



## A Pilot Study on Phytoconstituents in Leaves Extracts of *Cardiospermum halicacabum* and its Antimicrobial activity against Selective Pathogens

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### Abstract

Increasing interest is devoted to phytochemical for their role in plant immunity. Medicinal plant are rich source of these bionutrients. *Cardiospermum halicacabum* is a climbed plant variety with seeds and fruits. It has a unique biodiversity in occurrence and distributed globally. A special attention is given to screening of phyto compounds, antibacterial activity was determined by agar well diffusion method against selected bacterial strains. Phytochemical screening revealed the presence of Terpenoids, glycosides, Saponins, Proteins, Carbohydrates, Volatile esters, Fatty acids and Quinones. It act as a diuretic, laxative, rubefacient, antipyretic, antiulcer, antianxiolytic, adulticidal activity in mosquito, antifilaril and effective in treating itchy scalp and infertility.

**Key Words:** *Cardiospermum halicacabum* Linn. Phytochemical Studies. Antibacterial activity etc

### Introduction

*Cardiospermum halicacabum* is a medicinal plant fits into the *Sapindaceae* family. It is otwell-known as Ballon vine, Love in a Puff, Heart seed, Modakathon(in Tamil ) etc. It has the natural healer properties for hay fever, allergic reactions, sneezing and watery eyes. Balloon vine is considered to be a traditional medicinal herb and the leaves are used to treat diarrhea, dysentery, Oedema, Nephritis and Oliguria haemorrhoids, asthma, pyodermas and carbuncles, earaches, ophthalmias and muscular pains, rheumatism and arthritis. (Shekhawat *et al.*, 2013). It is a dioecious, woody perennial climber originated from the region of native tropical

America, distributed in the tropical and subtropical America. The plant grows up to the length of 2 to 4 m which are deltoid with two highly lobed leaflets. Each leaflet comprises of 2-ternate, lanceolate segment with inciso-serrate margins. Stem looks long and wiry. Flowers are white, small and hermaphrodite which are arranged in axillary, umbellate cymes. Two circinate tendrils also located at the top on a slender peduncles.

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## Material and Method

### Collection of plant material

Fresh *Cardiospermum halicacabum* leaves were collected from the local study area Korkadu, Puducherry, South India in month of January 2024 for our study. Taxonomic characteristics and expert consultation were done to authenticate the plant leaves.

### Processing of plant material

Fresh *Cardiospermum halicacabum* leaves were collected, washed, dried, and powdered, then stored in sealed glass bottles for analysis until needed (Subramanian *et al.*, 2020)

### Preparation of plant extract

The study used distilled water and cold maceration to extract plant leaves. The leaf powder was dissolved in the solvent, shaken, and left to stand for 24 hours. The resulting solution was distilled, collected in pre-weighed bottles, and stored at 4°C for analysis. The extraction of the plant leaves were carried out using chloroform and aqueous as extracting solvents. The cold maceration extraction method was used. 50g of leaf powder was weighed and dissolved in 1000mL of the extracting solvent inside a 2L conical flask and covered with parafilm. The flasks were shaken vigorously at 30 minutes interval and left to stand for 24 hours at room temperature. The resultant mixture was then filtered with Whatman's No.4 filter paper and cotton wool to remove particles of plant sample. The clear solution obtained was distilled at 65°C under low pressure on a steam bath. The semi solid concentrations of the extracts were then collected in sterile pre-weighed screw capped bottles and labeled accordingly. The extracts were stored at 4°C until when needed. (Ogunjobi *et al.*, 2007)

### Phytochemical analysis

The phytochemical tests were performed to identify active chemical constituents such as carbohydrates, phenolic compounds, steroids, alkaloids, proteins, terpenoids, flavonoids, cardiac glycosides, saponins, tannins, gums, and mucilages. (Waiganjo *et al.*, 2020)

## Qualitative Analysis

### Test For Carbohydrate

#### Molisch's Test

To 2mL of the extract, 2 drops of Molisch reagent was added and mixed. 2mL of concentrated Sulphuric acid was added to this solution. Occurrence of violet ring at the junction of the solution and its vanishing on addition of excess alkaline solution indicates the presence of carbohydrate. (Mohammed *et al.*, 2018).

#### Benedict's Test

To the test solution few drops of Benedict's reagent was added and heated on water bath. The presence of sugars was indicated by reddish precipitate formation. Depending on the concentration of the reducing sugars, the amount and colour of the precipitate produced varied. A positive Benedict's test appears green, yellow, orange, or red (Nagaraju *et al.*, 2014)

#### Fehling's Test

To 1mL of the extracts, 1mL of Fehling's A and 1mL of Fehling's B solution were added in a test tube and heated in a water bath for 10 minutes. Formation of red precipitate indicates the presence of a reducing sugar. The filtrate was reacted with a 1mL of Fehling's A and B and heated in water bath for 5-10 minutes. Appearance of reddish orange precipitate shows the presence of carbohydrates (Mahendra *et al.*, 2018).

### Test for Protein

#### Biuret Test

To 3 mL of extracts add equal volume of 4% sodium hydroxide and add a few drops of 1% solution CuSO<sub>4</sub> to form violet or pink color (Rangarajan *et al.*, 2015).

#### Millon's Test

Add 5mL of Millon's reagent to 3mL extract. Warm the solution till it boils. White precipitates formed where precipitate turns brick red color solution. (Sagar & Ashok, 2012)

#### Xanthoproteic's Test

Add few drops of Concentrated Nitric acid to

the walls of test tube containing 2mL of extract. Appearance of yellow color indicated the presence of proteins (Saima *et al.*.,2020)

### **Saponins**

#### **Foam Test**

Add 1ml of extract and 2mL of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins. (Them *et al.*,2019)

### **Cardiac Glycosides**

#### **Test for Cardiac Glycosides**

Add 2 mL of extract and glacial acetic acid and add a 2-3 drops of 5% Ferric chloride along with concentrated Sulphuric acid in a test tube and observed for a reddish brown coloration at the junction of two layers and the bluish green color in the upper layer denoted the presence of cardiac glycosides (Rahman *et al.*, 2017).

### **Quinones**

#### **Test for Quinones**

Take 1mL of extract in a clean test tube and add 5 mL of distilled water and observe for the turbidity(Farooque & Jadada,2021).

### **Test For Steroid**

#### **Liebermann Burchard Test**

Crude extracts were dissolved in 2mL of chloroform to which 10 drops of acetic acid and 5 drops of concentrated sulphuric acid were added in a test tube and mixed. Formation of red colour from blue to green indicated the presence of steroids (Kalpana *et al.*, 2018)

### **Test For Alkaloids**

#### **Wagner's Test**

2mL of crude extract filtrate was taken in a test tube and it was added with same quantity of Wagner's reagent. The formation of reddish-brown precipitate reveals the presence of alkaloids (Balamrigan *et al.*,2019)

### **Test For Terpenoids**

#### **Salkowski Test**

5mL of the extract was added to chloroform along with a few drops of concentrated

sulphuric acid. The mixture was shaken well and kept aside for some time. Appearance of red color in the lower layer indicates the presence of steroids and the formation of yellow color in the lower layer indicates the presence of terpenoids.(Krvavychet *et al.*, 2014).

### **Test For Phenolic compound**

#### **Ferric Chloride Test**

A little extracts was dissolved in distilled water. To this, 2 mL of 5% Ferric chloride solution was added. Formation of blue, green, or violet color indicates the presence of phenolic compounds. (Vimalkumar *et al.*, 2014).

#### **Ellagic Acid Test**

Add 5-6 drops of extract solution and add 5% Glacial acetic acid and 5% Sodium nitrate and mix well. Transformation of dark brown precipitate known as Niger brown indicates the presence of Phenolic compounds (Wanget *et al.*, 2019).

#### **Potassium dichromate Test**

To 1 mL of extract add 2-3 drops of Potassium dichromate solution. The solution colour will change into dark indicates the presence of phenolic compounds(Shaikh *et al* 2020)

### **Test For Phlobatannins**

#### **Haemolysis Test**

One drop of fresh human blood is taken on a glass slide and few drops of extract were added and mixed well. Formation of Zone of haemolysis indicates the presence of phlobatannins (Yusuf *et al.*,2014)

### **Test For Anthocyanins**

#### **Hydrochloric acid Test**

To 2 mL of extract, add 2 mL of 2N Hydrochloric acid solution and 5-7 drops of Ammonia solution was added. Pink-red solution which turns blue-violet after addition of ammonia solution indicates the presence of anthocyanins (Obouayeba *et al.*,2015)

### **Test For Coumarins**

In a clean glass test tube mL of extract was taken and added with 1.5ml of 10% Sodium

Hydroxide and was observed for the formation of yellow color which indicated the presence of coumarins(Saberi *et al.*,2013)

#### **Test For Cholesterol**

To 2mL of extract add 2mL of chloroform and add 2-3 drops of acetic anhydride along with 2-3 drops of concentrated Sulphuric acid. Transformation of vibrant red-rose color indicates the presence of cholesterol (Oyewole *et al.*,2011)

#### **Test For Flavanoid**

5 mL of dilute ammonia solution was added to a portion of the extracts followed by addition of concentrated sulphuric acid. Development of a yellow color in the extract designates the presence of flavonoids. After some time the yellow color will disappears (Mishra *et al.*, 2024)

#### **Test For Tannins**

##### **Braymer's Test**

Take 1mL of extract and treat it with 1ml of 10% alcoholic ferric chloride solution and observe for the formation of blue or greenish color. (Morsy *et al.*, 2014)

#### **Test For Gums and Mucilages**

##### **Gums and Mucilages Test**

To mL of distilled water the extract is mixed and dissolved and to this 2mL of absolute alcohol is added and further stirred constantly. The formation of white or cloudy precipitate indicates the presence of Gums and Mucilages(Kumar *et al.*, 2014)

#### **Determination of Antimicrobial Activity**

##### **Selection of Test organisms**

Invitro antibacterial activity was evaluated against five bacterial pathogens of clinical origin. Gram positive strain *Staphylococcus aureus*, Gram negative strains *Escherichia coli*, *Shigella* sp, *Alcaligenes faecalis* and *Pseudomonas aeruginosa* were used in the study. All the clinical strains were procured from Microbiology laboratory of Divine Mother College, Puducherry, India.

##### **Inoculum preparation**

Bacterial inoculum was prepared by inoculating a loopful of test organisms in 5 ml

of Muller Hinton Broth and incubated at 37°C for 3-5 hours till a moderate turbidity was developed. The turbidity was matched with 0.5 Mc Farland standards.

#### **Assessment of antibacterial activity**

Immediately after standardization, a sterile cotton swab was immersed in the bacterial suspension and swabbed aseptically on the surface of sterile Mueller-Hinton agar (Hi-media, Mumbai) plates. Wells of 8mm diameter were punched in to the agar medium and filled with 10µL, 50µL and 100µL of root extracts (100mg/mL of water). Well loaded with 100µL of Levofloxacin (5µg) served as positive control. The plates were incubated at 37<sup>o</sup> C for overnight in a bacteriological incubator. Anti-microbial activity was measured by measuring the zone of inhibition in mm around each well, negotiating the diameter of well. (Balourietal., 2016)

#### **Results and Discussion**

##### **Preliminary Phytochemical Analysis**

The phytochemical screening of Aqueous and chloroform Leaves extract of *Cardiospermum halicacabum* was analysed and it exhibited the presence of various phytoconstituents during the investigation.(Table.1)Phytoconstituents such as Carbohydrates, Proteins, Terpenoids, Amino acid, Saponins, Quinones, Coumarins and Alkaloids were detected. Tannins and Anthocyanins were absent in both the extract...Carbohydrates were present in both the aqueous and chloroform extracts.(Fig.1)Carbohydrates, essential for genetic material and energy, are crucial in living cells. Glucose, found in fruit juices and honey, is the most common carbohydrate found in polysaccharides like cellulose and starch.(Beta & Camire, 2018).

Both the extracts revealed the presence of Proteins during millon's and xanthoproteic test.But in biuret test only chloroform extract exhibited its presence and were absent in aqueous extract.(Fig.2) Plant proteins and amino acids were crucial for food and feed, absorbed by the intestine and converted into modified proteins. These bioactive proteins and peptides exert specific body actions. (Ghaly & Alkoaik 2010). Saponins were present in aqueous extract and

absent in chloroform extract. Saponins were naturally occurring amphiphilic glycosides widely distributed in the plant kingdom glycosides were used in treatment of atrial fibrillation, flutter, and they acts as emetics and as diuretics (Agidew, 2022). **Fig.3).**

Steroids were existed in chloform extract and in absent in aqueous extract.**(Fig.4).**Steroid glycosides should be used cautiously, as excessive dosage can cause severe heart damage, while small amounts can provide necessary stimulation. (Rana *et al.*, 2022)

**Table 1: Phytochemical Constituents of *Cardiospermum halicabubum***

SL.NO	PHYTOCHEMICAL ANALYSIS	AQUEOUS EXTRACTS	CHLOROFORM EXTRACTS
<b>1.</b>	<b>CARBOHYDRATE</b>		
	Molisch's Test	+	+
	Benedict's Test	+	+
	Fehling Test	+	+
<b>2.</b>	<b>PROTEIN</b>		
	Biuret Test	-	+
	Millon's test	+	+
	Xanthoproteic test	+	+
<b>3.</b>	<b>PHENOLIC COMPOUNDS</b>		
	Ferric Chloride Test	+	+
	Ellagic Acid Test	-	+
	Potassium Dichromate Test	+	+
<b>4.</b>	<b>STEROID</b>		
	Liebermann Burchard Test	-	+
<b>5.</b>	<b>ALKALOIDS</b>		
	Wagner's Test	+	+
<b>6.</b>	<b>FLAVONOIDS</b>		
	Ferric chloride Test	+	+
<b>7.</b>	<b>TERPENOIDS</b>		
	Salkowski Test	+	-
<b>8.</b>	<b>SAPONINS</b>		
	Foam's Test	+	-
<b>9.</b>	<b>TANNINS</b>		
	Braymer's Test	-	-
<b>10.</b>	<b>GLYCOSIDES</b>	+	-
<b>11.</b>	<b>QUINONES</b>	-	+
<b>12.</b>	<b>COUMARINS</b>	+	+
<b>13.</b>	<b>GUMS AND MUCILAGES</b>	+	+
<b>14.</b>	<b>ANTHOCYANINS</b>	-	-

15.	CHOLESTEROL	-	+
16.	PHLOBATANNINS	+	+

Alkaloids were present in both the extracts(Fig.4). Alkaloids are secondary compounds derived from amino acids or transamination, found in nature. They are diverse and categorized based on amino acid structure , with different biosynthetic pathways and biological activity. (Gul *et al.*, 2017)

Aqueous extracts demonstrated the prevalence of Terpenoids where as it was absent in Chloroform extracts( Fig.4).(Terpenoids, also known as isoprenoids, are diverse in natural products found in plants with over 25,000 compounds. They have strong odors and flavors, and were used in herbal remedies due to their varied actions.(Indumathi, *et al.*, 2014)

Both the extracts confirmed the existence of Phenolic compounds except in Ellagic acid test for Aqueous extract.(Fig.5). Phenolic compounds, abundant secondary metabolites of plants, consist of aromatic rings and hydroxyl groups. With over 8,000 structures, they produce natural color pigments, responsible for fruit color in plants.(Maria,*et al.*, 2018)

Flavanoids werefound existent in both the extracts.(Fig.6). Flavonoids were the active biological phytochemicals found in plants, used in herbal medicines, and essential in daily diets. Found in fruits, vegetables, grains, bark, stems, tea, and wine, they are synthesized by plants in response to microbial infections.The presence of flavonoids in human diet may control inflammation and play a significant role in cancer prevention which were already reported in our earlier studies in Piper betle (Jayarajan *et al.*, 2023).Tannins were not detected in both the extract).(Fig.6).(Pinheiro *et al.*, 2012.Tannins, plant polyphenols, exhibit binding properties to proteins, pigments, and large-molecular compounds, as well as anti-oxidant activities, distinguishing them from other plant polyphenol(Waiganjo, *et al.*, 2016)

Gums and mucilages were detected in both the extracts during the study (Fig.6). Plant-derived gums have been used by humans since ancient times for numerous application(Vasile, *et al.*, 2019). Natural coumarins play a significant role in cancer prevention and treatment. (Jain &

Joshi,2012). Both extracts comprised Phlobatannins were confirmed during the study(Fig.7). Phlobatannins has the property of ,anti inflammatory and analgesic..Phlobatannins, found in plants, protect the body by slowing artery hardening, maintaining blood pressure, and lowering cholesterol levels (Doss,2009). Anthocyanins were also not found in both the extract(Fig-8). Anthocyanins were extracted from plant materials using methanol with hydrochloric or formic acid, which lowers pH and prevents pigment degradation. However, concentrated acid during evaporation can cause degradation.(Obouayeba *et al.*, 2015). Cholesterol were identified only in chloroform extract and could not be identified in aqueous extracts. (Fig.8).Cholesterol was a vital molecule in human life, contributing to cell membrane structure and fluidity. It also play a significant role in synthesis of vitamin D, steroid hormones, and sex hormones. Cholesterol was also a constituent of bile salt, aiding digestion and fat-soluble vitamin absorption. (Negm El-Dein *et al.*, 2023)

**TEST FOR CARBOHYDRATE**

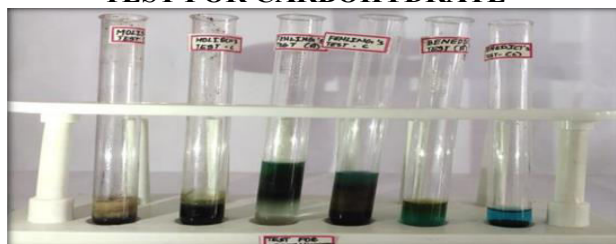


Figure.1 Shows the test results for Carbohydrate

**TEST FOR PROTEIN**

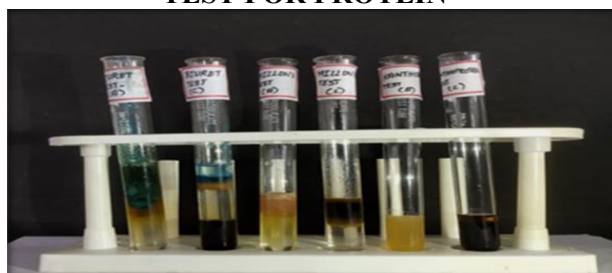


Figure .2. Shows the test results for Proteins

### TEST FOR SAPONINS, CARDIAC GLYCOSIDES AND QUINONES

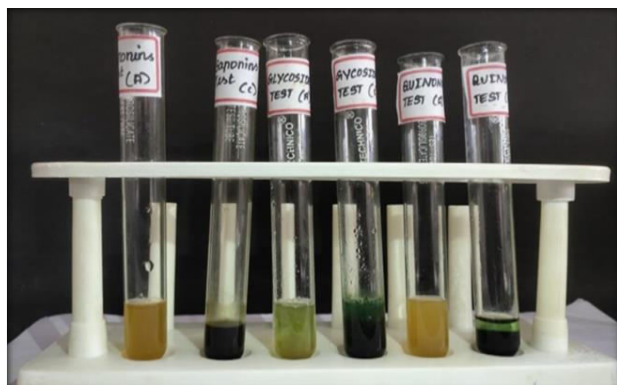


Fig.3. Shows the test result for Saponins, Cardiac Glycosides and Quinones

### TEST FOR FLAVONOIDS, TANNINS AND GUMS AND MUCILAGES

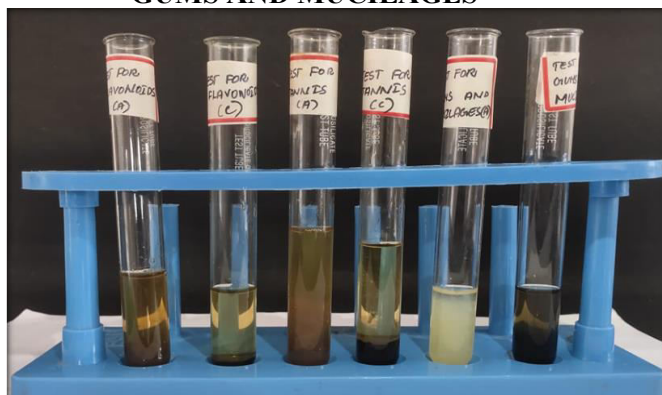


Fig.6. Shows the test results for Flavonoids, Tannins, Gums and Mucilages

### TEST FOR STEROIDS, ALKALOIDS AND TERPENOID

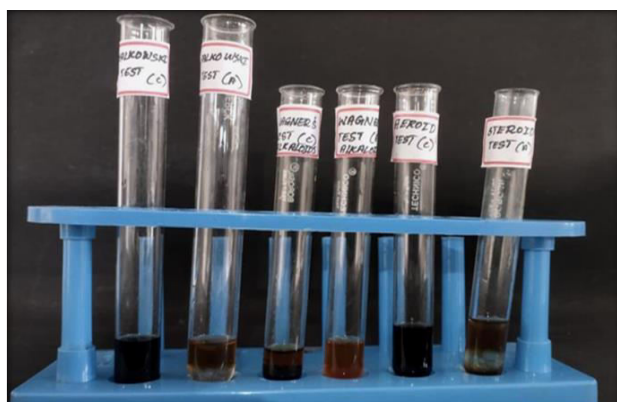


Fig.4. Shows the test results for Steroids, Alkaloids and Terpenoids

### TEST FOR PHLOBATANNINS (FIGURE -7)

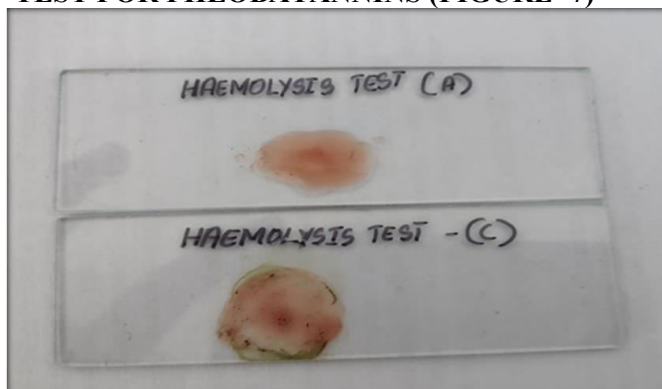


Fig.7 Shows the test results for Phlobatannins

### TEST FOR PHENOLIC COMPOUNDS

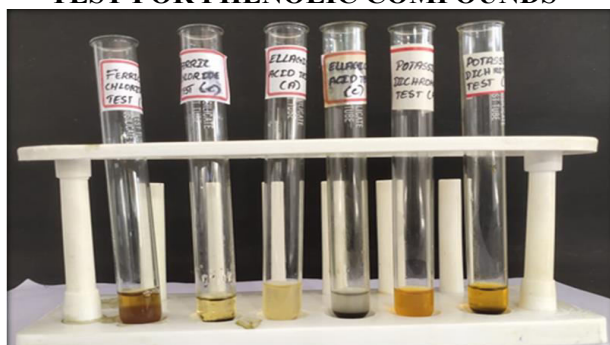


Fig.5. Shows the test results for Phenolic compounds

### TEST FOR ANTHOCYANINS, COUMARINS AND CHOLESTEROL

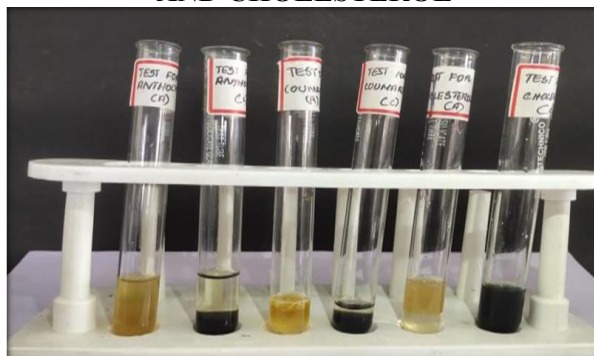


Fig.8. Shows the Test results for Anthocyanins, Coumarins and Cholesterol

**Antibacterial Activity**

Antimicrobial agents are the substances that possess the ability to either destroy or inhibit the growth of microorganisms. Evidences are increasing day by day from various regions around the world that medicinal plants have potential antimicrobial characteristics. It is expected that plant extracts will be more effective in targeting specific sites than standard antibiotics in combating drug-resistant pathogenic microorganisms. The phytochemicals which are biologically active compounds within the plants were responsible for their effective action against microbial pathogens.(Jayarajan *et al.*, 2023).

The antibacterial properties of Chloroform leaves extract of *Cardiospermum halicacabum* was assessed against bacterial pathogens using agar well diffusion method by measuring the diameter of growth inhibition zones with 10µL, 50µL, 100µL of suitable extracting solvents with Levofloxacin (commercial antibiotic) as control. The results illustrated that the chloroform extract possess antibacterial activity against the tested gram positive (*Staphylococcus aureus*) and gram negative (*Escherichia coli*, *Shigella* Sp, *Alcaligenes faecalis* and *Pseudomonas aeruginosa* bacterial strains.(Fig.9 & Table.2)

**Table. 2 Antibacterial Activity of *Cardiospermum halicacabum***

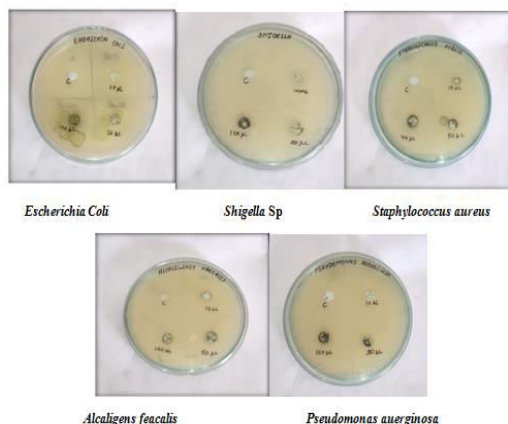
Test organisms	Zone of inhibition(mm)			
	Control (Levofloxacin) (10 µg)	Chloroform extract		
		10(µL)	50(µL)	100(µL)
<i>Staphylococcus aureus</i>		4	8	12
<i>Escherichia coli</i>		2	6	10
<i>Alcaligenes faecalis</i>		--	--	--
<i>Shigella</i> sp sp		4	9	15
<i>Pseudomonas aeruginosa</i>		--	--	--

In the present antibacterial assay the maximum activity was recorded against *Shigella* sp . Similarly least activity was recorded against *Escherichia coli*. Plant-based antibacterials have significant therapeutic potential due to their minimal side effects compared to synthetic ones. Due to the increasing failure of chemotherapeutics and antibiotic resistance, medicinal plants are

being screened for their potential antimicrobial activity. Further research and exploration of plant-derived antimicrobials is needed to develop new chemotherapeutic drugs and phytomedicines against microbes. Medicinal plants are crucial sources for developing potential chemotherapeutic drugs.(Silva *et al* 2010)



Fig.9 Antibacterial Activity of *Cardiospermum halicacabum*



### Conclusion

The invitro analysis for the presence of phytoconstituents, antibacterial activity of *Cardiospermum halicacabum* exposed that plant was medically potent has been demonstrated. The pharmacological effects exhibited by the phytoconstituents from *Cardiospermum halicacabum* would be an alternative for various health disorders. While some traditional applications have been supported by modern scientific studies, more pharmacological evaluations are needed to validate other traditional uses Bioassay-guided isolation, pharmacokinetic evaluations, and mode of action identification are recommended for future research on *Cardiospermum halicacabum* therapeutic drug leads.

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