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Antibacterial Activity of Solvent Extracts of *Cardiospermum halicacabum* against Clinical Pathogens

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Abstract : Antibacterial activity of *Cardiospermum halicacabum* was examined against clinical human bacterial pathogens. The butanol and methanol extract of *C. halicacabum* was endowed with plenty of phytochemical compounds. The susceptibility of *Micrococcus luteus* (33mm) was found pronounced followed by *Proteus vulgaris* as well as *Staphylococcus aureus* (32mm) against stem of acetone extract of *C. halicacabum.* The HPLC peaks at 2.267, 2.780 and 2.11 also describe the presence of active compounds, which are played key role in antimicrobial activity
Keywords: *Cardiospermum halicacabum*, Antibacterial, HPLC, solvents.

Introduction

So far diagnosed infectious human disease treatment and prevention all over the world[2] relies mostly on the phytomedicine and are now inevitable portion[9] in novel medicines. *Cardiospermum halicacabum* L. member of Sapindaceae family is prevalent in tropical and subtropical areas and grown in plains such as India, Pakistan, Bangladesh, Africa and America. It has been used as antimicrobial[7] and is also exert as anticancer activity[10]. Its role in antiparasitic as well as antimalarial and antifilarial has been known[12]. [1]Khunkitt *et al*. [5]Deepan *et al.*, observed the excellent antimicrobial activity of aqueous extract of *C. halicacabum* against *Escherichia coli*.. [3]Girish *et al*. observed the significant antibacterial activity of ethanolic extract of *C. halicacabum* against *Staphylococcus aureus,* the gram positive bacteria. Hence the present investigation, the antibacterial activity of *C. halicacabum* was examined against clinical human bacterial pathogens.

Materials and Methods

Collection of *Cardiospermum halicacabum*

Cardiospermum halicacabum plants were collected from Vallalapati, Madurai district, Tamil Nadu, India. The plants were brought to the laboratory after proper identification, fresh plant materials were washed under running tap water. Leaf, stem, flower, seed, seed coat, root parts were separated from the plants. They were separately shade dried for 5 days, pulverized in an electric mixer and each powdered plant parts were stored in air tight bottles.

Preparation of plant extract

The plant extracts were prepared by adopting the following procedures adopted by Vinoth *et al.*, (2012). 20 g of each powdered plant parts were percolated with 60 ml of different solvents such as butanol, acetone, methanol and petroleum ether. They were left under room temperature for two days with intermittent shaking. The percolate was filtered with Whatman's No 1 filter paper (Hi-Media, India). The resulting extracts were concentrated by evaporation at room temperature. The crude extracts were collected and stored in screw capped vials at 4 °C until further study.

Phytochemical composition of solvent extracts of *Cardiospermum halicacabum*

The solvents such as butanol, acetone, methanol and petroleum ether extracts of *Cardiospermum halicacabum* were subjected to preliminary phytochemical analysis by the following methods followed by Harborne[4] for the presence of alkaloids, flavonoids, phenols, tannins, saponins, terpenoids and glycosides.

Antibacterial assay

The antibacterial screening was done by using well diffusing assay[8] against eight bacterial strains such as *Streptococcus pyrogenes, Staphylococcus aureus, Pseudomonas aeruginosa, Proteus vulgaris, Bacillus subtillus, Salmonella typhimurium, Micrococcus luteus, E.col* obtained from Bose laboratory, Madurai district, Tamil Nadu.

Results and Discussion

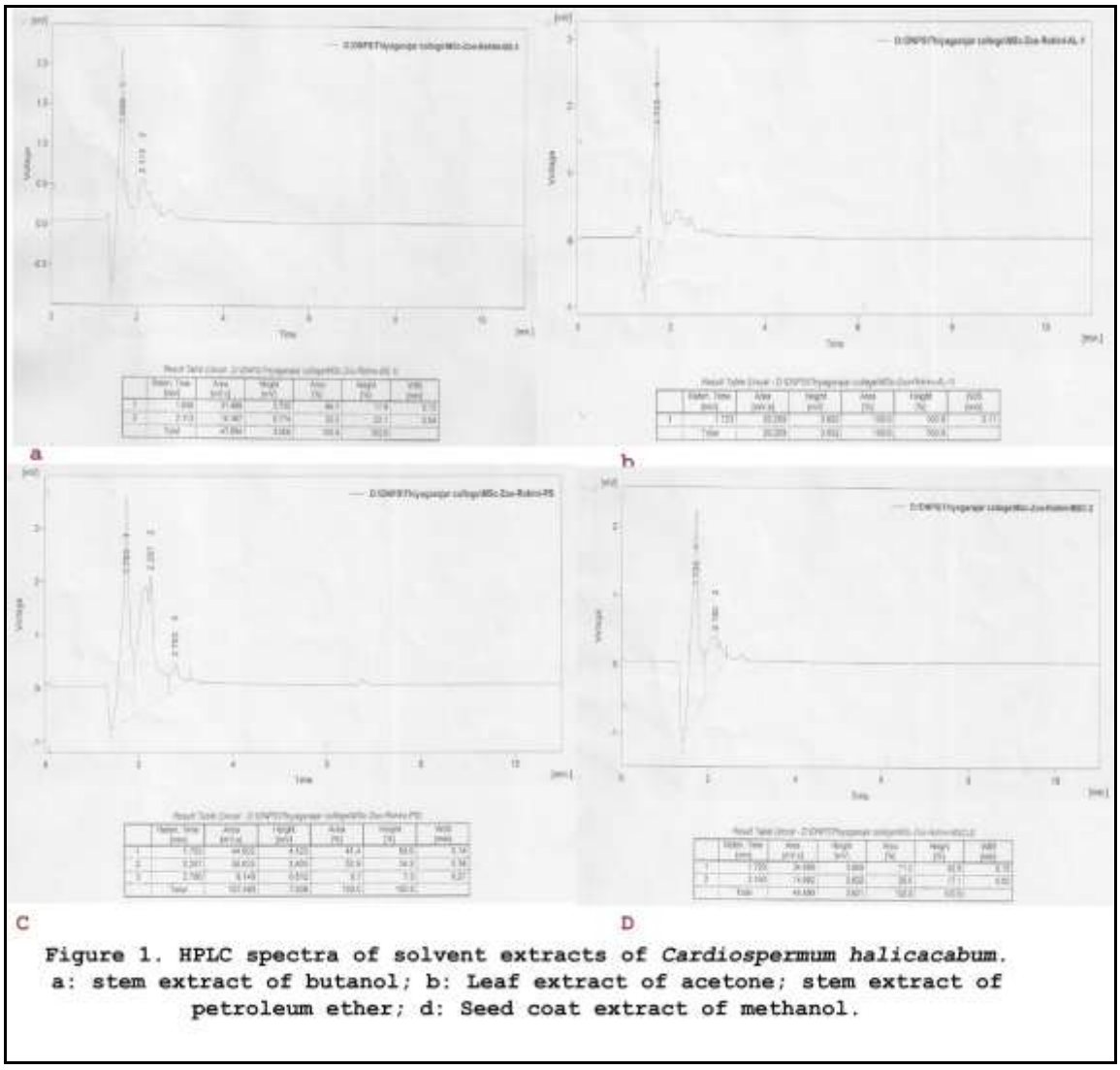

Figure 1. HPLC spectra of solvent extracts of *Cardiospermum halicacabum*. a: stem extract of butanol; b: Leaf extract of acetone; stem extract of petroleum ether; d: Seed coat extract of methanol.

Table 1. Phytochemical screening of solvent extracts of *Cardiospermum halicacabum* L.

Phytochemicals	Butanol extract						Acetone extract						Methanol extract						Petroleum Ether Extract					
	L	S	F	Se	SeC	R	L	S	F	Se	SeC	R	L	S	F	Se	SeC	R	L	S	F	Se	SeC	R
Alkaloids	-	+	-	-	-	+	+	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-
Flavonoids	+	+	+	-	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	-	-	-	-	-
Phenols	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-	-
Tannins	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-	-
Saponins	+	-	-	-	-	-	+	+	-	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+
Terpenoids	+	+	-	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Glycosides	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+

'+' = Presence of compound, '-' = Absence of compound, 'L' = Leaves, 'S' = Stem, 'F' = Flower, 'Se' = Seed, 'SeC' = Seed Coat, 'R' = Root.

Table 2. Antibacterial activity of solvent extracts of *Cardiospermum halicacabum* against clinical bacterial pathogens.

Test organisms	Zone of Inhibition (mm)																							
	Butanol extract						Acetone extract						Methanol extract						Petroleum Ether Extract					
	L	S	F	Se	SeC	R	L	S	F	Se	SeC	R	L	S	F	Se	SeC	R	L	S	F	Se	SeC	R
Streptococcus pyrogenes	23	21	15	19	18	19	27	27	11	-	13	13	22	21	17	17	25	17	-	-	-	-	-	-
Staphylococcus aureus	21	18	18	17	18	20	30	32	-	-	-	20	25	20	21	19	24	-	-	-	14	-	-	-
Pseudomonas eruginosa	17	18	-	17	17	-	30	31	-	-	-	21	23	23	24	19	25	19	-	-	24	-	22	-
Proteus vulgaris	17	18	12	-	17	15	27	32	-	-	-	21	21	22	19	19	25	19	-	-	-	-	-	-
Bacillus subtilus	20	18	13	13	17	16	29	26	-	-	-	22	24	22	18	19	24	-	18	19	15	15	18	12
Salmonella typhimurium	17	21	16	14	22	20	30	31	-	-	-	21	22	22	23	21	26	17	22	24	12	13	22	-
Micrococcus luteus	15	16	15	12	17	17	30	33	-	-	-	18	22	21	19	18	28	13	-	-	18	-	-	-
Escherchia coli	15	18	16	17	20	16	28	27	13	-	-	18	21	19	17	15	23	17	22	22	-	-	21	20

Cardiospermum halicacabum, reservoir of highly active chemical constituents. In the present study, antimicrobial activity of extracts of *C. halicacabum* was studied. The leave, stem, flower, seed, seed coat and root of the *C. halicacabum* was subjected to butanol, acetone, methanol and petroleum ether extraction and its phytochemical composition was presented in table 1. The butanol and methanol extract had composed of plenty of phytochemical compounds compared to acetone and petroleum ether extracts. Alkaloid was present only in stem and root of butanol extract, leaf and root of acetone extract and seed of methanol extracts. Phenol, tannin and glycosides were observed in Butanol and methanol extract of all the portion of the *C. halicacabum*. Table 2 reveals the antibacterial activity of extracts of *C. halicacabum* against clinical pathogens. The zone of inhibition (ZoI) of extracts namely butanol, acetone, methanol and petroleum ether were ranged between 12 to 23mm, 11 to 33mm, 13 to 28mm and 12 to 30mm respectively. Acetone extracts revealed excellent antibacterial activity followed by methanol and petroleum ether. Regarding the susceptibility, *Micrococcus luteus* (33mm) found pronounced followed by *Proteus vulgaris* as well as *Staphylococcus aureus* (32mm) against stem of acetone extract of *C. halicacabum.* Similar result was observed by Krishna Murthy Naik *et al*.[6]. As reported by Girish *et al*. (2008), the acetone extracts revealed excellent activity against *S. aureus.*

The HPLC results (Figure 1) revealed that, in addition to solvent peaks at 1.65 to 1.753 min, there are few compound response peaks obtained at 2.11, 2.267, 2.160 and 2.780 min with respect to butanol stem, acetone leaf, petroleum ether stem and methanol seed coat pigments accordingly. In the present study, HPLC is used to detail the chemical constituents present in the *C. halicacabum.* A rapid HPTLC densitometry method for simultaneous determination of flavonoids in important medicinal plants was reported by Bhandari *et al*. (2007). Apigenin and luteolin, secondary metabolites from *C. halicacabum* was estimated by (Bhandari *et al*., 2007) by using RP-HPLC. In the present investigation, except solvent peaks near around 1.6-1.7 min, peaks at 2.267, 2.780 and 2.11 also describe the presence of active compounds, which are played key role in antimicrobial activity. It would be further analyzed in FT-IR, NMR and GC-MS to reveal the actual chemical structure of the compound.

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