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Pharmacological And Phytochemical Study of *Cardiospermum Helicacabum Linn* Medicinal Plant

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ABSTRACT

The pharmacological and phytochemical studies on cardiospermum helicacabum linn medicinal plant have been carriedout by employing standard methods. The present research investigation is significantly focused on In vitro and In vivo anticancer activity of ethanolic and aqueous extracts of medicinal plant roots. From the present research study, it is clearly evident that the ethanolic extract and aqueous extracts of roots of medicinal plant exhibited the anti cancer activity. The phytochemical and anticancer activity of cardiospermum helicacabum roots has been evaluated on mice with novel approach.

Keywords: Cardiospermum helicacabum Linn, exracts, Phytochemical, Anticancer.

INTRODUCTION

Cardiospermum helicacabum Linn is belonging to family sapindeceae, commonly known as a heart seed it is a evergreen tree , which is widely distributed in India, Bangladesh, China, Srilanka, different part of the plant have been recommended in Ayuredic literature as a remedy for various ailments. Cardiospermum helicacabum Linn a root have described as a useful remedy for rheumatism, lumbago skeletal fractures, nervous diseases amenorrhea, and hemorrhoid, erysipelas the herb is used in hair oil for treating dandruff alopecia and for darkening hair. Cardiospermum helicacabum Linn root have been used analgesic, and anti-inflammatory and Cancer, It is used for the treatment of skeletal fractures in Srilanka; the juice of the herb is used to cure ear-ache and to reduce hard denned tumors'.Flavonoids are a group of polyphenolic compounds which are distributed throughout the plant kingdom. To date about 3000 varieties of flavonoids are known [1]. Flavonoids exhibit several biological effects such as anti-inflammatory, antihepatotoxic and anti-ulcer action [2,3]. The stem bark is reported to possess antitumor, antiulcer, antifungal and antidiarrhoeal activities [4-6]. Plants are theonly economic source of a numberof well-established and important drugs. [7]. The history of herbal medicine is as old as human civilization. [8]. The juice of the herb is used to cure ear-ache and to reduce hardened tumours, which is transient in nature. In vitro studies have revealed its antispasmodic and curative action confirming the use of the herb in Ayurvedic medicine [9]. Cardiospermum halicacabum linn.(Sapindaceae) is an herbaceous climber [10]. Commonly used in the treatment of rheumatism, lumbago, earache, fever [11&12]. Reports are available on analgesic, anti-

inflammatory and vasodepressant activities [13-15]. It exhibits significant analgesic anti-inflammatory and depressant activity. This is transient in nature in vitro studies revealed its Cancer.

MATERIALS AND METHODS

Materials used for the phytochemical investigation: Chloroform, methanol, ethanol. Petroleum ether, acetone, benzene and chloroform were procured from Ranbaxy Fine Chemicals Ltd., Mumbai, India.

Plant Material: The roots of *Cardiospermum helicacabum* was collected during January 27 from Tirupati Andhra Pradesh state, India. The samples were authenticated by Dr. Madhava chetty, Assistant Professor of Botany, S.V. University College, Tirupati, India.

Preparation of Plant Extracts

Petroleum ether extract: The coarsely powdered shade dried roots of *Cardiospermum helicacabum* (50g) was extracted with ether by hot extraction process (Soxhlet) for 4 h. After completion of extraction the solvent was removed by distillation and Concentrated.

Benzene extract: The marc left after the ether extraction was dried and extracted with benzene by hot extraction process (Soxhlet) for 4 h. After completion of extraction, the solvent was removed by distillation and Concentrated.

Chloroform extract*:* The marc left after benzene extraction was dried and extracted with chloroform by hot extraction process (Soxhlet) for 4 h. After completion of extraction the solvent was removed by distillation and Concentrated.

Ethanolic extract: The marc left after the acetone extraction was dried and extracted with 95% ethanol by hot extraction process (Soxhlet) for 4 h. After completion of the extraction the solvent was removed by distillation and Concentrated.

Aqueous extract (Chloroform water): The marc left after the ethanolic extraction was dried and extracted with chloroform water by hot extraction process (Soxhlet) for 4 h. After the completion of the extraction the solvent was removed by distillation and Concentrated.

RESULTS AND DISCUSSION

Preliminary phytochemical screening: Preliminary Phytochemical screening was done according to the phytochemical methods described by. Following chemical tests were carried out for different extracts of *Cardiospermum helicacabum* fruit to identify the presence of various phytochemical constituents.

Test for Carbohydrates

Molisch's test (General test): To 2-3mL. Aqueous extract, added few drops of alpha- Naphthol solution in alcohol, shake and added Conc. H_2SO_4 from sides of test tube. Violet ring is formed at the junction of two liquids.

Test for Proteins

Biuret test (General test): To 3mL test solution added 4% NaOH and few drop dos 1% $CuSO_4$ solution. Violet or pink colour appears.

Million's test (for proteins): Mix 3mL test solution with 5mL. Million's reagent. White precipitate is obtained; warm it then the precipitate turns to brick red or the precipitate dissolves giving red coloured solution.

Tests for Steroids

Liebermann – Burchard reaction: Salkowski reaction: To 2mL. of extract, added 2mL.chloroform and 2mL.Conc. H_2SO_4, shake well. Chloroform layer appears red and acid layer shows greenish yellow fluorescence. Mix 2 mL. Extract with chloroform. Add 1-2mL. acetic anhydride and 2 drops of Conc. H_2SO_4 from the sides of the test tube. First red, then blue and finally green colour appears.

Liebermann's reaction*:* Mix 3mL. Extract with 3 mL. Acetic anhydride. Heat and cool. Add few drops of Conc. H_2SO_4. Blue colour appears.

Tests for Volatile Oils: Hydro distillate material. Separate volatile oil from distillate and perform the following tests:

1. Volatile oils have characteristic odour.
2. Filter paper is not permanently stained with volatile oil.

Tests for Glycosides: Determine free sugar content of the extract. Hydrolyse the extract with mineral acid (Dil. HCl/Dil.H_2SO_4). Again determine the total sugar content of the hydrolised extract. Increase in sugar content indicates presence of glycoside in the extract.

Tests for Flavonoids:

a) Shinoda test: To dry powder or extract, added 5mL. 95% ethanol, few drops of Conc. HCl and 0.5g magnesium turnings. Pink colour is observed.

b) To small quantity of residue, add lead acetate solution. Yellow coloured precipitate is formed.

c) Addition of increasing amount of NaOH to the residue shows yellow colouration, which decolorizes after addition.

Tests for Alkaloids: Evaporate the aqueous, alcoholic and chloroform extracts separately. To residue, add dilute HCl. Shake well and filter. With filtrate, perform following tests:

a. Dragendroff's test: To 2-3mL. of filtrate, added few drops of Dragendroff's reagent. Orange brown precipitate is formed.

b. Mayer's test*:* To 2-3mL. of filtrate added few drops of Mayer's reagent which gives a precipitate.

c. Hager's test*:* To 2-3mL. of filtrate added Hager's reagent which gives a yellow precipitate.

d. Wagner's test: To 2-3mL. of filtrate added few drops of Wagner's reagent which gives reddish brown precipitate.

e. Murexide test for purine alkaloids: To 3-4mL. of test solution, added 3-4 drops of Conc. HNO_3. Evaporate to dryness. Cool and added 2 drops of NH_4OH. Purple colour is observed.

Tests for Tannins and Phenolic Compounds: To 2-3mL. of aqueous or alcoholic extract, added few drops of following reagents:

1. 5% $FeCl_3$ solution: Deep blue-black colour is observed.
2. Lead acetate solution: White precipitate is observed.
3. Gelatin solution: White precipitate is observed.
4. Acetic acid solution: Red colour solution is observed.
5. Potassium dichromate: Red precipitate is observed.

Qualitative chemical examination of various extracts of roots of *Cardiospermum helicacabum* has been tabulated **(Table 1).**

Table 1

S.No.	Test	Ethanol	Petrolem ether	Benzene	Chloro form	Ethyl acetate	Aqueous
1	Alkaloids	+	+	+	+	+	-
2	Carbohydrates	+	-	-	+	-	+
3	Phytosterols	+	-	+	-	-	-
4	Fixed oils and fats	-	-	-	+	+	+
5	Phenolic compounds and tannins	+	-	-	+	-	-
6	Proteins and amino acids	-	-	-	+	-	-
7	Volatile oil	-	-	-	-	-	-
8	Flavanoids	+	-	-	+	-	+
9	Glycosides	+	-	-	+	+	-

+ denotes the presence of the respective class of compounds.
- denotes the absence of the respective class of compounds

APPLICATIONS

Pharmacological potential of Cardiospermum helicacabum Linn family Sapindeceae

Preparation of Plant Extracts: The roots of the plant *Cardiospermum helicacabum* was shade dried and powdered. A weighed quantity of 2500g was taken for chemical investigation. The bark was dried in the shed and coarsely powdered. The powder was extracted with ethanol in a soxhlet apparatus for 72h. The ethanolic extract was evaporated in vacuo giving the residue (24%). The ethanolic extract obtained was suspended in distilled water in small amounts and was extracted successively and exhaustively with petroleum ether (60-80°C), benzene, chloroform and acetone in the order of increasing polarity. The left over fraction was considered as aqueous fraction. The extract and fractions were concentrated in a rotary evaporator at reduced pressure.

Experimental Pharmacology

In vitro cytotoxicity studies- Tryphan blue exclusion method: Tryphan blue exclusion test is based on the principle that living cell membrane has ability to prevent the entry of dye. Hence they remain unstained and can be easily distinguished from dead cells, which take the dye. This method used mainly to check whether the alcoholic extract having cytotoxicity and among the all fraction which fraction having high cytotoxicity. The ascitic carcinoma bearing mice (donor) was taken 15 days after tumor transplantation. The ascitic fluid is drawn using an 18-gauge needle into sterile syringe. A small amount tested for microbial contamination. Cells were counted using Haemocytometer. The Ascitic fluid was suitably diluted in normal saline to get a concentration of $2X10^6$ cells mL^{-1}. of tumor cell suspension. Equal volume of cell suspension was mixed with different concentrations of drugs and incubated for three hours in incubator at 27^oC. Then added equal volume of Tryphan blue were added and mixed thoroughly. The diluted suspension was charged into hemocytometer. The viable cells were (unstained) counted in WBC chamber under microscope and mean number of cells in four chambers was calculated s follows**:**
Total number of cells = Mean number of cells X Dilution factor (2) X 10^4.

***In vivo* Anticancer studies on mouse Ehrlich Ascites Carcinoma Acute toxicity studies-Selection of animals:** Albino mice of either sex weighing 20-30 g., bred in our own animal house were selected. They were individually housed in propylene cages, in well-ventilated rooms, under hygienic conditions. Animals

were given, water *ad libitum* and were fed with rat pellet feed (Hindustan Lever). Ethical committee clearance was obtained from IAE (Institutional Animal Ethics Committee) of CPCSEA (Ref. No./IAEC/XII/06/SIPS/2011-2012).

Preparation of drugs: Alcoholic, Petroleum ether and Acetone extracts of the plants were prepared in Suspension form in water by using suspending agent Corboxy methyl cellulose (CMC) and these were administered once daily in the volume of 0.1ml/10g mouse through intraperitoneal route for 9 days.

Acute toxicity studies: The acute toxicity of ethanolic extract and the aqueous extract of *Tephrosia purpurea* fruit extract was determined as per the OECD guideline no. 423 (Acute toxic class method).

Anti cancer studies- Experimental protocol
Group I- Tumor control.
Group II- Camptothecin.
Group III- Alcoholic Extract (100mg)
Group IV- Alcoholic Extract (200mg)
Group V- Aqueous extract (250mg).
Group VI. - Aqueous extract (500mg).

Selection of dose: The doses selected for the extracts were about $1/10^{th}$ of the safe dose found in acute toxicity studies. They were administered till 10^{th} day of tumor cell inoculation, once daily by intraperitoneal route.

Selection of animals: Swiss albino mice from an inbred colony maintained under controlled conditions of light (10:14 h light: dark), temperature (23 ± 3^0) and humidity were used to induce Ehrlich ascitic carcinoma. Mice were housed in sterile polypropylene cages containing sterile paddy husk as bedding material. The animals were fed on autoclaved mice feed and water. Six to eight weeks old female mice of weighing 25 ± 5 g were used.

Tumor Model: Ehrlich ascites carcinoma, procured from National cancer Institute, Mumbai, was maintained and propagated intraperitoneally by serial transplantation in adult female Swiss albino mice.

Preparation of drugs: Alcoholic extract, Petroleum ether fraction, and Acetone fraction were prepared were prepared by suspending in water by using C.M.C. These solutions were administered through intraperitoneal route.

***In vitro* cytotoxicity tests- Tryphan blue exclusion Method:** *In vitro* cytotoxic potential of different concentrations of extracts and fractions of *Cardiospermum helicacabum* was studied on EAC cell lines (**Table 2**). The results of the study showed that while the alcoholic extract at a concentration of 200 (µg/ml) produced 100 % cell death of EAC cell lines, Aqueous fraction produced 80 % cell death at a concentration of 250 (µg/ml). From this study it was concluded that the Alcoholic extract has the maximum cytotoxicity followed by the aqueous extract. Based on this data further *in vivo* studies were carried out.

Table 2.Effect of *Cardiospermum helicacabum* extracts over *in vitro* Cytotoxicity Using EAC Cell lines

Drugs	**Mean % Death after 3 hrs.**				
	50(µg/ml)	**100(µg/ml)**	**150(µg/ml)**	**200(µg/ml)**	**250(µg/ml)**
Alcoholic.ext	40	70	90	100	100
Aqueous.ext	25	35	50	75	80

***In vivo* studies**

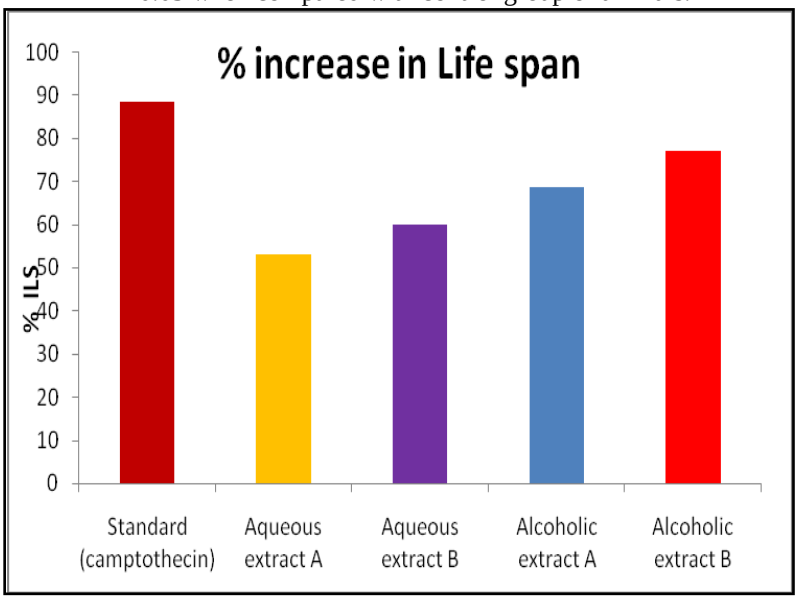
Liquid tumor model with EAC cell lines - Survival Time parameter: The effect of different extracts of *Cardiospermum helicacabum* on the survival of tumor bearing mice is shown in the **figure-1**, that the mean survival time (MST) for the control group was 17.5±0.22 days. This was significantly (P<0.05) increased by administration of Camptothecin, which showed highest % increase of life span (% ILS) of tumor bearing animals. The MST of extracts were 26.8 ± 0.33, 28 ± 0.19, 29.5 ± 0.33, 31 ± 0.16, days for Aqueous A, B, and alcoholic A, B fractions respectively.

In general if a compound shows 25% ILS, it is said to posses good antitumor activity. Thus, based on these findings, it could be said that the extracts possessed good antitumor activity **(Table-3).**

Table -3: Effect of different extracts of *Cardiospermum helicacabum* roots on survival time in EAC induced Liquid tumor model in mice.

	Dose mg/kg	**Mean survival time (days)**	**%ILS**	**% Increase in body weight**
Control		17.5 ± 0.22		20.2 ± 0.35
Standard (camptothecin)	10	33 ± 0.25	88.57	0.532 ± 0.22
Aqueous extract A	250	26.8 ± 0.33	53.14	12.6 ± 0.44
Aqueous extract B	500	28 ± 0.19	60	10.6 ± 0.25
Alcoholic extract A	100	29.5 ± 0.33	68.57	3.5 ± 0.33
Alcoholic extract B	200	31 ± 0.16	77.142	2.5 ± 0.23

Each value represents Mean ± SEM of six observations; All the values are significant at 0.05 Level. P<0.05 when compared with control group of animals.



All the values represent mean ± SEM of data obtained from 6 different animals.

Figure 1: Effect of different treatments on % Increase in Life Span (ILS) in tumor bearing mice

The phytochemical analysis of the ethanolic extract and aqueous extract showed the presence of steroids, alkaloids, carbohydrates, Phenolic compounds, glycosides, tannins and Flavonoids. Further the phytochemical analysis and chromatographic separation of benzene fraction yielded the two compounds. These two compounds have to be identified by spectral characterization. In the present research work, phytochemical and anti Cancer activity of *Cardiospermum helicacabum* roots has been evaluated on mice. *In vivo* anti cancer activity of ethanolic and aqueous extracts of roots were evaluated by using Ehrlisch ascites Carcinoma (EAC) cell lines. Acute toxicity studies were also performed initially in order to ascertain the safety of root extracts. The results showed that the ethanolic root and aqueous extracts were having significant anti cancer activity. The phytochemical investigation of the ethanolic root extract showed that the presence of Steroids, Glycosides, Flavonoids, Alkaloids, Phenolic compounds and Carbohydrates. Hence the anti Cancer activity of *Cardiospermum helicacabum* roots can be attributed to these phytoconstituents. The aqueous extract showed the presence of Flavonoids hence the anti cancer activity of aqueous extract can be attributed to the presence of Flavonoids.

CONCLUSIONS

Disease and their treatment must have also been contemporaneous with the dawn of human intellect. In our country, the science of Ayurveda had provided a system of medical treatment and most of the remedies for treating illness were taken from plants. Since disease, decay and death have always co-existed with life, the study of during the last few decades, much work has been done in the field of natural products. The development of the science of phytopharmaceuticals and the hopes for the remedies in chronic diseases has generated more new enthusiasm in research workers to develop herbal medicines.

In the present research work, phytochemical and anti Cancer activity of *Cardiospermum helicacabum* roots has been evaluated on mice. *In vitro* and *In vivo* anti cancer activity of ethanolic and aqueous extracts of roots were evaluated by using Ehrlisch ascites Carcinoma (EAC) cell lines. Acute toxicity studies were also performed initially in order to ascertain the safety of root extracts. The results showed that the ethanolic root and aqueous extracts were having significant anti cancer activity. The phytochemical investigation of the ethanolic root extract showed that the presence of Steroids, Glycosides, Flavonoids, Alkaloids, Phenolic compounds and Carbohydrates. Hence the anti Cancer activity of *Cardiospermum helicacabum* roots can be attributed to these phytoconstituents. The aqueous extract showed the presence of Flavonoids hence the anti cancer activity of aqueous extract can be attributed to the presence of Flavonoids.

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