# EVALUATION OF ANTIMICROBIAL POTENTIALS OF *Cardiospermum halicacabum* Linn.

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ABSTRACT

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## INTRODUCTION

*C. halicacahum* Linn belongs to the family Sapindaceae. It is commonly known as mudakattan in Tamil, Ballon vine or Heart's pea in English. This plant is distributed throughout India in the plains. The plant is pubescent or nearly glabrous, annual or perennial with slender branches, climbing by means of tendrillar hooks; leaves ternately bicomponent, leaflets acuminate at the apex; flowers white, small; fruits membranous, depressed, pyriform capsule winged at the angles; seeds black with a large white heart shaped aril.

The roots, leaves and seeds of the plant were used as herbal medicine (Warrier, 1996). The roots are diuretic, diaphoretic, emetic, mucilaginous, laxative and emmenago-gue. They are useful to cure strangury, fever, arthritis, amenorrhoea, lumbago and neuropathy. According to earlier research the roots are ineffective for chronic rheumatism. The leaves are rubifacient and are good for arthritis and fever. The plant has sedative action on the central nervous system (Nadkarni, 1996).

Antimicrobial activity of Cardiospermum halicacabum shoot extracts were studied on gram-positive bacteria such as Staphylococcus aureus, Bacillus substilis, and gram-negative bacteria such as E.coli, Proteus vulgaris, and fungus Candida albicans. Disc diffusion method was used to study the antimicrobial activity of aqueous, ethanol, chloroform and ether extracts of C. halicacabum. Ampicillin was used as reference standard at 10 µg/disc concentration. Extracts of C. halicacabum exhibited a significant antibacterial activity except the aqueous extract. Ethanolic extract was found to be very effective with maximum activity index (0.84). The ethanolic extract exhibited minimum inhibitory concentration (MIC) of 0.25 mg/mL against Staphylococcus aureus, Bacillus substilis, E. coli and Proteus vulgaris and 0.125 mg/mL against Candida albicans. The MIC of chloroform and ether extracts ranged between 0.25 and 1.0 mg/ml against the test organisms. All the extracts showed antifungal activity against Candida albicans.

Key words: antimicrobial activity, *Cardiospermum halicacabum* shoot system, solvent extraction,.

The antipyretic activity of was reported by Asha *et al.* Treatment of chronic dermatoses with plant pharmaceuticals was reported by Shakhtmeister *et al.* (1997). Having known these facts an attempt was made to evaluate the antimicrobial activity of *C. halicacabum* extracts on various gram-positive, gram-negative bacteria and fungi.

#### **METHODOLOGY** Plant material and extract

Good quality plant material of C. halicacabum was collected from in and around Tiruchirapalli city, Tamilnadu, India shade dried and powdered by making use of mechanical blender (Smith mixer). The powder was stored in an air tight container and was used for extraction. The coarse powder of C. halicacabum L. was subjected for (100 g) cold extraction with various solvents like water (chloroform ethanol, chloroform and ether. water), Different extracts collected were filtered, evaporated under vacuum (Kelmanson et al., 2000). The extracts were stored in a sterile container for further use.

S.No.	Test Organisms	Diameter of zone of inhibition in mm				
		Aqueous	Alcoholic	Chloroform	Ether	Standard
		extract	extract	extract	extract	(Ampicillin)
1	S.aureus	Nl	9±0.47	$15\pm0.52$	14±0.41	$27 \pm 0.51$
			(0.33)	(0.55)	(0.51)	
2	B.substilis	Nl	$10 \pm 0.43$	$11 \pm 0.65$	13±0.73	$22\pm0.47$
			(0.45)	(0.50)	(0.59)	
3	E.coli	N1	$13\pm0.27$	$11 \pm 0.47$	13±0.54	$31 \pm 0.75$
			(0.41)	(0.35)	(0.41)	
4	P.vulgaris	N1	8±0.36	$12\pm0.22$	9±0.32	$25\pm0.45$
	-		(0.32)	(0.48)	(0.36)	
5	C.albicans	8	$16 \pm 0.47$	$12\pm0.28$	$12 \pm 0.58$	19±0.29
		(0.42)	(0.84)	(0.63)	(0.63)	

Table I. Antimicrobial activity of C.halicacabum Linn

Note: NI = No Inhibition, Each value is a mean  $\pm$  standard error mean of 3 determinations.

Reference standard =Ampicillin ( $10 \mu g/disc$ ) (Himedia)

Values in parentheses indicate Activity Index

Activity index = Inhibition zone of test / Inhibition zone of standard.

Table II. MIC values of ex	tracts of C. halicacabum Linn
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S.No.	Test Organisms	Alcoholic extract (mg/mL)	Chloroform extract (mg/mL)	Ether extract (mg/mL)
1	S. aureus	$0.25 \pm 0.04$	$0.5 \pm 0.02$	1.0±0.1
2	B. substilis	$0.25 \pm 0.04$	$0.5 \pm 0.1$	$1.0 \pm 0.03$
3	E. coli	$0.25 \pm 0.02$	$0.5 \pm 0.04$	$1.0 \pm 0.05$
4	P. vulgaris	$0.25 \pm 0.06$	$0.25 \pm 0.02$	$0.5 \pm 0.03$
5	C. albicans	$0.125 \pm 0.04$	$1.0 \pm 0.08$	$1.0\pm0.03$

Each value is a mean  $\pm$  standard error mean of 3 determinations.

#### Microbial strains used

Gram-positive bacteria like *S.aureus, Bacillus substilis,* and gram-negative bacteria like *E.coli, P.vulgaris* and fungus *C.albicans* were used as test organism and were obtained from National Chemical Laboratory (NCL), Pune, India.

#### Preparation of inoculums

The selected bacterial and fungal strains were inoculated in nutrient broth and incubated at 37° C for 18-24 hours and used for antimicrobial activity testing of various extracts.

#### **Determination of antimicrobial activity**

Disc diffusion method (Bauer *et al.*, 1996) was followed to determine the antimicrobial activity of various extracts of *C. halicacabum* L. The test was performed in

triplicates and the zone of inhibition was measured by making use of antibiotic zone reader (Pathak Electrical Works, Mumbai). Sterile discs containing the respective solvents and ampicillin 10  $\mu$ g was used as control and reference standard respectively. The parameter Activity Index was calculated by using the formula as follows:

# Activity Index = $\frac{\text{Inhibition Zone of test}}{\text{Inhibition Zone of standard}}$

Sterile nutrient broth double strength (peptone broth and Sabouraud's Dextrose broth) was used to determine the minimum inhibitory concentration (MIC) of the extracts. Two-fold serial dilution technique was used (Dhar *et al.*, 1998). Plant extracts (20mg/mL) in PEG 200 was used for testing MIC. This solution (0.2mL) was added to 1.8 mL of

nutrient broth formed the first dilution. All culture tubes were incubated for 24 hours at 37° C. Following incubation the tubes were examined for sign of microbial growth. The lowest concentration inhibiting microbial growth was considered to be MIC.

## **RESULTS AND DISCUSSION**

Antimicrobial activity was showed by alcohol, chloroform and ether extracts of *C. halicacabum* L. against the test organisms tried, but the aqueous extract showed minimum inhibition against the fungus *C. albicans*. Selected Malaysian plants were studied for their antimicrobial activity by Ali *et al.* (1995).

Zone of inhibition was found maximum with chloroform extract (15mm) against *S.aureus* followed by alcohol and ether extracts (13 mm) against *E. coli* and *Bacillus substilis* (Table I)

All the extracts showed inhibition against the fungi *C.albicans* including the aqueous extract of *C.helicacabum* L. Maximum inhibition of *C.albicans* was observed (16mm) with alcohol extract followed by chloroform and ether (12 mm) and aqueous extract (8 mm).

These inhibitory effects were compared with the reference standard antibiotic under the same laboratory conditions and the activity index was calculated (Table I). The efficacy of this plant extracts is comparable with the efficacy of streptomycin antibiotic disc it may be noticed that the plant extracts used in this study are in crude form and further studies with different purified compounds of these extracts may help to understand the merits of this plant against bacterial cultures in comparison to streptomycin (Sasidharan *et al.*, 1998). Phytochemical examination of the *C. helicacabum* L. revealed the presence of alkaloids (Srinivas *et al.*, 1998).

Another observation in this study was that *C. helicacabum* L. in its aqueous extract showed no inhibitory activity against any of these bacterial species.

The results further showed that the extracts were having more potent anti fungal activity against *C.albicans* than the antibacterial effect against the bacteria under test. The results of this study confirm the traditional claim for the usefulness of this plant against various diseases. Since the alcohol, chloroform

and ether extracts were more effective than aqueous extract, these can be elucidated for its effectiveness by further study and they may become novel sources for antimicrobial compounds.

The extracts of *C. helicacabum* L. exhibited prominent antimicrobial activity. Table II gives the average values of MIC in mg/mL of various extracts of *C. helicacabum* L. used in this study. The MIC of alcoholic extract against all bacteria and fungi was found to be 0.25 mg/mL and 0.125 mg/mL respectively. The growth of *P.vulgaris* was greatly inhibited by chloroform and ether extracts with MIC of 0.25 and 0.5 mg/mL respectively. The chloroform and ether extracts exhibited MIC of 1.0 mg/mL against *C. albicans*.

Thus the alcoholic, chloroform and ether extracts exhibited potent antimicrobial activity. The alcoholic extract was significantly more potent compared to the chloroform and ether extracts. The aqueous extract was devoid of any antibacterial activity.

## CONCLUSION

This study leads to the conclusion that *C.halicacabum* L. showed significant antimicrobial activity against the organisms under test. Bio assay directed fractions of the active crude extracts to isolate and identify the compounds responsible for the antimicrobial activity could be investigated.

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