used in the treatment of rheumatism, nervous diseases, stiffness of the limbs and snakebite. Young leaves can be cooked as vegetables [7].Medicinal plants continue to be an important therapeutic aid for alleviating the ailments of humankind. Natural products are known to play an important role in both drug discovery and chemical biology. Although some therapeutic benefits can be traced to specific plant compounds, many herbs contain dozens of active constituents that, together, combine to give the plant its therapeutic value. Any part of the plant may contain active components [8,9, 10].

Phytochemical studies have shown that plants with antimicrobial activity contain bioactive constituents such as tannins, flavonoids, alkaloids and saponins [11]. Alkaloids & flavinoids have been used as antiviral, antibacterial, antiamoebial & anticancer agents. Phenolic and polyphenolic are the other group of secondary metabolites [12]. The uses of plant-derived products as disease control agents have been studied, since they tend to have low mammalian toxicity, less environmental effects and wide public acceptance [13].

MATERIALS AND METHODS

Plant collection: The wild plants were directly collected along the road sides of Manamedu – Trichy Districts, Tamil Nadu, India.

Strains collection: The microbial strains were collected from the K.A.P. Viswanatham Government Medical College, Trichy-20. Medicinal plants were tested against the bacteria such as *Pseudomonas, Escherichia coli, Aeromonas, Salmonella, Staphylococcus aureus,*

Sterilization of Plant Materials: The disease free and fresh leaves of plant were selected. About 2 grams of fresh and healthy leaves were taken, and washed with tap and distilled water for three times. Surface sterilization was done with 0.1% mercuric chloride for 2minutes. Again the plant materials were washed thoroughly with distilled water for three times.

Preparation of Plant extracts: The whole plants along with leaf, stem and seed coat were dogged out from their carefully. Plant was collected from nearby places, and the leaf, stem and seed coat were separated and washed under running tap water. Thoroughly washed leaf, stem and seed coat 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.

Preliminary Phytochemical analysis: *Cardiospermum halicacabum L.* diethyl ether, chloroform, acetone extract of leaf stem and seed coat analysed. [14], preliminary qualitatively screened for phytochemicals as per standard biochemical procedure. The crude extract was diluted with diethyl ether, chloroform, acetone to the concentration of 1mg/ml. The qualitative phytochemical analysis of crude extract was performed to determine the presence of tannin, saponin, flavonoid, steroid, terpenoids, cardiac glycosides, alkaloids, and anthraquinones. The samples were crushed into fine powder and dissolved separately in 100ml of solvent. The solution was kept at room temperature for seven days to allow the extraction of compounds from seeds. The solution of each sample was stirred after every 24 hrs using sterile glass rod. After 7 days the solution was filtered through whatman filter paper No-1 and a greenish filerate was obtained. The solvent was evaporated and sticky cumbrances obtain that was stored in the refrigerator and suspended in 10% dimethyl sulfoxide prior to use. Preliminary phytochemical tests were carried out on diethyl ether, chloroform, acetone extract and on the powdered specimen using standards procedures to identify the constituents as described.

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

Bioassay: The bioassay used as the standard Agar Disc Diffusion assay adapted from [15]. 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 37º plant parts such as leaf, stem and seed coat were tested against each organism in triplicate. Diethyl ether 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 diethyl ether, chloroform, acetone extracts of leaf, stem and seed coat of *Cardiospermum halicacabum L.* was analysed for the compounds such as tannin, saponin, flavonoid, steroid, terpenoids, cardiac, dlycosides, alkaloids, anthraquinones. Acetone, chloroform and diethyl ether of leaf, seed coat and stem were used for phytochemical studies indicated that the leaf, seed coat and stem contain a broad spectrum of secondary metabolites. Flavonoid, Terpenoids and cardiac glylosides were predominantly found in all the three tested solvent extracts of leaf followed by Tannin, Flavonoid, Terpenoids and cardiac glylosides (Acetone, chloroform and Diethyl ether). Likewise, Tannins, Flavonoid, Terpenoids and cardiac glylosides and anthrquinonus were predominantly found in all the tested solvent extracts of the stem. Then seed coat followed by Tannins, Flavonoids and Terpenioids were predominantly found in all the tested solvent in plant extracts. Saponins were not found in any of the solvent extract of stem, leaf and seed coat (Tables -1, 2 and 3)

Table 1: Preliminary Phytochemical analysis of diethyl ether, chloroform, acetone extract of leaf of *Cardiospermum halicacabum L.*

S. No	Test	Leaf acetone extract	Leaf diethyl ether extract	Leaf chloroform extract
1	Tannins	+	+	-
2	Saponins	-	-	-
3	Flavanoids	+	+	+
4	Steroids	-	-	-
5	Terpenoids	-	+	+
6	Cardiac glylosides	-	-	+
7	Alkaloids	-	-	-
8	Anthraquinonus	-	-	-

Table 2: Preliminary Phytochemical analysis of diethyl ether, chloroform, acetone extract of stem of *Cardiospermum halicacabum L.*

S. No	Test	Stem acetone extract	Stem diethyl ether extract	Stem chloroform extract
1	Tannins	+	+	-
2	Saponins	-	_	-
3	Flavanoids	+	+	+
4	Steroids	-	-	-
5	Terpenoids	+	+	+
6	Cardiac glylosides	+	+	+
7	Alkaloids	+	+	-
8	Anthraquinonus	-	-	+

Table 3: Preliminary Phytochemical analysis of diethyl ether, chloroform, acetone extract of seed coat of *Cardiospermum halicacabum L.*

S. No	Test	Seed coat acetone extract	Seed coat diethyl ether extract	Seed coat chloroform extract
1	Tannins	+	+	-
2	Saponins	-	-	-
3	Flavanoids	+	+	+
4	Steroids	-	-	-
5	Terpenoids	-	+	-
6	Cardiac glylosides	-	-	+
7	Alkaloids	-	-	-
8	Anthraquinonus	-	-	-

Antibacterial Activity

The antibacterial property of diethyl ether, chloroform, acetone extract of leaf, stem and seed coat of *Cardiospermum halicacabum.L* was analysed against bacterial pathogens using ofloxacis as control. Out of these five bacterial pathogens four were found to be gram negative (*Escherichia coli*, *Pseudomonas*, *Aeromonas*, *Salmonella)* and one were gram positive (*Staphylococcus aureus*). Disc diffusion method was used to evaluate the antibacterial activity of taken samples. After twenty-four hours the minimum inhibitory zone of diethyl ether leaf, stem and seed coat extract of *Cardiospermum halicacabum.L* and control were measured (Table: 4)

The leaf, stem and seed coat extracts of *Cardiospermum halicacabum.L* were found tested for their *pseudomonas* was found to be more susceptible towards the diethyl ether, chloroform and acetone extracts of seed coat with a maximum inhibitory zone (6mm each), followed by chloroform in stem with a maximum inhibitory zone (5mm). Chloroform and diethyl ether in the following inhibitory zone of maximum leaf and stem (4mm each), acetone and diethyl ether extracts of stem and leaf with a maximum inhibitory zone (3mm each).

Escherichia coli were tested in *Cardiospermum halicacabum.L* were found to be acetone and diethyl ether extracts of seed coat with a maximum inhibitory zone (6mm each), followed by acetone (4mm), maximum inhibitory zone in acetone and diethyl ether (3mm), and the chloroform extracts did not show any inhibition against of *E.coli.*

Aeromonas was found to be more susceptible towards the acetone extracts seed coat maximum inhibitory zone (5mm), chloroform and diethyl ether extracts in stem inhibitory zone (4mm each), acetone, chloroform and diethyl ether extracts seed coat, stem and leaf inhibitory zone (3mm).

Staphylococcus aureus was found to be more susceptible towards the acetone and diethyl ether extracts of seed coat maximum inhibitory zone (6mm), followed by acetone stem (5mm), inhibitory zone acetone and chloroform leaf and stem (2mm,3mm), and chloroform extracts did not show any inhibitory against in *staphylococcus aureus* at leaf and seed coat.(Figure-1,2 and 3).

Salmonella was found to be more susceptible towards the acetone and diethyl ether extracts of seed coat with a maximum inhibitory zone (6mm each), chloroform in seed coat (5mm), followed by stem (4mm), acetone, chloroform and diethyl ether extracts of stem, leaf maximum inhibitory zone (3mm each). The results obtained are encouraging as the acetone, chloroform and diethyl ether extracts have shown considerable antibacterial activity against the tested organisms. 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.

Medicinal plants are important source for the development of potential, new chemotherapeutic drugs and the in vitro antibacterial test form the basis [16, 17, 18].The plant leaf possesses several flavonoids such as apigenin, pinitol and luteolin [19], which are reported as the antidiabetic principles. Apigenin, a component of CHE, was also isolated from *Myrcia multiflora* leaves [20], and found to possess an inhibitory effect on the aldose reductase enzyme. Because of the intracellular accumulation of sorbitol, the chronic complications (such as neuropathy, retinopathy and cataracts) of diabetes can occur. Apigenin and luteolin were shown to possess antihyperglycaemic [21,22] and antioxidant activity[23]. The phlogistic agent induced inflammation in rats and it was used to examine the anti-inflammatory activity of *Cardiospermum halicacabum L.* This method is not only simple and reliable but also affords rapid evaluation of peripheral type of anti-inflammatory action[24]. Finally it is concluded that the whole plant extract of *Cardiospermum halicacabum* L. may be possibly a great potential source of active antimicrobial agents due to the presence of number of chemical constituents which can be the part of new and novel bioactive compounds. The antibacterial effect of Diethyl ether, chloroform, acetone solvents revealed no activity against bacteria studied. The maximum antibacterial activity was shown by *Cardiospermum halicacabum* respectively. The Diethyl ether extracts of the investigated plants showed maximum antibacterial activity against gram-negative ancylobacter, similar results were also reported by [25].Most chemotherapy drugs targets pathways that are essential to dividing cells [26]. Several studies have now documented the importance of reactive oxygen metabolites (ROM) in cisplatin and gentamycin induced renal damage [27]. The present study conclude that antibacterial activity against the gram negative and gram positive organisms showed the maximum zone of inhibition.

Table: 4 Antibacterial activity of *Cardiospermum halicacabum L.* plant extract

S. No	Number of organisms	Acetone			Chloroform			Diethyl ether		
		S	L	S C	S	L	S C	S	L	S C
1.	*Pseudomonas*	3mm	3mm	6mm	5mm	4mm	6mm	4mm	3mm	6mm
2.	*E.coli*	4mm	3mm	6mm	Nil	Nil	Nil	3mm	3mm	6mm
3.	*Aeromonas*	3mm	3mm	5mm	4mm	3mm	3mm	4mm	3mm	3mm
4.	*Staphylococcus aureus*	5mm	3mm	6mm	2mm	Nil	Nil	4mm	3mm	6mm
5.	*Salmonella*	3mm	3mm	6mm	4mm	3mm	5mm	3mm	3mm	6mm

S – Stem. L- Leaf. C- Seed coat

Fig-1 Zone of inhibition of various concentrations *Cardiospermum halicacabum L.* in Acetone

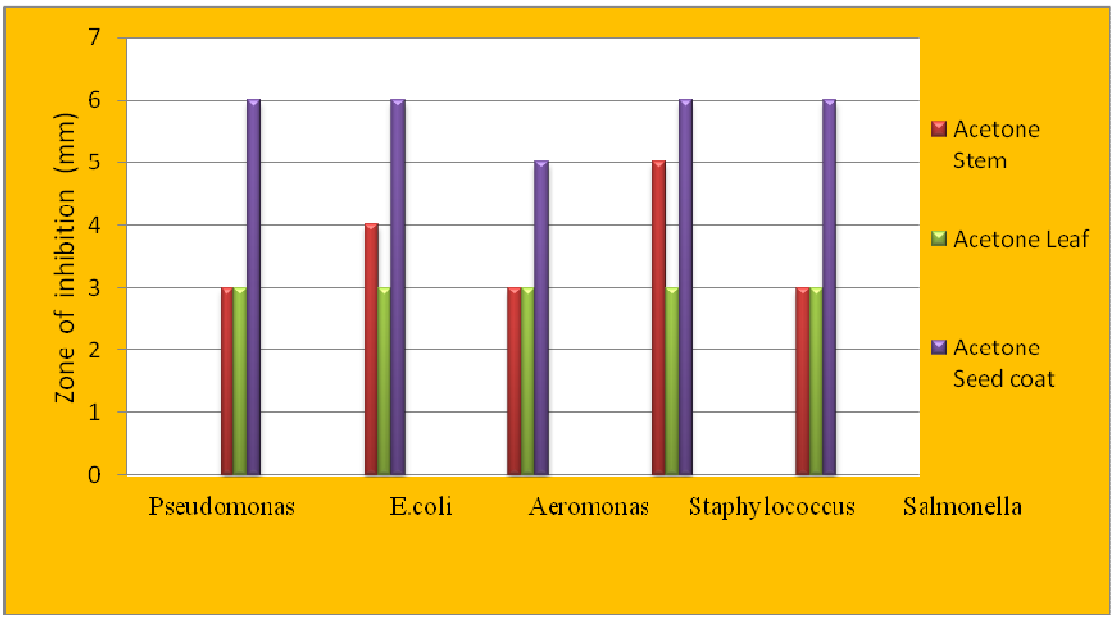


Fig- 2 Zone of inhibition of various concentration of aqueous extract of *Cardiospermum halicacabum L.* in Chloroform

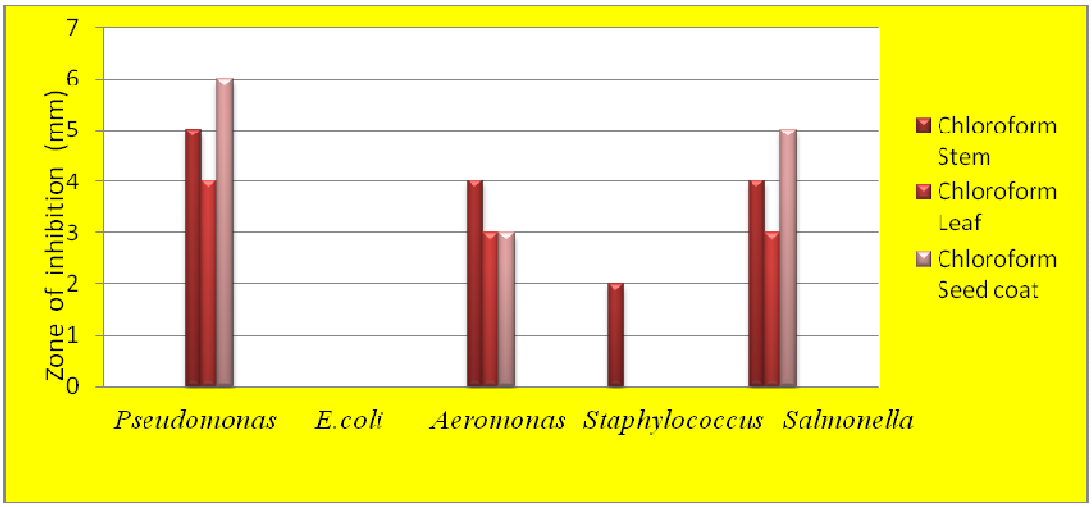
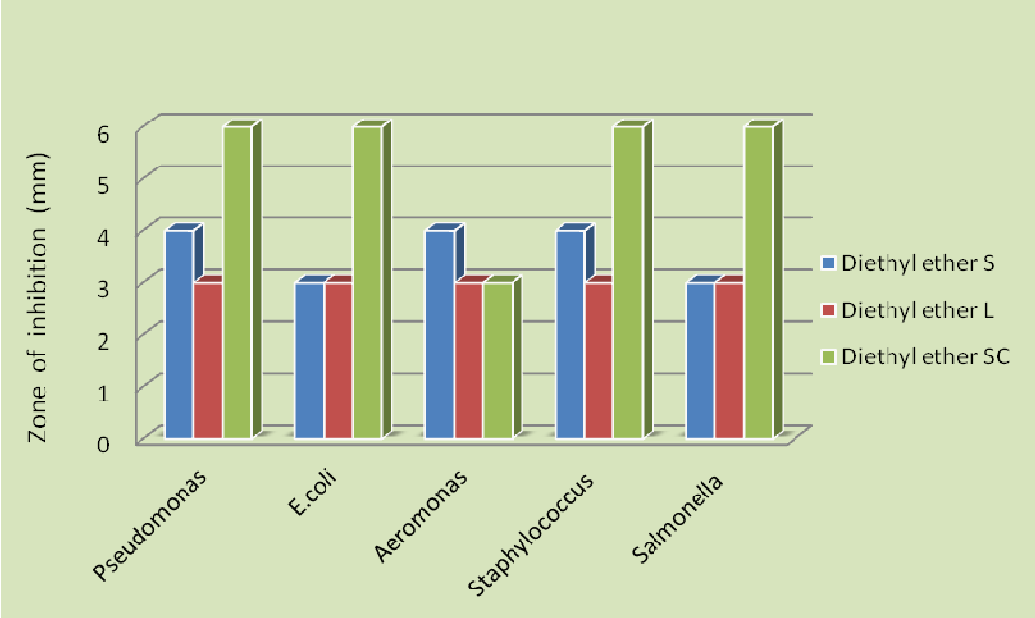


Fig-3 Zone of inhibition of various concentrations of aqueous extract of *Cardiospermum halicacabum L.* in Diethyl ether



CONCLUSION

The plant extractive studied could be an answer to the people seeking for better therapeutic agents from natural sources which is believed to be more efficient with little or no side effects when compared to the commonly used synthetic chemotherapeutic agents. The present study verified the traditional use of *Cardiospermum halicacabum L.* for human ailments and partly explained its use in herbal medicine as rich source of phytochemicals with the tannin, flavonoid, terpenoid, cardiac glycosides, alkaloids, and anthrax quinones. Thus this plant can be utilized as an alternative source of useful drugs. Further studies are needed with this plant to isolate, characterize and elucidate the structure of the bioactive compounds of this plant for industrial drug formulation. Finally it is conceded that the whole plant extract of potential source of active antimicrobial agents due to the presence of number of chemical constituents which can be the pant of new and novels bioactive Compounds.

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