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Phytochemicals analysis and antibacterial activity of *Cardiospermum halicacabum* against clinical MDR Pathogens

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Abstract
The MDR pathogen to effect on *Cardiospermum halicacabum* plant extract from to treated on medicinal field. The plant leaves and stem extract are used in antibacterial activity. For using a microbial strains are *Escherichia coli, Streptococcus pyogenes, Staphylococcus aureus, Pseudomonas aeruginosa and Klebsiella pneumoniae* in antibacterial activity. To the secondary metabolites present in plant extract for isolated in the phytochemical analysis and TLC method are followed. Carbapenemase producing an important mechanism responsible for MDR pathogen in Carbapenemase resistance. To activity on a beta - lactam activity to produce a bacterium.

Keywords: Antibacterial activity, TLC, Phytochemical analysis, carbapenemase resistance

Introduction
The discovery of penicillin in 1928 was followed by the discovery and commercial production of many other antibiotics. We now take for granted that any infectious disease is curable by antibiotic therapy. Antibiotics are manufactured at an estimated scale of about 100,000 tons annually worldwide, and their use had a profound impact on the life of bacteria on earth. More strains of pathogens have become antibiotic resistant, and some have become resistant to many antibiotics and chemotherapeutic agents, the phenomenon of multidrug resistance [1]. Gram-negative bacteria pose a therapeutic problem not only in the hospital settings, but also in the community as they have acquired resistance to multiple antibiotics. Emergence of carbapenemases in Enterobacteriaceae and non-fermentative bacteria poses a serious therapeutic problem in hospitals because carbapenems are often antibiotics of last resort for the treatment of serious infections caused by multidrug-resistant Gram- negative bacteria. These bacteria have the potential to spread rapidly within the hospital environment and also across the continents. Resistance to carbapenem is mostly due to production of enzymes-carbapenemases that hydrolyze carbapenems and other β-lactams [2].

Herbal medicine is also called, botanical medicine or phy`tomedicine which refers to using a plant's seeds, berries, roots, leaves, bark, or flowers for medicinal purposes. Herbalism has a long tradition of use outside of conventional medicine. It is becoming more main stream as improvements in analysis and quality control along with advances in clinical research show the value of herbal medicine in the preventing disease. The MDR pathogen to effect on *Cardiospermum halicacabum* plant extract from to treated in medicinal field. Eco-friendly and bio-friendly plant based commodities for the avoidance and treatment of various human infections including microbial diseases throughout the world **[3]**. This herb act as a diaphoretic, diuretic, emetic, laxative, refrigerant, stomachic and sudorific and has antibacterial [4,5], anti-diarrheal [6], antioxidant activities [7], suppresses TNF production [8], exhibits anticancer [9], vaso depressant [10] effect.

Methodology
Collection of Plant
Cardiospermum halicacabum plants were collected from Tiruvonikovil, Trichy District, Tamil Nadu, India. Fresh plant materials were washed under running tap water. The leaf, steam, seed, flower, coat, root parts were separated from the plant. From the plant parts are used in the experimental for leaf and steam and were dried a plant material in one week. In pulverized in an electric mixer and each powdered plant parts were stored in air tight bottles. The identified plant material was authenticated with the specimen deposited at RAPINAT herbarium, Department of Botany, St. Joseph's college, Trichy.

Extraction of plant materials
The plant leaf material was washed with water to remove shade dried at room temperature. Extracts were prepared from the method of [11]. 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.

Preparation of plants extract
The plant leaf powder was mixed with 50 g of plant powder in 200ml of Chloroform, Hexane, Methanol, and Butanol. It was then allowed to soak in it for 24 to 48 hours at room temperature. The soaked powder was then filtered and purified and the content were allowed for condensation at 50ºC and evaporated and the extract was collected.

Phytochemical screening
The crude extracts and the extracts obtained from fractionation of the crude methanolic extracts were screened for the

presence/absence of phytochemicals such as steroids, flavonoids, tannins, saponins, alkaloids, terpenoids and glycosides using the method described by Harborne [12].

Isolation of clinical organisms

The clinical samples were collected from the Viswanathan Government medical College, Trichy. Then it was isolated by performing serial dilution and spread plate in Nutrient agar medium and incubated for 24 hours at 37ºC the organisms were then differentiated through selective and differential media and confirmed through biochemical tests. Then clinical samples were collected from **government hospital, srirangam**. In urinary tract infection and respiratory tract infection patient sample also collected.

Isolation of MDR pathogen organisms

The clinical samples were collected from the infected patient. Then it was isolated by performing serial dilution and spread plate in Nutrient agar medium and incubated for 24 hours at 37ºC. The organisms were then differentiated through selective and differential media and confirmed through biochemical tests.

Identification of isolates

Selected colonies from selective and differential media were subjected to microscopy and biochemical tests for identification.

Medicinal plants and their antimicrobial activity

Antimicrobial activity test

Test microorganisms

Escherichia coli, Streptococcus pyogenes, Staphylococcus aureus, Pseudomonas aeruginosa and *Klebsiella pneumoniae.*

Preparation of discs

Known quantity of extracts was dissolved in DMSO. It was then filter sterilized by making use of sortorious syringe filter of pore size 0.22 µm. Sterile discs of 6 mm diameter Hi-Media) were loaded with various concentrations of extracts was dried. Dried discs were stored in sterile containers till use. Solvent loaded discs were also prepared and used as negative control. Loaded Chloramphenicol Hi-Media discs were used as positive control.

Preparation of inoculum

The isolates were inoculated in nutrient broth and incubated at 37^0C for 4 hours in a shaker Orbitech, Scigenics, India) and was used for anti - bacterial activity test and to look for the MIC of various extracts and fractions.

Determination of Antibacterial activity

Disc diffusion method was followed to determine the anti - bacterial activity of various extracts and fractions. Petriplates containing 20 ml of Mueller Hinton agar were seeded with 4 hours old fresh cultures. By making use of template drawn extracts loaded discs were dispensed on the solidified Mueller Hinton agar with test organisms. Cifproflaxine antibiotic disc obtained from M/s Hi-Media laboratories Ltd, Mumbai was used as a positive control and solvent loaded discs were used as a negative control. This was incubated at 37˚C for 24 hours in an incubator Rands SBC). The test was performed in triplicates. The zone of inhibition was measured by making use of Antibiotic zone scale Hi - media). In another disc diffusion method was followed to determine the anti- bacterial activity of various antibiotics and fractions of the microbes in using for selective antibiotic are used. These antibiotics are shown in Table 4).

Combined disk test

Metallo-β-lactamase production was detected by CDT method on disk containing EDTA as per the method used by Yong *et al*., [13] briefly, a 0.5 M EDTA Hi-Media) solution was prepared by dissolving 18.61 g of EDTA in 100 ml of distilled water and adjusted it to pH 8.0 by using NaOH and sterilized by autoclaving. Turbidity of 0.5 McFarland standards was made from overnight subculture of test organism and spread as lawn culture on the surface of a Muller Hinton agar plate. Two IPM disks 10µg) Hi-media) were placed on the agar, and 10 µl 0.5M EDTA solution was added to one of the disks. Inoculated plates were incubated overnight at 35°C. An organism demonstrating a zone diameter around the disk containing IPM-EDTA ≥7 mm than the zone diameter around the IPM disk alone was considered as an MBL producer.

Result and Discussion

Natural crops either extract or pure compounds provide infinite prospects for the progress of new drugs due to availability of chemical diversity.The antimicrobial activities of plant extracts have created the starting point of many applications, including raw and processed food formation, pharmaceuticals, alternative medicine and natural therapies.

The generation of drugs in plenty from natural sources with more efficacy, low cost of production and low or negligible side effects has become a prime focus of the pharmacological industry [14]. The development of synthetic antibiotics have been successful in eliminating these organisms to an extent but pose the limitations like development of drug resistance by microorganisms [15], high cost and adverse side effects on the host.

For phytochemical screening by chemical tests, four different extracts viz Chloroform, Butanol, Hexane and Methanol extracts were used. Presence of carbohydrate, tannins, saponin, terpenoids, lignin, protein and flavonoids were detected in all the four extracts used Table 1).

Table 1: Phytochemical Screening of Cardiospermum halicacabum Stem and Leaf extract

Test	Leaf Chloroform	Leaf Methanol	Leaf Butanol	Leaf Hexane
Coumarin	+	-	+	+
Terpenoids	+	+	+	+
Flavonoids	+	+	+	-
Inulin	+	+	+	+
Saponins	-	-	-	+
Carbohydrates	-	-	+	+
Tannin	+	+	+	+
Alkaloids	+	-	-	+
Lignin	+	+	+	-
Protein	+	+	+	+

+) - Present, -) – Absent

Table 2: TLC - R/f Calculation

Phytochemical Test	Leaf chloroform	Leaf methanol	Leaf Butanol	Leaf hexane
TLC Method	0.85	088533	0.816666667	0.81666667

Table 3: Collection of clinical sample

Total number of sample collected	Number of bacteria isolated
16	5

Table 4: Various Antibiotic Using and Mcg

S. no.	Antibiotic disc	Mcg	*Staphylococcus Aureus*	*Pseudomonas Aeruginosa*	*Klebsiella pneumoniae*	*Streptococcus Pyogenes*	E.coli
1.	Cefotaxmine cephotaxime)-CTX	30mcg.	S	S	S	S	S
2.	Carbenicillin-CB	100mcg	S	S	R	S	S
3.	Cefoperazone-CPZ	75 mcg	S	R	R	R	S
4.	Cefixime-CFM	5mcg	R	S	R	R	R
5.	Oxacillin-OX	1mcg	S	S	R	R	R
6.	Imipenum-IPM	10 mcg	S	S	S	S	S
7.	Cephlotoxin –CPT	30mcg	S	S	R	S	S
8.	Tetracycline	10mcg	S	S	R	S	S

S- Sensitivity, R-Resistant)

Positive control antibiotic are using to the MIC of the bacteria in zone are formed *Staphylococcus aureus* 34mm), *Pseudomonas aeruginosa* 40), *Escherichia coli 39mm), Klebsiella pneumoniae* 10mm),and *Streptococcus pyogenes 44mm).*

Table 5: Antibacterial activity of Chloroform, Butanol, Hexane and Methanol extract of *Cardiospermum halicacabum Leaf against* Clinical isolates µg /µl.

S. NO	Clinical isolates	Concentration of extract /Zone of inhibition mm)				
		chloroform	Butanol	Hexane	Methanol	Positive disk
1	*E.coli*	10	16	11	15	39
2	*Klebsiella pneumoniae*	Nil	13	Nil	14	10
3	*Pseudomonas aeruginosa*	10	13	Nil	11	40
4	*Staphylococcus aureus*	3	9	Nil	10	34
5	*Streptococcus pyogenes*	11	3	Nil	10	44

Thin layer chromatography is carried out using solvents viz Table1, 2). Similar result has been reported as, Flavonoid, Terpenoids and cardiac glycosides were predominantly found in the tested solvent extracts of leaf followed by Tannin, Flavonoid, Terpenoids and cardiac glycosides. Likewise, Tannins, Flavonoid, Terpenoids and cardiac glycosides and anthraquinones were predominantly found in all the tested solvent extracts of the stem and leaf [16].

The clinical microorganism's characteristics features were identified by their colony morphology and the growth characters and individualism in various selective and differential Medias. The isolated organisms were identified by the biochemical tests based on their biochemical characters. Depicts that 16 clinical urine and sputum samples were collected and the five species *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Pseudomonas aureus, Klebsiella pneumoniae,* and *Streptococcus pyogenes* which are the common urinary tract, respiratory tract infections causing bacteria, were isolated from the urine, sputum samples Table 3).

The antibacterial property of Methanol, Butanol, Hexane, chloroform, extract of leaf *Cardiospermum halicacabum* was analysed against bacterial pathogens using ciprofloxacin as positive. Out of these five bacterial pathogens four were found to be gram negative *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae*) and two were gram positive Staphylococcus *aureus,* Streptococcus pyogenes). Disc diffusion method was used to evaluate the antibacterial activity of taken samples. After twenty-four hours the minimum inhibitory zone of leaf extract of *Cardiospermum halicacabum* and control were measured.

Carbapenemase production could be detected by and CDT to the MIC inhibition zone of 12> mm on IPM and EDTA-IPM.MDR pathogen microbes in MIC of zone of inhibition for tested bacteria in *Klebsiella pneumoniae* Table 4). Positive control antibiotic are using to the MIC of the bacteria in zone are formed *Staphylococcus aureus* 34mm), *Pseudomonas aeruginosa* 40), *Escherichia coli* 39mm*), Klebsiella pneumoniae* 10mm), and *Streptococcus pyogenes* 44mm).

The zone of inhibition was noted which varies according to the solvents. Table 5) the antibacterial activity of the plant leaf chloroform extract against clinical species. The extract concentration varies from 60 to 100µg/µl where the highest range Zone of inhibition was identified at 100µg/µl for all species, 10mm for *Escherichia coli,* 10 mm for *Pseudomonas aeruginosa*, 3 mm for *Staphylococcus aureus* 11mm for *Streptococcus pyogenes,* not zone are formed in *Klebsiella pneumoniae*. It denotes that the higher concentration of plant extract only active against the clinical pathogens. Butanol extract against clinical species. The highest range of zone of inhibition was identified at 100µg/µl for all species, 16 mm for *Escherichia coli, 13 mm* for *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, 9mm for Staphylococcus *aureus* and 1mm zone of inhibition was found to be observed for Streptococcus pyogenes. The lower concentration of 60 and 70 µg/ml of plant extract formed minimum zone against *Escherichia* coli and *Pseudomonas aeruginosa* only. It denotes that the higher concentration of plant extract only active against the clinical pathogens.

Hexane extract against clinical species. The highest range Zone of inhibition was identified at 100µg/µl for all species, 11mm for *Escherichia coli,* not zone are formed in *Pseudomonas*

aeruginosa, *Staphylococcus aureus*, *Streptococcus pyogenes* and *Klebsiella pneumoniae.* It denotes that the higher concentration of plant extract only active against the clinical pathogens. Methanol extract against clinical species. The highest range zone of inhibition was identified at 100µg/µl for all species, 15 mm for *Escherichia coli*, 11 mm for *Pseudomonas aeruginosa*, 10 mm for *Staphylococcus aureus*, 10mm for Streptococcus pyogenes,14mm for *Klebsiella* pneumoniae. It denotes that the higher concentration of plant extract only active against the clinical pathogens. The studied plant was most active against *Escherichia* coli. The methanol extracts of the investigated plants showed maximum antimicrobial activity against Gram negative *Klebsiella pneumoniae* showed similar results were also reported by Parekh *et al* [17].

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. The results of phytochemical screening of extracts of leaf 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 extracts of *Cardiospermum halicacabum* is highly recommended for the herbal preparations to the traditional medicinal prescription and for the pharmaceutical industries for the mass scale extractions of the therapeutic agents.

Conclusion

Medicinal plants are considered to be most important structures in the Indian medicine. The medicinal plant plays a vital role in the traditional medicines such as Ayurveda, Unani, Siddha and Homeopathy. Based on this the present study was done to identify the Antimicrobial activity of the leaves extract of Cardiospermum halicacabum against the common urinary tract and respiratory tract pathogens which includes Staphylococcus aureus, Pseudomonas aureus, Escherichia coli, Klebsiella pneumoniae and Streptococcus pyogenes.

The antimicrobial activity in Chloroform, Butanol, Hexane and Methanol extracts of plant leaves and stem of Cardiospermum halicacabum were tested. The pharmacognostic, phytochemical and antimicrobial activity were identified through the standard procedures. All the solvents showed goo d zone of inhibition and it is found to be fit for Ayurvedic pharmacopeia of India. It has been confirmed that the antimicrobial activity is due to the presence of the phytochemical components such as carbohydrate, tannins, saponins, terpenoids, lignin, protein and flavonoids were detected. The phytochemical analysis and antibacterial activity of *Cardiospermum halicacabum* showed positive in all aspects. This may be due to the presence of various compounds present in *Cardiospermum halicacabum* and this may be the source for the wide activity of the plant against MDR pathogen such as *Staphylococcus aureus, Pseudomonas aureus, Escherichia coli, Klebsiella pneumoniae and Streptococcus pyogenes*. The present study reveals, the potentiality of the plant taken for the study extensive study with a compound identification of focus in future may form a basis for a new drug discovery.

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