PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY OF MEDICINAL PLANT *CARDIOSPERMUM HELICACABUM* LINN.

***Maluventhan Viji[1], Mani Sathiya[2], Sangu Murugesan[3]**

1. Department of Plant Morphology and Algology, School of Biological Sciences, Madurai Kamaraj University, Madurai – 625 021. India.
2. Department of Bioenergy, School of Energy, Environment and Natural Resources, Madurai Kamaraj University, Madurai – 625 021. India.
3. Department of Botany, Saraswathy Narayanan College, Perungudi, Madurai – 625 022. India.

Summary

The crude extracts from leaf and stem of *cardiospermum helicacabum* in different solvent, were subjected to pharmacognostic and fluorescence analysis, phytochemical and antimicrobial screening against selected Gram positive and Gram negative bacteria. Acetone, alcohol, benzene, chloroform and aqueous extracts of leaf and stem were used for phytochemical screening and antimicrobial activity. Phytochemical studies indicated that the leaf and stem contain a broad spectrum of secondary metabolites. Phenol, tannins and saponins were predominantly found in all the five tested solvent extracts of leaf followed by steroids, sugars, flavonoids and terpenoids (Benzene and acetone). Like wise, phenol, tannin, amino acids were predominantly found in all the tested solvent extracts of the stem. Triperpenoids were not found in any of the solvent extracts of stem. All the extracts showed varying degree of inhibitory potential against all the tested bacteria. Acetone and chloroform extracts of leaf had higher inhibitory action against *Salmonella typhi* and *Streptococcus subtilis* respectively. Acetone extracts of stem showed maximum inhibitory action against *S. typhi* and benzene extracts of stem had moderate inhibitory action against *Escherichia coli*.

Keywords: *Cardiospermum helicacabum*, fluorescence characteristics, pharmacognostic studies, phytochemical screening, antibacterial activity.

***Corresponding author**
Maluventhan Viji,
Research Scholar,
Department of Plant Morphology and Algology,
School of Biological Sciences,
Madurai Kamaraj University,
Madurai – 625 021. India.
Email: vijisp08@gmail.com

Introduction

The use of plants by man to treat common ailments is time immemorial and many of the traditional medicines are still included as part of the habitual treatment of various maladies (1). About 60 % of the total global population remains dependent on traditional medicines for their healthcare system (2). In India thousands of species are known to have medicinal values and the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient times (3). Medicinal plants are valuable natural resources and regarded as potentially safe drugs and have been tested for biological, antimicrobial and hypoglycemic activity also play an important role in the modern medicine (4-5). It is well known that even the most synthetic drugs have their origin from plant products (6). Recently scientific interest in medicinal plants has burgeoned due to the increased efficiency of plant derived drugs and raising concern about the side effects of modern medicine. The efficacy of current antimicrobial agents has been reduced due to the continuing emergence of drug resistant organisms and the adaptations by microbial pathogens to commonly used antimicrobials. There fore the search for new drugs from plants continue to be a major source of commercial drugs. Plant based antimicrobials represent a vast untapped source of medicines even after their enormous therapeutic potential and effectiveness in the treatment of infectious disease hence, further exploration of plant antimicrobials need to occur (7). The screening of plant extracts and their products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototypes (8). The selection of crude plant extracts for screening programs is potentially more successful in initial steps than the pure compounds (9). Such screening of various plant extracts has been previously studied by many workers (10-11). Eventhough hundreds of plant species have been tested for antimicrobial properties, the vast majority of them have not yet been evaluated (12).

C. helicacabum 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. Leaves ternately compound, leaflets membranous, depressed, pyriform capsule wrangled at the angles. Seeds black with a large white shaped aril. It has been widely used in traditional medicines for curing various human ailments. This plant exhibit a wide range of biological and pharmacological properties. It is well known that active constituents contributing extracts and powders from the leaves, roots and seeds of this plant are used in the preparation of shrubs and infusions in traditional medicine against diabetics and arthritis. The roots are diuretic, diaphoretic, emetic, mucilaginous, laxative and emmenagogue. They are useful in strangury fever, arthritis, amenorrhea, lumbago and neuropathy and rheumatism, stiffness of limbs and snake bite, nervous disorders and piles. The leaves are rubefacient and are good for arthritis and piles. The plant has sedative action on central nervous system. Phytochemical examination of the extracts of this plant showed the presence of glycosides, steroids, flavones and reducing sugars. Considering this an attempt has been made to investigate the phytochemical, antimicrobial and fluorescence characters of benzene, chloroform, acetone, ethanol and aqueous extracts from leaf and stem of *C. helicacabum*. This study will also hopefully exposes new frontiers by improving the current applications of this plant and provides a scientific basis for the traditional claims of this ethnic medicinal plant.

Materials and methods

Preparation of plant extracts

Fresh Plant of *Cardiospermum helicacabu*m L. was collected from Saraswathi Narayanan College campus; they were identified with the help of Gamble's flora. The plant material was washed with water to remove shade dried at room temperature. Extracts were prepared from the method of (13). The dried plant materials were ground into fine powder in an electric blender and subsequently sieved for obtaining fine powder. The soaked plant powder was filtered and used as such for qualitative, phytochemical analysis and antimicrobial assays.

Analysis of fluorescence pharmacognostic characters

Fluorescence analysis was carried out with powders prepared from shade dried plants as well as in acetone, alcohol, chloroform, benzene and water extracts as described by Thomas *et al*. (14). The powders were treated separately with 1N aqueous NaOH, 1N ethonolic NaOH, 1 N H_2SO_4 and 1N HNO_3. The supernatants were examined under ultraviolet light and ordinary day light. Pharmacognostic characters of *Cardiospermum helicacabum* were analyzed by employing standard method as described in Pharmacopeia of India.

Phytochemical screening

Phytochemical screening was carried out to assess the qualitative chemical composition of crude extracts using commonly employed precipitation and coloration to identify the major natural chemical groups such as steroids, reducing sugars, alkaloids, phenolic compounds, saponins, tannins, flavonoids, amino acids and anthracene glycosides. General reactions in these analysis revealed the presence or absence of these compounds in the crude extracts tested (15). Crude extracts of the plants previously prepared and stored in a refrigerator were used for the phytochemical tests.

Collection of microorganisms and preparation of media

Stock cultures such as *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeroginosa*, *Klebsiella pneumoniae*, *Citrobacter freundi*, *Streptococcus aureus* and *Salmonella typhi* were obtained. The growth media employed in the present study included nutrient agar and nutrient broth. The medium was adjusted to pH 7.4 and sterilized by autoclaving at 120 °C for 15 min.

Screening for antibacterial potential

Antibacterial activity was determined by disc diffusion method as described by Langfeild (16). The standard inoculum suspensions were swabbed over the surface of media. The oven dried discs impregnated with 20 µl of the leaf and stem extracts (1mg/ml) were placed on the surface of the medium. After the incubation period the diameter of inhibition zone around the plant extract saturated discs were measured as the difference in diameter between the discs (6 mm) and growth free zone.

Results

Fluorescence analysis and quantitative determination of pharmacognostic characters

The results of Fluorescence analysis of the powder and extracts in visible and UV range has been shown in Table1. The results of quantitative determination of pharmacognostic characters of *C. helicacabum* were presented in Table 2 and were helpful in evaluating the pharmacognostic value of the medicinal plant. The moisture, total ash, acid insoluble ash, water soluble ash contents were found to be 73.6 % and 75.1 %; 88.9 % and 92 %; 17.33 % and 15.33 %; 10 % and 9.33 % for leaf and stem extracts respectively. Higher amount of water soluble ash was recorded in leaf (10 %) than stem (9.33 %). Higher extractive value was found in ethanol extract of leaf, stem when compared to other solvents.

Table 1. Analysis of fluorescence characters of leaf and stem powders and extracts of *Cardiospermum helicacabum* L. in different solvents

Sl. No.	Treatment	Under Day Light		Under UV Light	
		Leaf	Stem	Leaf	Stem
1.	Powder	Green	Green	Green	Green
2.	Powder + 1N NaOH	Light green	Pale green	Dark green	Dark green
3.	Powder + 1N NaOH (ethanolic)	Brownish yellow	Reddish brown	Blackish red	Brownish yellow
4.	Powder + 1N HCl	ale green	Light yellow	Yellow	Yellow
5.	Powder + H_2SO_4	Yellowish green	Yellow	Blackish green	Greenish yellow
6.	Powder + HNO_3	Yellow	Yellow	Yellowish green	Greenish yellow
7.	Acetone	Yellowish green	Light green	Brownish green	Dark green

8.	Alcohol	Dark green	Dark green	Blackish green	Dark green
9.	Benzene	Pale green	Pale green	Dark green	Dark green
10.	Chloroform	Brownish yellow	Brownish yellow	Dark green	Brownish yellow
11.	Water	Light yellow	Light green	Dark yellow	Yellow

Table 2. Pharmacognostic characters of leaf and stem of *Cardiospermum helicacabum* L.

Parameters tested	**Percentage Yield (%)**	
	Leaf	**Stem**
Loss of weight on drying	73.6	75.1
Total ash	88.9	92
Acid soluble ash	10.5	15.33
Water soluble ash	17.33	9.33
Percentage of extractive yield values		
Acetone	60	56
Ethanol	96	73
Benzene	73	75
Chloroform	83	86
Water	90	92

Phytochemical screening

Phytochemical evaluation of the various extracts of the leaf and stem of *C. helicacabum* were done for the presence of steroids, triterpenoids, sugars, alkaloids, phenols, saponins, amino acids, tannins, flavonoids and anthracene glycosides and results were presented in Table 3.

Table 3: Results of phytochemical screening of leaf and stem extracts of *Cardiospermum helicacabum* L.

Phyto-chemicals	Solvent extract used									
	Benzene		Chloroform		Ethanol		Acetone		Water	
	L	S	L	S	L	S	L	S	L	S
Steroids	+	+	+	+	+	+	+	+	-	-
Triterpenoids	-	-	-	-	-	-	+	-	-	-
Sugars	+	+	-	+	-	-	+	+	+	+
Alkaloids	+	+	+	+	-	-	+	+	-	+
Phenols	+	+	+	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+	+	+	+
Aminoacids	+	+	-	+	+	+	+	+	-	+
Tannins	+	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	-	+	+	+	-	-	-	-
Anthracene glycosides	+	+	+	+	+	+	-	-	-	-

Symbols + and – represent the presence or absence of the respective phytochemicals.

Antimicrobial activity

The leaf and stem extracts of *C. helicacabum* were tested for their antimicrobial activity against *S. aureus, B. Subtilis, C. freundii, E. coli, P. aeroginosa, S. typhi, K. pneumoniae* and the results are presented in Table 4.

Table 4. Antibacterial activity of various extracts of leaf and stem of *Cardiospermum helicacabum* L.

Bacterial strains	Solvent extract used									
	Benzene		Chloroform		Ethanol		Acetone		Water	
	L	S	L	S	L	S	L	S	L	S
Stepto-coccus aureus	2	2	0	1	3	3	1	2	0.5	0
Bacillus Subtilis	1.5	1	2	2	3	1	1.5	2	1	2
Citro-bacter freundii	1.5	1	0	0	2	1.5	1.5	2	0.5	0.5
Escherichia coli	1	2.5	0	0	1	2	1	1	1	0
Pseudo-monas aeroginosa	2	2	0	0	1	0	2	1	0.5	2.5
Salmonella typhi	2	1.5	0.5	0	2	2	3	3.5	0.5	0

Values presented indicate the zone of inhibition formed around the discs (mm).

Streptococcus aureus was found to be more susceptible towards the ethanolic extracts of leaf and stem with a maximum inhibitory zone (3 mm each) followed by benzene (2 mm each), acetone (1 mm, 2 mm), Chloroform (0 mm, 1 mm) and aqueous (0.5 mm, 0 mm). *Bacillus subtilis* was found to be more sensitive to the ethanolic extracts of leaf and stem with a maximum inhibitory zone (3 mm, 1 mm) followed by chloroform (2 mm each), acetone (1.5 mm, 2 mm), benzene (1.5 mm, 1 mm) and aqueous extract (1 mm, 2 mm). *Citrobacter freundii* was found to be more susceptible towards the ethanolic extracts of leaf and stem with a maximum inhibitory zone (2 mm, 1.5 mm), acetone (1.5 mm, 2 mm), benzene (1.5 mm, 1 mm), aqueous (0.5 mm, 0.5 mm) and the chloroform extracts did not show any inhibition against *C. freundii*. *E. coli* was found to be sensitive to benzene with a maximum inhibitory zone (1 mm, 2.5 mm), followed by ethanol (1 mm, 2 mm), acetone (1mm, 1 mm), aqueous (1 mm, 0 mm) and the chloroform extracts did not show any inhibition against *E. coli*. *Psuedomonas aeroginosa* was found to be more susceptible to benzene (2 mm, 2 mm) followed by acetone (2 mm, 1mm), ethanol (1 mm, 0 mm), aqueous (0.5, 0.5) and the chloroform extracts did not show any inhibition against *P. aeroginosa*. *Salmonella typhi* was more susceptible to acetone extracts (3 mm, 3.5 mm) followed by ethanol (2 mm, 2 mm), benzene (2 mm, 1.5 mm), chloroform (0.5, 0 mm) and aqueous extracts (0.5 mm, 0 mm). *Klebsiella pneumoniae* was sensitive towards acetone extracts with a maximum inhibitory zone of 1 mm, 2 mm followed by benzene (1.5 mm, 1 mm), ethanol (1 mm, 1 mm), chloroform (0 mm, 1 mm) and aqueous (1 mm,0 mm). The results obtained are encouraging as the benzene, ethanolic and chloroform extracts have shown considerable antibacterial activity against the tested organisms.

Discussion

The antimicrobial activity have been screened because of their great medicinal relevance with the recent years, infections have increased to a great extent and resistant against antibiotics, becomes an ever increasing therapeutic problem (17-18). The presence of antifungal and antimicrobial substances in the higher plants is well established as they have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health. Phytomedicine have been used for the treatment of diseases as in done in cases of Unani and Ayervedic system of medicines, a natural blueprint for the development of new drugs. Much of the exploration and utilization of natural product as antimicrobial arise from microbial sources. Present study was conducted to analysis the pharmacognostic, phytochemical, fluorescence characteristics and antibacterial potential of leaf and stem extracts of *C. helicacabum*.

Florescence analysis of powders and crude extracts of different parts of medicinal plants (leaf, stem, root, bark and fruit) gives a clue if powder and extracts are in adulteration, thus can be used as a diagnostic tool for testing the adulteration. Such studies were done previously in *Morinda tinctoria* (19), and *Abutilon indicum* (3).

Knowledge of the phytochemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of such economic materials as tannins, oils, gums, flavonoids, saponins, essential oils precursors for the synthesis of complex chemical substances (20). The results of phytochemical screening of extracts of leaf and stem indicate the strength of active principle depends on the use of a suitable solvent besides the type of the plant species to achieve positive results. Hence leaf and stem extracts of *C. helecacabum* is highly recommended for the herbal preparations to the traditional medicinal practioners and for the pharmaceutical industries for the mass scale extractions of the therapeutic agents. Similar studies by previous workers showed the presence of steroids and anthocyanin in the seeds of *Boerrhhavea orellana* and alkaloids and steroids in *Cardiospermum officinalis* (21); Terpenoids, tannins and guaabins from *Psidium guajava* and polygalactorunases in *Mangifera indica* (22); alkaloids, tannins, steroids, flovonoids from the ethanolic and aqueous extracts of stem and bark of *Picralima nitida* (23); lenolinic acid in *Ocimum sanctum* (24); phenolic compounds, flavonoids, cyclobutane in *Combretum alpopunctatum* (25); diterpenes, flavonoids, andrographolates and polyphenols from *Andrographis paniculata* (26-27) and the presence of tannins, alkaloids, phenols and saponins in twelve Indian medicinal plants (28).

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 (29-30). Many of the studies were useful in identifying the active principle responsible for such potentials and to develop clinically important therapeutic drugs for mankind. Hence an attempt has been made to identify the antibacterial activity of leaf and stem extracts of *C. helicacabum* against seven clinically important Gram positive and Gram negative bacteria. Few studies have showed the antiviral, antibacterial, antifungal, antihelminthic, antimolluscal, anti-inflammatory, antidiarrhoeal and insecticidal potential of this traditional medicinal plant (31-33). Previously such studies have been done in several medicinal plants (34). Ethanolic extracts of *Holarrhenea antidyssentaria* seeds showed antibacterial activity against *E. coli*. Previous screening studies by earlier workers proved the antibacterial and antifungal potential of *Holarrhenea antidyssentrica* (25); *Nerium oleander* (35); *Tapinthus senssilifolius* (36); *Rauelfia tetraphylla* and *Physalis minima* (37); *Achillea santolina, Salvia dominica* and *Salvia officinalis* (4); *Vitex doniana* and *Shigella dyssentriae* (38); *Psidium guajava* and *Mangifera indica* (22) and *Salicornia brachiata* (39) against several bacterial strains including *E.coli*, *Bacillus subtilis, Streptococcus aureus*, *Psuedomonas aerogenosa* and *Candida albicans*.

Many plants have limitless ability to synthesize secondary metabolites of which at least 12000 have been isolated. These substances serve as plant defense mechanism against predation by microorganisms, insects and herbivores (40). Many plants and their extracts

used against microbial infections due to the presence of secondary metabolites such as phenols (41); essential oils (42-43); terpenoids (44-45); alkaloids (46) and flavanoids (47).

Plants are used medicinally in different countries and are a source of many potent and powerful drugs (30-48). Natural products either extract or pure compounds provide unlimited opportunities for the development of new drugs due to the availability of chemical diversity (49). To overcome the problem of antibiotic resistance ethnic medicinal plants have been extensively studied as an alternative treatment for diseases due to their ability to produce a variety of compounds of known therapeutic properties (50-51) and much attention has been paid to plant extracts and their biologically active compounds (52). The screening of natural products has been the source of innumerable therapeutic agents (53). Higher plants as a source for new potential drugs is still largely unexplored and only a small percentage of them has been subjected to phytochemical investigation and the fractions submitted to pharmacological screening is very low. Such screening of various natural organic compounds and identifying active agents is a need of the hour as due to successful prediction of lead molecule and drug like properties at the onset of drug discovery will pay of later in drug development.

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 preset study verified the traditional use of *C. helicacabum* for human ailments and partly explained its use in herbal medicine as rich source of phytochemicals with the presence of tannins, phenols, saponins, steroids, flavinoids and terpenoids. 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.

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