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Research Article

Phytochemical and Antibacterial Activity of *Cardiospermum halicacabum* Against Wound Pathogens

Sathish Kumar Karuppannan, Mohammed Junaid Hussain Dowlath, Mohamed Khalith S B, Darul Raiyaan G I, Sundarapandian Subramanian, Kantha Deivi Arunachalam*

ABSTRACT

Background: Plants serve as an important source for curing various medical ailments for a wide variety of human and animal diseases. It is therefore necessary to prove the biological activities of the selected plants scientifically using modern technology. The current study focuses on the use of *Cardiospermum halicacabum* in their wound healing applications. *Cardiospermum halicacabum* is a well-known plant that has antibacterial, anti-inflammatory, anti-rheumatic properties and it is also used to treat joint pains, muscle tears, back pain, etc. **Materials and Methods:** In this study methanolic extracts of the active compounds from *Cardiospermum halicacabum* were tested for its phytochemical attributes by qualitative method, GC-MS, and the antioxidant properties were also assessed. The bactericidal activity and Minimal Inhibitory concentration (MIC) of the plant extract has been evaluated in both Gram +ve and Gram -ve microorganisms using the disc diffusion method. **Results:** The results obtained showed the presence of significant antibacterial and antioxidant activity. The plant extract was found to be more active against Gram positive microbes compared to Gram negative microbes. The extract has the radical scavenging activity of about 77%. And the GCMS results showed the presence of different phytocompounds which are greatly known for their pharmacognistic activities.

Key words: *Cardiospermum halicacabum,* Antibacterial Activity, Phytochemicals, Antioxidant activity.

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INTRODUCTION

Plants are the source for biologically active compounds and a thorough investigation of numerous plant species is still needed for unravelling their medicinal properties.[1] Identification of pharmacologically important compounds further helps in the availability of the compounds and biological studies. World-wide 60% of people rely on plant-based drugs (natural products) for treatment of various diseases and to reduce or avoid adverse effects which could accompany many modern medicines.[2] In developing countries, 80% of people use traditional medicines for primary health care. It is known that antibiotics are primarily derived from the microorganisms.[3,4] The antibiotics threatened by the emergence of multidrug-resistant by indiscriminate usage of commercial antibiotics. The antibiotics some time causes adverse effects like immunosuppression, allergic reaction and hypersensitivity. So, researchers are now looking for novel antimicrobial agents with various chemical structures and new mechanism.[5]

Plant-based drugs or antimicrobials agents are mitigating side effects of synthetic antimicrobials. Several reports are available on herbal extracts against multidrug-resistant pathogens. Plant secondary metabolites (alkaloids, steroids, phenols, and tannins) exert their action by resembling endogenous metabolites, signal transduction, ligands, hormones and neurotransmitters. Plant also provide boundless prospects for the discovery of new drug via different ways depending on the availability of chemical diversity. The plants are a source of novel antibiotics and people choose herbal medicines because of the adverse side effects associated with synthetic antibiotics.[6,7]

Cardiospermum halicacabum (CH) is an annual or perennial herbaceous climber about 200-400 cm height and is found throughout tropical as well as subtropical regions of Asia and Africa, which is consumed as a green vegetable in Indian peninsula. CH has its application for the treatment of lumbago, rheumatism, nervous diseases, as a demulcent in orchitis, and in edema.[8,9] The plants have shown activities such as vasodepressant, antipyretic, antimalarial, antioxidant, antiulcer, and suppress the production of TNF α in mononuclear cells of human blood, anti-inflammatory and LPS induced COX 2, TNF α, nitric oxide production.[10,11] The plant has also been widely used for the treatment of skeletal fractures, ear-ache, reduce hardened tumors, hyperthermia, anti-hyperglycaemic, antispasmodic, rheumatism, lumbago, snake bite.[8,9] With this background, the current research was carried out to analyse the chemical constituents, antioxidant and antibacterial efficiency of methanolic extracts of *Cardiospermum halicacabum* (MECH) leaves.

MATERIALS AND METHODS

Plant collection and processing

The plants were collected from Kanavaipatti, Namakkal, Tamilnadu, India. Collected plants were

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identified by Prof. P. Jayaraman, Plant Anatomy Research Centre (PARC/2020/4249), Chennai. The leaves were washed thrice with distilled water to remove unwanted debris and the washed leaves were dried at room temperature (RT) for 3 to 4 weeks. The dried leaves were powdered by blender and sieved using mesh. The powder was the stored in an airtight bottle for future analysis in RT.

Preparation of extract

The leaf Powder (50g) was dissolved in 500ml of methanol. The mixture was then incubated on a rotary shaker at 100rpm for 3 h followed by keeping the mixture in an ultrasonic water bath (Labman LMUC-9) at 50°C for 50 min. The mixture was then filtered through a No.42 Whatman filter paper and the filtered solvent was concentrated using a rotary evaporator (Buchi R-100) at 40°C. The plant extracts were stored in a refrigerator till further analysis.

Fourier Transform Infrared Spectroscopy (FTIR)

Fourier transform infrared spectroscopy SHIMADZU IRTracer-100 was used to identify the functional group in the MECH. The spectrometric measurement was taken in the frequency ranging between 4000 and 400 cm^{-1}.

Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS analysis of MECH were done using Shimadzu QP-2010 Plus with Thermal Desorption System TD 20. The ionization energy of 70 eV was used and Helium gas was used as a carrier with a flow rate of 1.20 ml/min. 1µl of the sample was injected. The GC injector temperature was maintained at 230°C and MS transfer line temperature was maintained at 280°C. The temperature for ion source was set at 300 °C. Initial oven temperature was set to 50 °C with a hold time of 60 sec. Then, it was raised to 300 °C (at 5 °C/min) with the hold time of 5 min and to 235 °C (at 10 °C/min) with the hold time of 10 min. The resulting peaks were analysed using various in inbuilt MS libraries such as WILEY8.LIB and NIST05.LIB.[12-14]

Preliminary phytochemical screening

The MECH was tested for alkaloids, phenols, terpenoids, flavonoids, steroids, saponins, tannins, reducing sugars, carbohydrates, volatile oils, and amino acids.[15]

Antioxidant activity

The antioxidant activity was characterized by utilizing DPPH (2, 2-Diphenyl-1-picrylhydrazyl) assay[8]. Briefly, different concentrations of plant extract was prepared in methanol. 200µl of different concentrations of plant extract was mixed with 100 µl of 1mM DPPH solution (Sigma, India). The mixture was kept for incubation with shaking at room temperature in dark for 30 min. Then the absorbance was measured at 517 nm. The scavenging ability of the MECH was calculated using the following equation.

DPPH scavenging Activity (%) = (Abs control – Abs sample) / Abs control x 100

Where Abs control represents absorbance of DPPH without sample; Abs sample represents absorbance of DPPH with sample.

Antimicrobial activity of CH

The clinical pathogens *Staphylococcus aureus (S. aureus)*, Methicillin-Resistant *Staphylococcus aureus* (MRSA), *Escherichia coli (E. coli)* and *Pseudomonas aeruginosa* (PA*)* were obtained from the Department of Microbiology, SRM Medical College Hospital and Research Centre, Chennai. The pathogens were maintained in nutrient agar (NA). The bacterial culture was grown in nutrient broth overnight at 37°C. The grown bacterial culture suspension was adjusted to visually comparable turbidity of 0.5 Macfarland standard (Hi Media, India) which is equal to 1-2 x 10^8 CFU/ml. The antimicrobial activity of methanolic extract of CH was determined by disc diffusion assay.[3] Briefly, the nutrient agar was prepared and taken in sterile disposable petri plates and using sterile cotton swabs bacterial suspension was spread uniformly on the surface of NA. Commercially available sterile empty and antibiotic disc were purchased (Hi Media, India) and 20µl MECH was added in an empty sterile disc without dilution. Methanol was used as a control. For positive control, separate antibiotic discs were used against pathogens, for *E. coli* and PA – gentamicin 10mcg, *S. aureus*, MRSA- ciprofloxacin 5 mcg. The plates were then incubated at 37°C for 24 h to find the bacteriostatic effect.

Minimal Inhibitory Concentration (MIC)

The minimal inhibitory concentration (MIC) of the plant extract was assessed by the broth dilution method.[16] Briefly, different concentrations of MECH were prepared and added in a sterile 96 well plate to which 100µl of inoculum was added and incubated at 37°C for 24 h in triplicates. After 24h, the plates were read in an Elisa reader (Automated Elisa Reader and Washer, Thermo Fisher Scientific Inc.) and the OD was taken at 625nm. The lowest concentration of each extract was taken as minimal inhibitory concentration and the MIC activity was confirmed.

Statistical analysis

Analysed data were expressed in mean ± standard deviation. The statistical analysis performed by using Microsoft Excel and Origin 2020 software was used to plot the graphs.

RESULTS AND DISCUSSION

Mankind has understood the significance of plant derived metabolites in the past few decades owing to their health benefits.[2] The plant bioactive compounds have been extracted by different methods such as Soxhlet extraction, cold maceration, ultrasound-assisted extraction, microwave-assisted extraction, etc. The ultrasound assisted extraction technique is an easy and effective method to extract phytochemicals from plants. The surface contact between the solvents, samples and permeability of cell walls is increased by the acoustic cavitation generated ultrasound. While subjecting to ultrasound, the physical and chemical properties of the materials are altered and which disrupts the plant cell wall; facilitates the compounds release and enhances the bulk transport of solvents into the cells.[17]

The selection of a suitable solvent is important for the recovery of bioactive compounds. Methanol is a suitable solvent for the extraction of polyphenols, carbohydrates, terpenes, and organic compounds.[18] Qualitative phytochemical analysis of compounds in MECH showed the occurrence of reducing sugars, flavonoids, saponins, and triterpenes (Table 1) and this observation on the presence of all these bioactive compounds could be attributed to the high polarity of methanol.[19] Vinoth and Manivasagam perumal, 2013 evaluated the phytochemical content in *Cardiospermum halicacabum* ethanolic extract, the presence of phytochemicals was correlated with our results.[20]

Table 1: Phytochemical analysis of MECH.

S. No.	Test	Positive / Negative
1	Reducing Sugar	Positive
2	Glycosides	Positive
3	Tannins	Positive
4	Flavonoids	Positive
5	Terpenoids	Positive
6	Phenols	Positive
7	Saponins	Positive

The FTIR spectra of crude methanol extract of CH is shown in Figure 1. Strong and broad absorption in 3247 cm^{-1} indicated the OH stretching vibration indicating of alcohols, 2938 $cm^{-1,}$ and 2364 cm^{-1} indicates the strong absorption of CH stretching, 1641 cm^{-1} stretching indicates NH amines which is evident with the presence of flavonoids, 1437 cm^{-1} is the presence of O-H carboxylic acid, 1048 cm^{-1} stretching indicates the C-F, 866 cm^{-1} stretching of CH indicates aromatics, 523 cm^{-1} stretching is of C-Br. The FTIR results correlate with the results of the qualitative phytochemical, which indicates the availability of phenols, flavonoids, terpenoids, tannins, saponins, etc. These compounds have been widely reported for different properties such as antibacterial, antiviral, antifungal, immunomodulating, hepatoprotective, and anti-inflammatory properties.[3]

Free radicals have the ability to induce biological damage and various pathological events like ageing, carcinogenesis and inflammation. Huang et al, 2011 reported phenolic compounds to be actively efficient against inflammation.[8] The highest concentration of standard (ascorbic acid) showed about 89.63% radical scavenging activity. Hydrogen-donating ability of MECH showed about 77.25% at higher concentrations. As the concentration of the extract increases, the scavenging effect of MECH also increases. The ascorbic acid has relatively more pronounced than that of MECH (Figure 2). The antioxidant activity of the MECH was evident with the presence of polyphenolic compounds evidenced by FTIR and phytochemical results. Flavonoids are widespread groups of phenolic compounds in plants, which widely reported for their antioxidant potential.[21] The

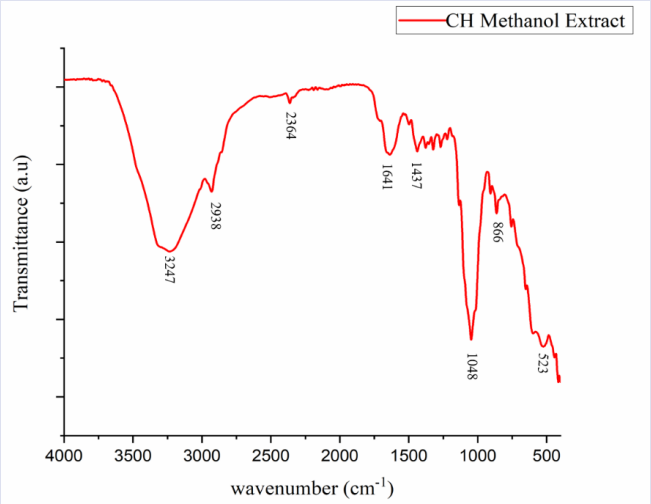

Figure 1: FTIR analysis of MECH.

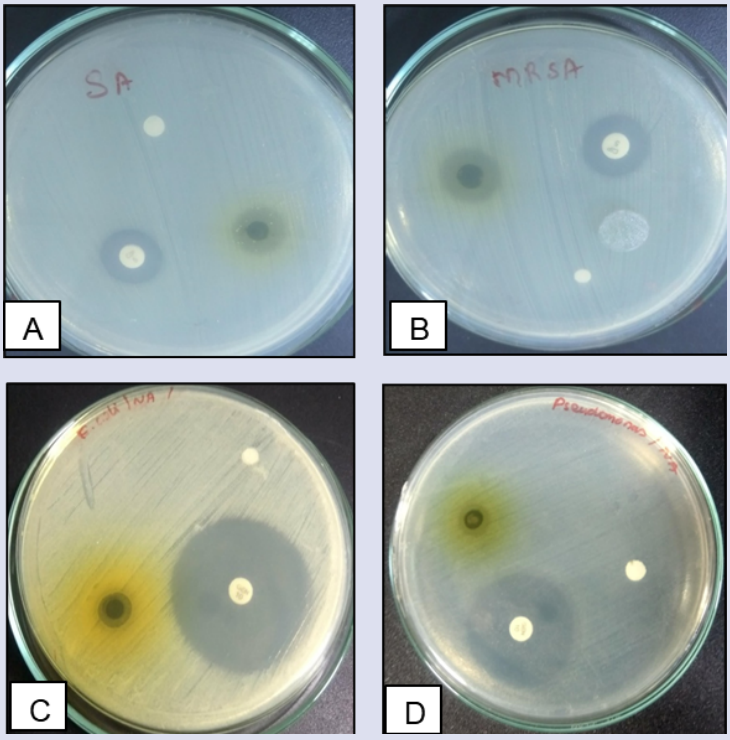

Figure 2: Antibacterial activity of MECH - A-*Staphylococcus aureus*, B- Multidrug resistant *Staphylococcus aureus*, C- *Escherichia coli*, D-*Pseudomonas aeruginosa*.

reducing power is directly correlated between the antioxidant activities. The reducing power is mainly the presence of reductones, which breaks the chain of free radicals by donating hydrogen atoms and preventing peroxide formation. Antioxidants activity in plants can be through several mechanisms such as chain initiation prevention, binding of transition metal ion compounds, peroxides decomposition, inhibition of continued hydrogen abstraction, reductive ability, and scavenging of radicals.[22,23]

Chemical composition of the MECH was confirmed by GC-MS analysis. 71 different compounds were present in the methanolic extract. In that some of the compounds were separated based on highest percentage such as (5h)-furanone, 5-methyl (4.16%), 3-pyrrolidinopropionitrile (1.37%), 1,5-anhydro-6-deoxyhexo-2,3-diulose (5.65%),(4-Aminophenyl)(2-methylpiperidin-1-yl) methanone (7.17%), undecanoic acid (2.5%), 2(4h)-benzofuranone, 5,6,7,7a-tetrahydro (1.86%), Neophytadiene (5.24%), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (1.15%), Hexadecanoic acid, methyl ester (2.72%), 9,12-Octadecadienoic acid, methyl ester (1.54%), 9-octadecenoic acid (z)-, methyl ester(8.09%), Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) (5.24%), 1,2-benzenedicarboxylic acid, dioctyl (1.35%), 9,12-Octadecadienoic acid (Z,Z)-2-hydroxy-1 (1.84%), Methyl (Z)-5,11,14,17-eicosatetraenoate (2.14%), Squalene (8.1%), vitamin E (3.05%). The GC-MS results exposed the occurrence of various bioactive compounds which has antimicrobial and antioxidant properties (Table 2). Jayadevi *et al,* 2013 analysed the phytochemicals of ethanolic extract of *Cardiospermum halicacabum* by GC MS analysis compared to this MECH extract has a greater number of phytocompounds.[3]

Synthetic drugs are used to kill a wide range of infection-causing microbes. Improper or over use of these antibiotics leads to the development of multidrug-resistant pathogens and adverse side effects. Multidrug-resistance is a major threat and challenge in the medical world. To overcome this issue, researchers are now focusing on developing plant-based drugs. These natural products both standardized extracts and purified compounds due to their matchless availability of diverse chemicals gives innumerable opportunities for the development of new drugs. Plant-derived therapeutics decreases the side effects connected with synthetic drugs.[24,25]

A wound can occur by trauma, accidents surgical procedures, and chronic disease conditions such as leprosy and diabetes mellitus. The wound provides a suitable environment for microbial colonization, proliferation, and infection. The wounds are most commonly

Table 2: Major bioactive compounds in MECH by GC MS analysis.

S. No	R. Time	Area%	Mol. Weight	Formula	Compound Name	Applications
1	6.244	4.16	98	C5H6O2	2(5H)-FURANONE, 5-METHYL	Antimicrobial and cardiac activity[36]
2	8.103	1.37	124	C7H12N2	3-Pyrrolidinopropionitrile	Antibacterial activity[37]
3	10.99	5.65	144	C6H8O4	1,5-ANHYDRO-6-DEOXYHEXO-2,3-DIULOSE	Anhidrotic property[37]
4	12.823	7.17	218	C13H18N2O	(4-Aminophenyl)(2-methylpiperidin-1-yl) methanone	Antibacterial, antifungal activity[37]
5	18.44	2.5	186	C11H22O2	UNDECANOIC ACID	Antifungal agent, antioxidant activity[38]
6	22.403	1.86	196	C11H16O3	2(4H)-BENZOFURANONE, 5,6,7,7A-TETRAHYDRO6-HYDROXY-4,4,7A-TRIMETHYL-, (6S-CIS)-	Antimicrobial and Antitubercular activity[39]
7	22.843	5.24	278	C20H38	Neophytadiene	analgesic, antipyretic, anti-inflammatory, antimicrobial, and antioxidant compound[40]
8	23.188	1.15	296	C20H40O	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Antimicrobial, anticancer, anti-inflammatory and anti-diuretic, Antioxidant[41, 42]
9	24.116	2.72	270	C17H34O2	Hexadecanoic acid, methyl ester	Antioxidant, decrease blood cholesterol, anti-inflammatory, nematicide, pesticide, antiandrogenic flavor, 5 alpha-reductase inhibitor[41,43,44]
10	26.414	1.54	294	C19H34O2	9,12-Octadecadienoic acid, methyl ester	Hypocholesterolemic, nematicide, anti-arthritic, hepatoprotective, anti antrogenic, 5 alpha-reductase inhibitors, antimicrobial, anticoronory[42]
11	26.494	8.09	296	C19H36O2	9-OCTADECENOIC ACID (Z)-, METHYL ESTER	Antioxidant, anticancer[45]
12	31.537	5.24	330	C19H38O4	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ETHYL ESTER	Hemolytic, pesticide, flavor, antioxidant[46]
13	31.662	1.35	390	C24H38O4	1,2-BENZENEDICARBOXYLIC ACID, DIOCTYL ESTER	Antimicrobial, antifungal and anti-malarial[39]
14	33.451	1.84	354	C21H38O4	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-11-(hydroxymethyl)ethyl ester	Anti-inflammatory, Hypocholesterolemic Cancer preventive, Hepatoprotective, Nematicide Insectifuge, Antihistaminic Antieczemic, Antiacne, 5-Alpha reductase inhibitor Antiandrogenic, Antiarthritic, Anticoronary, Insectifuge[43]
15	33.532	2.14	318	C21H34O2	Methyl (Z)-5,11,14,17-eicosatetraenoate	Antibacterial[47]
16	34.499	8.1	410	C30H50	Squalene	antimicrobial and antioxidants, anticancer, immunostimulatory activity[44]
17	38.528	3.05	430	C29H50O2	Vitamin E	Antioxidant, antimicrobial, analgesic, antidiabetic, anti-inflammatory, anti dermamatitic, antileukemic, anticancer, hepatoprotective, and antispasmodic[41]

contaminated by *S. aureus, P. aeruginosa,* and *E. coli.* Among these microbes, higher percentage of *S. aureus* was isolated from wounds.[26] It causes numerous health problems in humans such as skin infections, pneumonia, sepsis, etc. MRSA causes community-acquired and nosocomial bacterial infections.[27] This limits the treatment of many patients infected with these organisms and creates resistance against antibiotics by different mechanisms. *S. aureus, P. aeruginosa* produces the biofilm in the wound area, which makes it difficult to treat with standard antibiotics. The bacteria create resistance against various antibiotics by various virulence factors.[28]

In the present study, the antibacterial activity and MIC of MECH extract against clinical pathogens was evaluated and the plant extracts showed a wide range of inhibition against gram-positive organisms compared to gram-negative organisms (Figures 3 and 4). The highest zone of inhibition was observed in the Gram-positive organism such as *S. aureus* (14 ± 0.5 mm), MRSA (12 ± 1.0 mm). The zone of inhibition was significantly comparable with commercial antibiotic ciprofloxacin. The least antibacterial activity was found against gram-negative organisms such as *P. aeruginosa* (7 ± 0.8 mm), *E. coli* (9 ± 0.7 mm). The antibacterial activity of MECH was correlated with previous studies.[29,30] MIC value MECH against pathogens were 15 mg/ml to 50 mg/ml concentration (Table 3). MIC value of *S. aureus* was 15 mg/ml, MRSA was 20 mg/ml, *E.coli* was 35mg/ml and *P. aeruginosa* was 50 mg/ml. MIC values differed from previous studies which could be attributed to various factors including the influence of chemical composition, quantity, and quality of plant compounds such as time of harvest, extraction method, geographical and environmental factors.[19] The bioactive compound present in methanol extract inhibit microbial growth by binding to their cell surfaces. The bioactive compound adsorbed by bacterial cell membrane leads to the generation of hydroperoxides, disruption, and then cell leakage.[31] For instance, the cell wall synthesis of microbes was inhibited by tannins by irreversible complexes with proline rich proteins.[32] Leakage of protein and some enzymes is caused by the saponins.[33] Terpenoids weakening the membranous tissue of microbes, which results in the dissolution of cell wall.[34,35] Flavonoids inhibit microbial growth by forming a complex with extracellular, soluble proteins, and cell walls. Gram-negative bacteria cell wall is complex compared gram-positive organisms, which act as a diffusion barrier and make less susceptible to plant extract.[29] FTIR, qualitative phytochemical analysis and GC- MS results confirmed the presence of pharmaceutically and therapeutically important flavonoids and phenolic compounds. Moreover, it is also recommended that these compounds in minor quantities might have involvement in some synergistic types with the active compound.[18]

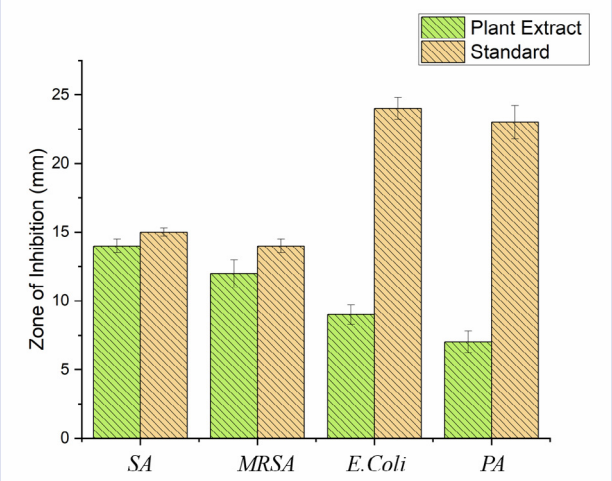

Figure 3: ZOI of MECH SA-*Staphylococcus aureus* (14 ± 0.5 mm), MRSA- Multidrug resistant *Staphylococcus aureus* (12 ± 1.0 mm), PA-*Pseudomonas aeruginosa* (7 ± 0.8 mm), E. Coli- *Escherichia coli* (9 ± 0.7 mm).

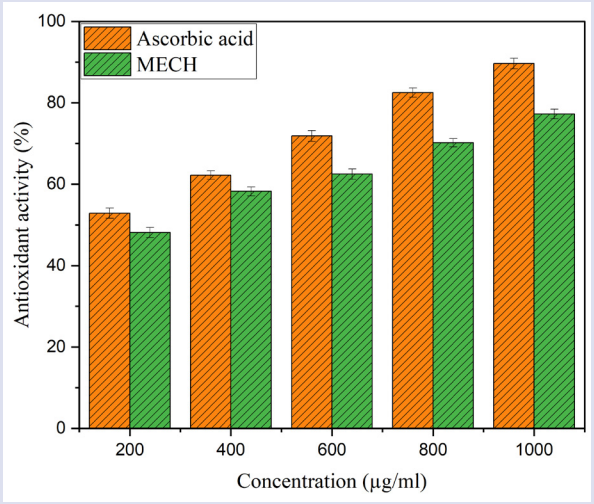

Figure 4: DPPH activity of MECH.

Table 3: MIC of MECH.

S. No	Microorganism	MIC (mg/ml)
1	*Staphylococcus aureus*	15
2	Multidrug resistant *Staphylococcus aureus*	20
3	*Escherichia coli*	35
4	*Pseudomonas aeruginosa*	50

CONCLUSION

This present study showed the presence of potential antioxidant and antibiotic activity of MECH. The results thus obtained are also in significant with previous studies as discussed. This plant can be further investigated for its wound healing activity as combating against the commensal pathogens which play crucial role in the wound healing processes. Being a potent antibiotic agent, this plant can be further studied for the identification of representative compounds of antibiotics present in it.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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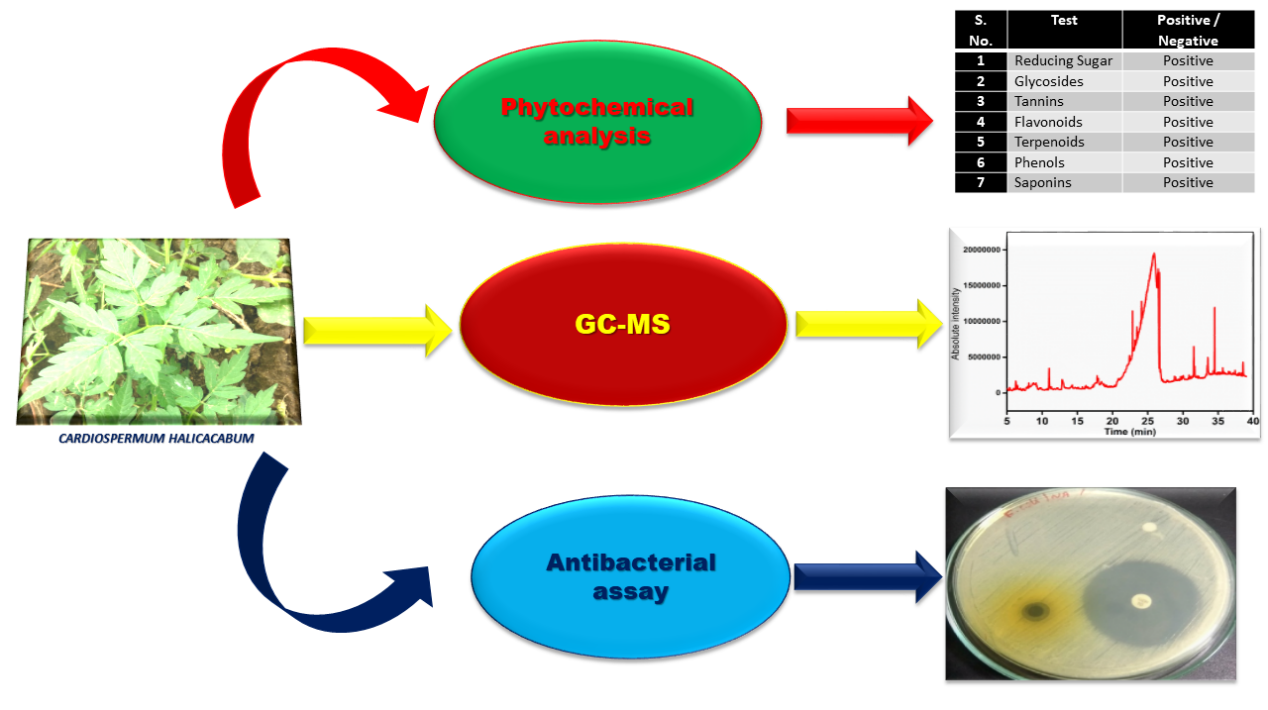
GRAPHICAL ABSTRACT



S. No.	Test	Positive / Negative
1	Reducing Sugar	Positive
2	Glycosides	Positive
3	Tannins	Positive
4	Flavonoids	Positive
5	Terpenoids	Positive
6	Phenols	Positive
7	Saponins	Positive

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