PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY OF CARDIOSPERMUM HALICACABUM LINN

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ABSTRACT

Objective: The use of plant as medicine is as old as human civilization. Exploration of this traditional knowledge for cures to common diseases is an attractive prospect. Cardiospermum halicacabum Linn commonly known as ‘Balloon vine’ has many associated utilities, including a remedy for joint pain.

Methods: To evaluate the antibacterial activity of cardiospermum halicacabum leaf extracts against four microorganisms, viz. Escherichia Coli, Pseudomonas aeroginosa, Staphylococcus aureus and Salmonella typhii. Phytochemical analysis of the leaf in solvents of varying polarity; viz., Aqueous, Ethanol and chloroform were also carried out. Well diffusion method was used to assess the antibacterial effect of the extracts on both Gram positive and Gram negative micro-organisms.

Results: The ethanolic extract was active against Salmonella typhii, and Staphylococcus aureus whereas the aqueous extract exhibited an inhibitory effect on Staphylococcus aureus only. The phytochemical screening indicated the presence of phenolics, flavonoids, tannins, glycosides etc., in the extracts. We believe that the higher reducing power of the aqueous extract could be due to the better solubility of the antioxidant components in water whereas the predominant antibacterial activity in organic solvent extracts as compared to aqueous extracts.

Conclusions: Indicates that the active components responsible for the bactericidal activity are more soluble in organic solvents. These studies provide an evidence to support traditional medicinal uses of the plant.

INTRODUCTION

Plant-based drugs have been used worldwide in traditional medicine for the treatment of various diseases. Approximately 60% of world’s population still relies on medicinal plants for their primary healthcare. According to a survey by NCI, USA, 61% of the 877 small-molecule new chemical entities introduced as drugs worldwide during 1981-2002 were inspired by natural products [1] Plant species still serves as a rich source of many novel biologically active compounds, as very few plant species have been thoroughly investigated for their medicinal properties [2]. Thus, there is renewing interest in phytomedicine during last decade and nowadays many medicinal plant species are being screened for pharmacological activities [3].

Cardiospermum halicacabum (Linn), family Sapindaceae, is a deciduous, branching, herbaceous climber, which is distributed throughout the plains of India. The whole plant has been used for several centuries in the treatment of rheumatism, stiffness of limbs, snake bite; its roots for nervous diseases, as a diaphoretic, diuretic, emetic, emmenagogue, laxative, refrigerant, stomachic and sudorific; its leaves and stalks are used in the treatment of diarrhea, dysentery and headache and as a poultice for swellings. Phytochemical constituents such as flavones, aglycones, triterpenoids, glycosides and a range of fatty acids and volatile ester have been reported from the various extracts of this plant [4]. Most likely confused with: Physalis spp. (ground cherry), Clematis occidentalis, Clematis virginiana, Campsis radicans, Adlumia fungosa.

The aim of the study was to show that leaves of cardiospermum halicacabum linn…have antimicrobial activity and preliminary phytochemical analysis was also evaluated.

MATERIALS AND METHODS

Plant Collection and Identification

The experiment was conducted in the year 2011 in the college laboratory. Leaves were collected from the C. halicacabum Linn leaves in the college campus. It was ensured that the
The plant was healthy and uninfected. The leaves were washed under running tap water to eliminate dust and other foreign particles and to cleanse the leaves thoroughly and dried.

**Preparation of leaf extracts**

20-30 grams of fresh leaves were boiled with 200 mL of solvent for 1 hour. The extract was filtered using Whatmann filter paper No. 1 and then concentrated in vacuum at 40°-50°C using a rotary evaporator. Evaporation of solvent in the rotary evaporator affords a crude extract of the soluble components and these extracts were subjected to the qualitative phytochemical analysis and antibacterial studies.

**Phytochemical Analysis**

The extracts were analyzed by the following procedures [5] to test for the presence of the alkaloids, saponins, tannins, Terpenoids, flavonoids, glycosides, volatile oils and reducing sugars.

**Saponins**

Saponins were detected using the froth test. 1g of the sample was weighed into a conical flask in which 10ml of sterile distilled water was added and boiled for 5 minutes. The mixture was filtered and 2.5 ml of the filtrate was added to 10ml of sterile distilled water in a test tube. The test tube was stoppered and shaken vigorously for about 30 seconds. It was then allowed to stand for half an hour. Honeycomb froth indicated the presence of saponins.

**Tannins**

To a portion of the extract diluted with water, 3-4 drops of 10% ferric chloride solution is added. A blue color is observed for gallic tannins and green color indicates for catecholic tannins.

**Reducing Sugars**

To 0.5ml of plant extracts, 1ml of water and 5-8 drops of Fehling’s solution was added and heated over water bath. Brick red precipitate indicates the presence of reducing sugars.

**Glycosides**

25ml of dilute sulphuric acid was added to 5ml extract in a test tube and boiled for 15 minutes, cooled and neutralized with 10%NaOH, then 5ml of Fehling solution added. Glycosides are indicated by a brick red precipitate.

**Alkaloids**

2ml of extract was measured in a test tube to which picric acid solution was added. An orange coloration indicated the presence of alkaloids.

**Flavonoids**

4ml of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red color was observed for flavonoids and orange color for flavones.

**Volatile oils**

2ml of extract was shaken with 0.1ml dilute NaOH and a small quantity of dilute HCl. A white precipitate is formed if volatile oils are present.

**Terpenoids**

Four milligrams of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet color was observed for terpenoid.

**Aqueous Extract**

Plant material (100 g) was crushed in sterile water (250 ml) for preparation of aqueous extract [6]. The extract was separated using sterile muslin cloth and filter through sterile Whatman filter paper (no. 02).

**Ethanol Extract**

*cardiospermum halicacabum* leaves (100 g) were ground into fine powder [6] using a stainless-steel grinder, and deep in100% ethanol (200 mL) for overnight. The ethanol fraction was separated using sterile muslin cloth and filter through sterile Whatman filter paper (no. 02). The filtered extract was concentrated by a rotary film evaporator.

**Chloroform Extract**

For preparation of chloroform extract ground plant sample (100 g) was added in chloroform respectively (200ml each case) and left for overnight at room temperature [7]. The extracts were separated using sterile muslin cloth and filter through sterile Whatman filter paper (no. 02).

**Source of microorganisms**

The organisms used were *Escherichia Coli, Pseudomonas aeroginosa, Staphylococcus aureus* and *Salmonella typhii*. The organisms were obtained from the Microbial Lab of Department of Microbiology, A.V.C. College, Mayiladuthurai, Tamilnadu, India.

**Determination of Antibacterial Activity**

The antibacterial activity of the leaf extracts was determined using agar well diffusion method by following the known procedure. Nutrient agar was inoculated with the given microorganisms by spreading the bacterial inoculums on the media. Wells were punched in the agar and filled with plant extracts. Control wells containing neat solvents (negative control) were also run parallel in the same plate. The plates were incubated at 37°C for 18 hours and the antibacterial activity was assessed by measuring the diameter of the zone of inhibition. The antibacterial potential of the different extracts was evaluated by comparing their zones of inhibition.

**RESULT**

The antibacterial activity of chloroform, ethanol and aqueous extracts was investigated using agar well diffusion method, against the selected human pathogens such as *Escherichia Coli, Pseudomonas aeroginosa, Staphylococcus aureus* and *Salmonella typhii*. All the examined extract showed varying degrees of antibacterial activities against the pathogens. The Phytochemical test was done to find the presence of active chemical constituents such as glycosides, alkaloids, tannins, flavonoids, terpenoids, saponins. The Table 1 showed the antibacterial activity of chloroform extract of *Cardiosperm halicacabum* showed maximum zone of inhibition (0.5 mm) against *Salmonella typhii*. The
antibacterial activity of chloroform extract of *Cardiospermum halicacabum* showed No zone of inhibition against

Table 1 Antimicrobial activity of Chloroform, Ethanol, Distilled Water extract of medicinal plants against human pathogens.

<table>
<thead>
<tr>
<th>Plants</th>
<th>Extracts</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Escherichia Coli</td>
</tr>
<tr>
<td><em>Cardiospermum halicacabum</em></td>
<td>Distilled Water</td>
<td>-</td>
</tr>
<tr>
<td>Ethanol</td>
<td>15</td>
<td>8</td>
</tr>
<tr>
<td>Chloroform</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*C* – Chloroform, *E* - Ethanol *W* – Water

Table 2 Qualitative Phytochemical Analysis of the extracts of *Cardiospermum halicacabum* Leaf

<table>
<thead>
<tr>
<th>Solvents used for extraction</th>
<th>Alkaloid</th>
<th>Reducing sugar</th>
<th>Flavonoid</th>
<th>Saponin</th>
<th>Tannin</th>
<th>Volatile oil</th>
<th>Glycoside</th>
<th>Terpenoids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ethanol</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Chloroform</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

(*) indicates presence while (–) indicates the absence of the components

**Pseudomonas aeroginosa, Staphylococcus aureus and Escherichia Coli.** The antibacterial activity of ethanol extract of *Cardiospermum halicacabum* showed maximum zone of inhibition (15 mm) against *Salmonella typhii* and *Escherichia Coli* showed the minimum inhibitory zone (8 mm) against *Pseudomonas aeroginosa*. The antibacterial activity of aqueous extract of maximum inhibitory zone (9 mm) against *Pseudomonas aeroginosa* showed the minimum inhibitory zone (7 mm) against *Staphylococcus aureus*. The antibacterial activity of aqueous extract of No inhibitory zone against *Salmonella typhii* and *Escherichia Coli*.

The phytochemical analysis of plant extracts using chloroform, ethanol and aqueous was showed in Table 2. From the phytochemical analysis catecholic tannin and saponin were found in *Cardiospermum halicacabum* in the solvents such as chloroform, ethanol and aqueous. The ethanol and water extract of *Cardiospermum halicacabum* showed the presence of flavanoids, tannins, glycosides, Reducing sugar and saponin presence of ethanol and aqueous extract. Alkaloids were observed only in chloroform extract of *Cardiospermum halicacabum*. In all plant extracts do not found in volatile oil and terpinoids of *Cardiospermum halicacabum*. Saponin were observed in the chloroform, Ethanol and aqueous extract of *Cardiospermum halicacabum*. Glycosides were observed in the ethanol and chloroform extract of *Cardiospermum halicacabum*. The ethanol, aqueous and chloroform all extract of *Cardiosperm halicacabum* showed the absences of volatile oil and terpenoids.

**DISCUSSION**

The findings of the preliminary Phytochemical investigations and the results of antibacterial activity were depicted in the respective Tables. The preliminary phytochemical tests performed were of qualitative type and from the phytochemical investigations it was observed that alkaloids, tannins, flavonoids, terpenoids, saponins Glycoside and compounds reducing were present in the extracts.

*Maluventhan viji et al., [8]* studied that ethanol, chloroform and aqueous extract of *Cardiospermum halicacabum* leaves shows the presence of flavonoids, tannins, steroids and glycosides, which were similar to our results. Antibacterial activity of *Cardiospermum halicacabum* was studied by same workers reported that ethanol extract was active against *Streptococcus aureus* followed by *Salmonella typhi, E. coli & P. aeroginosa*. It is also related to our results.

The ethanol, chloroform and aqueous extract showed considerable activity against *Salmonella typhi*. The ethanol extract was more active than the standard against *Salmonella typhii*. Previous study conducted by [9] suggests that the essentialoil of *O. majorana* possess antibacterial activity. The work conducted by [10] reveals that the leaves of marjoram have antimicrobial activity against *Escherichia Coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Salmonella typhii*. Similarly antimicrobial activity of ethanol, chloroform and water extract of *Marrubium vulgare*, was further assessed against, *Salmonella typhi, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*, were recorded [11].

*Vimalraj et al. [12]* tested the antibacterial activity of aqueous and alcoholic extract of stem bark of *C. fistula* with disc diffusion and MIC methods. Alcoholic extracts recorded greater inhibition against *S. aureus* compared with aqueous extract. Zones of inhibition of alcoholic and aqueous extracts were in the range of 7.0.
to 12.0 mm and 7.0 to 11.6 mm, respectively. MIC values of the alcoholic extracts against S. aureus were in the range of 0.78 to 6.25 mg/ml. Our finding was almost coinciding with this study.

S.K. Panda et al., [13] studied the antibacterial activity and phytochemical screening of ethanol; chloroform and extract of vitex negundo were similar to our results. Antibacterial activity on vitex negundo tested by [14] and [15] reported negative results. On the other hand, [16] reported positive results against B.subtilis, S. epidermis, E.coli & P. aeruginosa.

Napoleon et al., [17] also reported Enterobacter spp, S.aureus, P.aeruginosa, S.typhi and E.coli to be sensitive to ethanol, chloroform and aqueous extract of Moringa olifera leaf at concentration of 200 mg/1. phytochemical analysis were similar report of our results.

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 preset study verified the traditional use of C. halicacabum 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.

References


