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STUDIES ON PHYTOCHEMICAL AND ANTIMICROBIAL ACTIVITY IN *Cardiospermum helicacabum L.*

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ABSTRACT

In the present study was carried out on phytochemical screening and antimicrobial activity of Cardiospermum helicacabum L. leaves and stems extract against some clinical pathogens. Phytochemical compounds were carried out on the different extract the powdered specimens using standard procedures. Soxhlet apparatus were used for the extracting antimicrobial active compounds from the plant leaves and stem powered. In this investigation tannin, flavonoids and terpenoids showed positive results in Cardiospermum helicacabum L. leaves and stem aqueous extract and ethanol extract showed positive results in terpenoids, saponins, steroids, and carbohydrates. Antimicrobial susceptibility test was determined by Bauer et al., 1966 method. Antimicrobial activity against were analyzed against Bacillus subtilis Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia Pseudomonas aeruginosa, Aspergillus niger and Aspergillus flavus. Ethanol and methanol extract was most effective followed by other aqueous extract. Staphylococcus aureus was more sensitive for ethanol and methanol extract of leaves and stem of the tested plants. In this investigation aqueous, acetone and methanol extracts from the leaves and stem of Cardiospermum helicacabum L. exhibit antifungal activity against Aspergillus niger and Aspergillus flavus. The crude extract as well as the isolated compounds found to be active in this study could be useful for the development of new antimicrobial drugs.

KEYWORDS : Phytochemical, Antimicrobial activity, Cardiospermum helicacabum L.

INTRODUCTION

Herbal medicine, also known as botanical medicine or phytomedicine refers to the use of any part of plant medicinal purposes about 25% these medicinal plants are known to be useful for the cure for gastrointestinal disorders ranging for peptic ulcer and abdominal ramps to diarrhea and dysentery (Hill, 1952). Traditional medicines are used by about 60 percent of the worlds, population. There are about 45,000 plants species in India with concentrated hotspots in the region of Eastern Himalayas, Western Ghats and Andaman and Nicobar Island. The traditional medicinal system of the ayurveda is ancient health care system and is practiced widely in India. In factious diseases caused by the microbes are major health hazards all over the cord. Several synthetic antibiotics and drugs are employed in the treatment of the microbial infection and communicable disease. But the microbial pathogens develop resistance to the synthetic antibiotics.

Cardiospermum helicacabum L. Mudakattan, Kottavan, Modikkottan is a climbing, many branched plant, has stems 2 to 4 m long (Johnston et al., 1979). The species has been used as a traditional medicinal plant over thousands of various ailments. Its fruits are eaten to treat liver dysfunction to break fever to counteract the putrefaction of blood while roots are used to improve digestion. Fruits are very rich source in iron and vitamin c. Therefore, ethnomedicallythe fruits are used for curing anemia as an astringent an antiscorbutic, and as a remedy for biliousness. Its leaf decoction is used against fever, diarrhea, and a stomachic, vermifuge, remedy for itches, and insect repellent Cardiospermum helicacabum L. (F: Sapindaceae) a genus of about 32 species distributed mostly in the warmer parts of the 8 Indian species 3 are economic importance the plant is native and common throughout much of Indian, Srilanka, Java, Malaysia, Myanmar and Pakistan (Afolayan and Meyer, 1997). The Cardiospermum helicacabum L. leaves and stem are used in traditional certain in India as remedy against cough, intestinal disorder and bacterial infection. Numbers of studies have been conducted in different countries to prove such efficiency. In the present study carried on phytochemical constituents and antimicrobial activity of Cardiospermum helicacabum L. leaves and stem.

MATERIALS AND METHODS

Collection and Preparation of Plant Material

The Cardiospermum helicacabum L. plant material were collected from Vallam, Thanjavur District, Tamil Nadu. India in January 2017. The collected plant materials (leaves and stem) were air-dried. After dried sample was ground in a grinding machine made for the laboratory. Exposure direct sunlight was avoided to prevent the loss of active components. These powdered materials were used for further analysis.

A soxhlet apparatus were used for the extracting antimicrobial active compounds from the plant materials (leaves and stem). 20g of the plant powder was ground and soaked with 50ml aqueous water, acetone and methanol (separately) in a 250ml conical flask. The flask was covered with cotton wool the present the solvent from escaping the extract was filtered in filter paper the plant extract were prepared by using soxhlet apparatus collected and stored in a vial for further studies.

Qualitative analysis of phytochemical compound

Chemical tests were carried out on the aqueous, acetone and methanol extracts and on the powdered specimens were using standard procedures to identify the constituents are described by Sofowara (1993) Treas and Evans (1989) and Harborne (1973).

Quantitative analysis of Phytochemical Compounds

Tannins and Flavonoids

The plant extracts extracted were 2ml added in test tube. Then 5ml of Folin-Denis reagent added inside test tube after 10ml of sodium carbonate solution add, few drops water mixed and the solution shaken well. The solution read at 700nm of spectrophotometric. The 0.5g the sample was weighed and extracted repeatedly with 100mlof 80% aqueous methanol at room temperature. The whole solution was filtered through whattman filter paper NO4. The filtrate was evaporated into dryness over a water bath and weighed to a constant weight the percentage flavonoids was then calculated.

Saponins

The extract was dissolved in 80% methanol, 2ml of phenolic aldehyde in ethanol was added than 2ml of 72% sulphuric acid mixed well and heated on a water bath at 60°C for 10min.Absorbance was measured at 544nm against reagent blank. Diosgeninis used as a standard material and compared the assay with Diosgninequivalents.

Carbohydrate

The 100mg of plant sample was hydrolysed in a boiling tube with 5ml of 2.5N HCl in a boiling water bath for a period of 3 hours. It was cooled to room temperature and solid sodium carbonate was added until effervescence cerses, the contents were centrifuged and the supernatant was made to 100ml using distilled water from this 0.2ml fruit sample was pipetted out and mode up the volume to 1ml with distilled water then 1.0ml phenol reagent was added followed by 5.0ml of sulphuric acid. The tubes were kept at 25˚-30ºC for 20min. The absorbance was read at 490nm.22.

Assay of Antibacterial Activity

Disc preparation and Collection of Test Organisms

The eight sterile disc in a disc preparation then various solvents extract disc and control disc were prepared. The test human pathogenic bacteria such as Bacillus subtilis Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae and Pseudomonas aeruginosa were collected from Microbial Type Culture Collection Center (MTCC). The fungal strains of Aspergillus niger, and Aspergillus flavus were obtained from Microbial Type culture Collection Centre (MTCC), Chandigarh.

Antimicrobial Susceptibility Test

Disc diffusion method (Bauer et al., 1966) was adopted for evaluation of antimicrobial activity of Cardiospermum helicacabum L. plant part. Muller Hinton Agar was prepared and autoclaved at 121°C pressure for 20 minutes and cooled to sterile petriplates and allowed for solidification. The plates with media were seeded with the respective microbial suspension using swab. The various solvents extract prepared discs individually were placed on the each petriplates and also placed on the each petriplates and also placed control and standard (Ampicillin for Bacteria and Amphotericin B for fungi) discs. The plates were incubated at 37ºC for 24hrs for bacteria and 28ºC for 72 hrs for fungi. After incubation period the diameter of the zone formed around the paper disc were measured and expressed in mm.

Statistical Analysis

The results obtained in the present investigation were subject to statistical analysis like Mean (*x*) and Standard Deviation (SD) by Zar (1984).

RESULTS

Qualitative analysis of phytochemical compounds

The analysis of Tannin compounds brownish green colour developed to indicate the presence of tannin aqueous extract. Similarly based on the presence or absence of colour change indicate positive and negative results. In these investigation flavonoids, saponins, and carbohydrate, showed positive results in Cardiospermum helicacabum L. plant leaves aqueous extract and acetone extract showed positive results in Tannin and saponins. Similarly phyotochemical compounds investigated in Cardiospermum helicacabum L. plant leaves methanolic extracts. The methanolic extracts showed positive result in Tannin, flavonoids and saponins (Table – 1).

Cardiospermum helicacabum L. plant stem phytochemical constituents were qualitatively analyzed (Table – 2). In the present study saponins, Phlobatannins and carbohydrate were positive results in Cardiospermum helicacabum L. plant stem aqueous, acetone and methanol extract. Tannin was present in acetone and methanolic extracts.

Quantitative analysis of phytochemical compounds

Cardiospermum helicacabum L. leaves and stem phytochemical constituents were quantitatively estimated. In the present study, carbohydrates (46.56±1.47, %) maximum amount were noted in Cardiospermum helicacabum L. leaves aqueous, extracts compared than other compounds such as tannins and flavonaids. The maximum level of sapoins was present in all the plant leaves extract. The minimum level of tannins (6.99±1.75 and 5.93±1.54 %) was noted in Cardiospermum helicacabum L. leaves acetone and methanol extracts (Fig. – 1).

In this investigation, Cardiospermum helicacabum L. stem aqueous, acetone and methanol extracts carbohydrates maximum amount were noted (49.33±1.53, 44.04±1.47 and 33.89±1.80 %) compared than other compounds such as tannins and flavonaids. The minimum level of tannins (1.60±2.15 and 11.97±1.42 %) was presented in Cardiospermum helicacabum L. stem acetone and methanol extracts (Fig. – 2).

Antibacterial activity of Cardiospermum helicacabum L.

In this investigation aqueous, acetone and methanol extracts from the leaves and stem of Cardiospermum helicacabum L. exhibit antibacterial activity against Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus and Pseudomonas aeruginosa (Table – 3). Acetone and methanol extract was most effective followed by aqueous extracts. Staphylococcus aureus was more sensitive for Cardiospermum helicacabum L. acetone and methanol extracts of leaves of the tested plants. Aqueous extracts show no inhibition against the tested organism compared to acetone and methanol extracts. The Staphylococcus aureus (16±1.4; 18±1.0 mm in diameter) exhibit relatively higher zone of inhibition compared then other test organisms.

Antifungal activity of Cardiospermum helicacabum L.

In this investigation aqueous, acetone and methanol extracts from the leaves and stem of Cardiospermum helicacabum L. exhibit antifungal activity against Aspergillus niger, and Aspergillus flavus. Acetone and methanol extract was most effective followed by aqueous extracts of leaves and stem (Fig. – 3).

DISCUSSION

Health is the real wealth of nation. Nature has provided all necessary things for survival. Medicinal plants are nature's best gift to cure a number of diseases for men and women. This present study was carried out on phytochemical and antimicrobial activity of Cardiospermum helicacabum L. leaves and stem extracts against some clinical pathogens. The investigated results were discussed with previous theoretical and statically.

In this investigation terpenoids, steroids, flavonoids, tannins, and phlobatannins, cartioac glycosides were present. Many of them are known to have different therapeutic. Tannins possess antibacterial, antiviral, moluscicidal and antitumoral properties (Scalbert, 1991). While steroids, also present in Cardiospermum helicacabum L. is recognized to have anticancer, antiviral and antihemorrhagic properties (Hegde and Joshi, 2009). The results of present research highlights, the fact that the organic solvent extracts exhibited greater antimicrobial activity because the antimicrobial principles were either polar or non-polar and they were extracted only through the organic solvent medium (Britto, 2001).

On the basis of the results obtained in this present investigation, conclude that the extract of Cardiospermum helicacabum L. leaves and stem had significant in vitro antibacterial activity. In this investigation aqueous extract was most effective followed by acetone and methanol extracts. Staphylococcus aureus was more sensitive for Cardiospermum helicacabum L. all leaves extracts. Methanol extracts was low inhibition against the tested organism compared to aqueous extracts. Similarly supported by Singh, (1986) has been studied Ethanol and aqueous extracts of Acalypha indica, Abutilon indicuma, Cassia auriculata, Eclipta alba, Mentha arvensis and Phyllanthus niruri against Bacillus subtilis revealed that ethanolic root extract seem to be more active compared to aqueous extract.

Many substances may be antimicrobial, but only a few of them will be potential therapeutic agents for the simple reason that mammalian cells are more sensitive to chemical inhibition than microbial cells (Sivakumar and Alagesaboopathi, 2006). Moreover

emphasized the need for toxicity testing of drugs derived from medicinal plants because the crude products obtained from such cheaper sources are often associated with a large number of compounds that have discomforting abilities (Ramdas et al., 2006). Thus the ethnobotanical approach will be like a search for molecular diversity subjecting a wide variety of new molecules from plant sources and testing them with as many different tests as possible (Muhammad and Muhammad, 2005).

CONCLUSION

From this study it is clear that Cardiospermum helicacabum L. indeed exhibits an antimicrobial activity. More research needs to be done to unravel the inhibitory effect of this plant. Since this herb had been used for ages traditionally and effectively, it is presumed that side effects should be less. Use of herbs by Indian (south) community is a well known fact; there is a treasure of herbs that we use daily in our food or in other forms customarily, even without knowing their medicinal benefits. Such use of plant material has always been a tradition, mostly community based that is passed on from one generation to another. In general, lesser known or used herbs and plant materials have to be researched further to study their medicinal properties especially their antibiotic nature. This will enable the use of our own local, rich plant heritage as effective medicines with probably fewer side effects.

ACKNOWLEDGMENTS

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Table – 1 Qualitative analysis of Phytochemical Compounds in Cardiospermum helicacabum L. Leaves extracts

S. No.	Phytochemical Compounds	Observa tion			
		Control	Aqueous	Acetone	Methanol
1	Tannins	-	-	+	+
2	Flavonaids	-	+	-	+
3	Terpenoids	-	-	-	-
4	Sapoins	-	+	+	+
5	Phlobatannins	-	-	-	-
6	Steroids	-	-	-	-
7	Carbohydrates	-	+	-	-
8	Glycosides	-	-	-	-
9	Cournarins	-	-	-	-
10	Proteins	-	-	-	-
11	Emodins	-	-	-	
12	Anthraquinones	-	-	-	-
13	Anthocyanins	-	-	-	-
14	Leucoantho cyaninsturns	-	-	-	-

+ indicate present; - indicate absent

Table – 2 Qualitative analysis of Phytochemical Compounds in Cardiospermum helicacabum L. Stem extracts

S. No.	Phytochemical Compounds	Observation			
		Control	Aqueous	Acetone	Methanol
1	Tannins	-	-	+	+
2	Flavonaids	-	+	-	-
3	Terpenoids	-	-	-	-
4	Sapoins	-	+	+	+
5	Phlobatannins	-	+	+	+
6	Steroids	-	-	-	-
7	Carbohydrates	-	+	+	+
8	Glycosides	-	-	-	-
9	Cournarins	-	-	-	-
10	Proteins	-	-	-	-
11	Emodins	-	-	-	
12	Anthraquinones	-	-	-	-
13	Anthocyanins	-	-	-	-
14	Leucoantho cyaninsturns	-	-	-	-

+ indicate present; - indicate absent

Fig. – 1 Quantitative analysis of Phytochemical Compounds in Cardiospermum helicacabum L. Leaves extracts

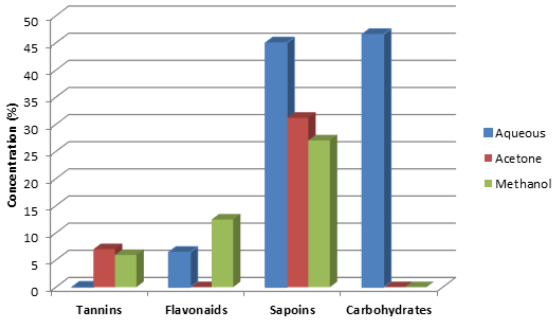

Fig. – 2 Quantitative analysis of Phytochemical Compounds in Cardiospermum helicacabum L. Stem extracts

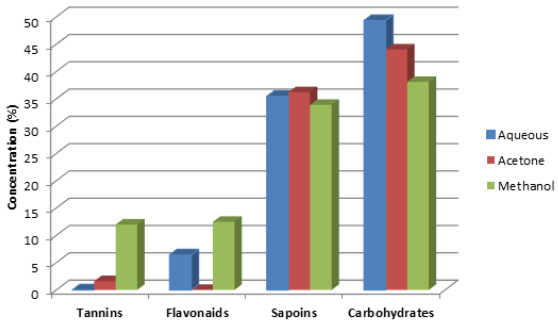

Table – 3 Antibacterial activity of Cardiospermum helicacabum L. Leaves extracts

S. No.	Bacteria	Zone of Inhibition (mm in diameter)				
		C	S	Aqueous	Acetone	Methanol
1	Bacillus subtilis	-	14±1.4	16±1.8	11±1.2	14±1.0
2	Escherichia coli	-	20±0.8	13±1.0	12±0.8	13±1.5
3	Klebsiella pneumoniae	-	14±1.5	10±1.2	13±1.0	13±1.2
4	Staphylococcu s aureus	-	18±1.0	-	16±1.4	18±1.0
5	Pseudomonas aeruginosa	-	15±1.0	-	17±0.8	15±1.8

Values are expressed in Mean ± Standard deviation; n=3
*Standard - Ampicillin (10 mg/disc)

Table – 4 Antibacterial activity of Cardiospermum helicacabum L. stem extracts

S. No.	Bacteria	Zone of Inhibition (mm in diameter)				
		C	S	Aqueous	Acetone	Methanol
1	Bacillus subtilis	-	10±1.4	12±1.1	11±1.7	11±0.9
2	Escherichia coli	-	12±0.6	14±1.5	14±1.8	13±1.2
3	Klebsiella pneumoniae	-	12±1.2	11±1.7	11±1.6	12±1.0
4	Staphylococcus aureus	-	10±1.6	13±1.0	14±0.8	12±1.5
5	Pseudomonas aeruginosa	-	10±1.2	08±1.6	08±1.2	-

Values are expressed in Mean ± Standard deviation; n=3
*Standard - Ampicillin (10 mg/disc)

Table – 5 Antifungal activity of Cardiospermum helicacabum L. Leaves extracts

S. No.	Fungi	Zone of Inhibition (mm in diameter)				
		C	S	Aqueous	Acetone	Methanol
1	Aspergillus niger	-	15±1.75	16±1.42	28±1.82	24±1.98
2	Aspergillus flavus	-	17±1.98	18±1.65	26±1.37	22±1.54

Values are expressed in Mean ± Standard deviation; n=3
*Standard - Amphotericin b (10 mg/disc)

Table – 6 Antifungal activity of Cardiospermum helicacabum L. Stem extracts

S. No.	Fungi	Zone of Inhibition (mm in diameter)				
		C	S	Aqueous	Acetone	Methanol
1	Aspergillus niger	-	18±1.89	18±1.75	29±1.46	24±2.93
2	Aspergillus flavus	-	12±2.36	11±0.86	22±1.98	22±1.21

Values are expressed in Mean ± Standard deviation; n=3
*Standard - Amphotericin b (10 mg/disc)

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