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Research Article

PRELIMINARY PHYTOCHEMICAL ANALYSIS, HPTLC FINGER PRINTING AND *IN VITRO* ANTI CANCER SCREENING OF EXTRACTS OF AERIAL PARTS OF *CARDIOSPERMUM HELICACABUM*

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ABSTRACT

Objective: The objective of the research is to subject Preliminary phytochemical analysis, HPTLC finger printing and *in vitro* anti cancer screening of extracts of aerial parts of *Cardiospermum helicacabum*

Methods: The aerial parts of *Cardiospermum helicacabum* was extracted using ethanol, chloroform and N-hexane. The extracts were subjected to preliminary analysis, TLC, HPTLC, total Phenolic content and total Flavonoid content. The anticancer activity was evaluated by MTT assay method.

Results: Ethanolic extract gave IC_{50} value of 1.90mg/ml whereas chloroform extract and n-hexane extract gave IC_{50} values of 2.47 and 2.595 mg/ml respectively, for potential *in vitro* anti cancer activity.

Conclusion: From the results, it is evident that *C. halicacabum* is a biologically significant plant material and can be further studied for developing synthetic analogs.

Keywords: *Cardiospermum helicacabum,* Anticancer

INTRODUCTION

Crude drugs have been replaced by pure chemical drugs and the developed countries have experienced a decline in popularity of medicinal plant therapy. The modern medicinal system has grown phenomenally as manifested by global pharmaceutical sales which have increased to 7% in 2006 fueled by strong international demand for cancer treatments and robust growth in the US market. In the current study, Cardiospermum genus from Sapindaceae family is considered. The name *Cardiospermum helicacabum* Linn is of Greek origin kardia "heart" and sperma "a seed" referring to the shape of the seed or to a heart shaped spot on the seeds and halikkabos. It is used in the treatment of Rheumatism, cough, hyperthermia, lumbago, nervous illness and amenorrhea [1]. The whole plant is diaphoretic, diuretic [2], emmenagogic[3], laxative [4], rubefacient [5] and stomachic [6]. The leaf juice has been used as treatment of earache [7]. The whole plant of *Cardiospermum helicacabum* contains saponins, traces of alkaloids, flavonoids, proanthocyanidin, apigenin and phytosterols. The leaves of plant contain β- sitosterol, D-glucose, oxalic acid, (+) pinitol, 7-*o*-glucuronides of apigenin, chrysoeriol and luteolin. The root portion contains β- sitosterol, phlobaphene, phloba-tannin and pro anthocyanidine. The seed portion contains fixed oil, fatty acid [8]. Considering these pharmacological actions and chemical constituents of the plant the present research is planned to do the phytochemical analysis of aerial parts of *Cardiospermum helicacabum*.

MATERIALS AND METHODS

Collection, identification and extraction

The aerial parts of the plant *Cardiospermum helicacabum* were collected from Kancheepuram and authenticated by Dr. P. Jayaraman, Ph.D., Director, Plant Anatomy Research Centre, Medicinal Plants Research Unit, Tambaram, Chennai-45. A portion of the sample was kept in the department museum for further reference (PARC/2010/579). All the chemicals and solvents used were of high purity. The plant material was dried and coarsely powdered. Coarsely powdered plant material of *Cardiospermum helicacabum* were extracted with various solvents like n-hexane, chloroform and ethanol by using Soxhlet apparatus. The process is continued until the drug is completely extracted and the extract in the flask is then filtered. The extracts were distilled and concentrated to three fourth of the volume under vacuum drier

Phytochemical screening

The extracts were subjected to various preliminary phytochemical analyses to test for the presence or absence of various phyto constituents, preliminary tests for alkaloids, steroids, flavonoids, saponins and cardiac glycosides. The extracts were then separated by TLC method. The TLC plates were made with silica gel and activated. The extracts were spotted by means of a micro pipette and dried, developed in solvent systems 1, 2 and 3 separately. The solvent system used were hexane: ethyl acetate (8:2), chloroform, toulene: ethyl acetate: formic acid (4.5 :4.5 :1). The different spots were identified using standard UV light.

Development of HPTLC finger print

The ethanol extract of *Cardiospermum helicacabum* were applied in a concentration of 10µl and standard quercetin at about 5µl were applied using CAMAG Linomat IV sample applicator on aluminium sheets pre coated with silica gel merck 60F 254 0.2mm layer thicknesses 5x10cm were used as stationary phase in two different tracks. The plate was developed in the mobile phase, toluene: ethyl acetate: formic acid (4.5:4.5:1) to a distance of 120 mm for developing the chromatogram. The development was carried out in CAMAG twin trough glass chamber. The width of the band was maintained as 6mm and the bands were applied on the plate at a distance of 6mm. The two different tracks were scanned using CAMAG densitometer scanner 3 VI.13 equipped with CATS V 4.04 software, at a wavelength of 366 nm using deuterium lamp and the finger print profile were recorded. The amount of Quercetin present in the extract was calculated by using the formula.

$$\text{Amount present} = \frac{\text{Concentration of unknown extract X 100}}{\text{Amount of extract taken}}$$

Determination of Total Phenolic Content

Total phenolics were determined using Folin-Ciocalteu's reagent (FCR) with slight modifications. This method depends on the reduction of FCR by phenols to a mixture of blue oxides. The total phenolic content was expressed as Gallic acid equivalents (GAE) in mg/g extract from the calibration curve of Gallic acid standard solution. For the gallic acid, the curve was established by plotting concentration (mg/ml) versus absorbance (nm) [9].*2.5 Determination of Total Flavonoid Content (TFC)*

Total flavonoids content in the extract was determined by aluminium chloride colorimetric method, using catechin as a standard. Total flavonoid content was measured by the aluminium chloride colorimetric assay. Total flavonoid content of extract was expressed as mg catechin equivalents CE/100 mg fresh mass. [10]

MTT Assay

After incubation, remove the medium from the wells carefully for MTT assay [11]. Add 200µl of MTT concentration of (5mg/ml). Incubate for 6-7 hours in 5% carbon dioxide incubator for cytotoxicity. After incubation, 1ml DMSO was added in each well and mixed by pipette and left for 45 sec. If any viable cells present, formazan crystals are visible. The color formed was purple. The optical densities were read at 595nm taking DMSO as blank. A graph is plotted by taking concentration of the drug on X axis and relative cell viability on Y axis Cell viability % = Mean OD of test/ Mean OD of control X 100

Statistical Analysis

Data were analyzed using Student's T test. The results were compared with standard. The analysis was carried out using Prism software. (version 3, GraphPad software, Inc). $P<0.05$ was used to determine significant difference.

RESULTS AND DISCUSSION

The plant *Cardiospermum helicacabum* is a well recognized plant. Upon phytochemical analysis, the major constituents like alkaloids, carbohydrates, cardiac glycosides, phytosterol, saponins, tannins, flavonoids and triterpenoids were found in ethanol and chloroform extract. N-hexane extract contained only phytosterol and saponins. The results of phytochemical screening was shown in table 1.

Table 1: Phytochemical screening of *Cardiospermum halicacabum*.linn.

S. No.	Test	Ethanol extract	Chloroform extract	n-Hexane extract
1.	Alkaloids	+	+	-
2.	Carbohydrates	+	+	-
3.	Cardiac glycosides	+	+	-
4.	Phytosterol	+	+	+
5.	Saponins	+	+	+
6.	Tannins	+	+	-
7.	Flavanoids	+	+	-
8.	Triterpenoids	+	+	+

Note: + indicates present; - indicates absent

The ethanol extract was subjected to HPTLC fingerprinting. The ethanol extract showed 6 peaks as represented where as the standard quercetin showed 1 peak. Table 2 shows the number of peaks formed in the HPTLC finger printing with sample and standard volume in the range of 5µL. The figure 1 depicts the HPTLC finger printing of ethanolic extract.

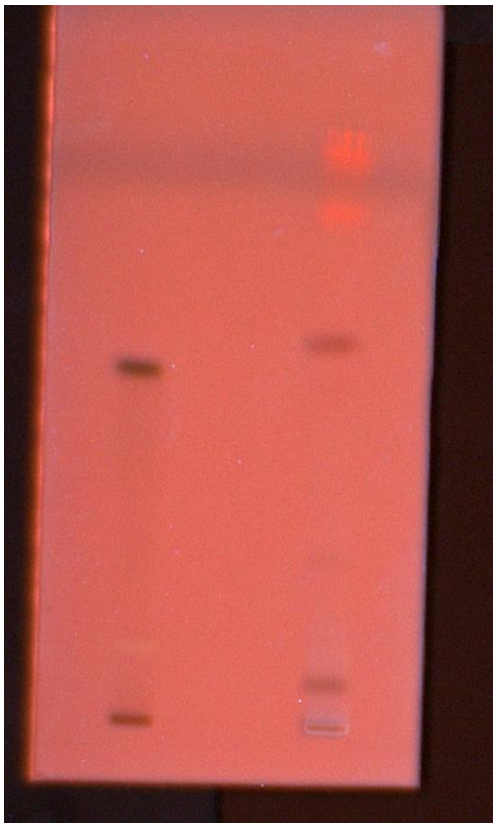

Fig. 1: HPTLC of ethanolic extract

The amount of extract present is 1.44µg. Figure 2 and figure 3 show the densitogram of test and quercetin respectively.

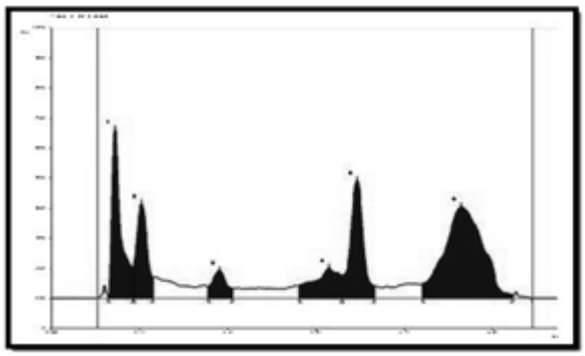

Fig. 2: Densitogram of test

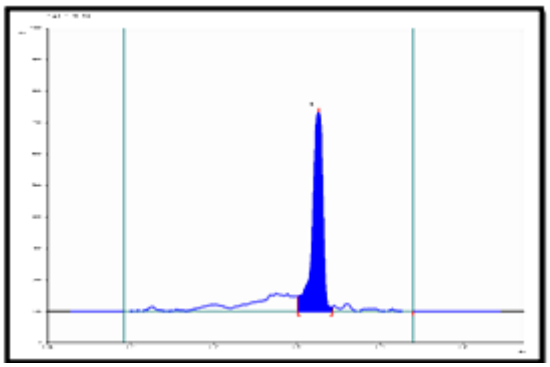

Fig. 3: Densitogram of quercetin

The results are represented data above in Table 2.

Table 2: HPTLC finger printing data showing number of peaks formed by standard quercetin and ethanol extract.

Sample	Solvent system	Volume	Number of peaks
Quercetin	Toluene: ethyl acetate: formic acid	5µL	1
Ethanol extract	Toluene: ethyl acetate: formic acid	5µL	6

The amount of quercetin present in the sample was found to be 0.001mg/ml as compared with standard quercetin. The results are given in table 3.

Table 3: Amount of sample extract taken from the specified concentration

Concentration of extract	Weight of extract taken	Amount taken
0.7230 µg	50 µg	1.44 µg

Table 3 shows the amount of extract taken from the given concentration of extract. It represents a chorded data by which the specific amount is used for further investigation. Total phenolics were determined by using Folin-Ciocalteu's reagent (FCR) with slight modifications was found to be 42% in ethanol extract and 36% in chloroform extract. 1mg/ml of ethanol extract of *Cardiospermum helicacabum* contains 480µg of Phenolic compounds. That is 42% equivalent to Gallic acid standard. 1mg/ml of Chloroform extract of *Cardiospermum helicacabum* contain 360µg of phenolic compounds. That is 36% equivalent to Gallic acid standard. The plant extracts were screened for *in vitro* anti cancer activity. There are several methods being used for the purpose. Among all the methods, MTT is found to be simpler and advantageous. So the plant extracts were evaluated for cytotoxicity using MTT assay [12, 13]. The data obtained signifies that, *C.halicacabum* is a potent material for treatment of cancer. IC_{50} values indicate that when compared to standard Doxorubicin, the ethanolic extract was more potent yielding a greater value, signifying its ability to inhibit fifty percent of the response, in a better mode alongside Doxorubicin. Table 4 and Table 5 shows, *in vitro* results for anti cancer activity specifying IC_{50} values comparing with Doxorubicin.

Table 4: *In vitro* anticancer activity of ethanolic extract of C.halicacabum by MTT assay method

S. No.	Name of extract	Concentration (mg/ml)	Absorbance	% inhibition	IC_{50} (mg/ml)
1.	Ethanolic Extract	5	0.10	81.13	1.90
		2.5	0.19	64.15	
		1.25	0.24	54.71	
		0.625	0.27	49.05	
		0.3125	0.34	35.84	
		0.15625	0.39	26.41	
		0.078125	0.46	13.20	
		0.039	0.49	7.54	

Table 5: *In vitro* anticancer activity of Doxorubicin standard by MTT assay method

S. No.	Name of extract	Concentration (mg/ml)	Absorbance	% inhibition	IC_{50} (mg/ml)
1.	Doxorubicin	5	0.08	85.45	1.503
		2.5	0.14	74.54	
		1.25	0.22	60.00	
		0.625	0.26	52.72	
		0.3125	0.33	40	
		0.15625	0.38	30.90	
		0.078125	0.45	18.18	
		0.039	0.49	10.90	

CONCLUSION

Since cancer is recognized as one of the most dangerous disease, the research focus was put on it. This effort led us to the findings of *in vitro* anti cancer activity of extracts of *Cardiospermum helicacabum*. This will be helpful in developing Pharmacopoeia standards for global acceptance. Finally, it is to be said that herbal medicine has been used up to 80% of the population in developing countries. Now

it is the right time to start consuming safe herbal standards for benefit of the recipient so that the context "Nature preserves human life" will be justified.

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