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Original Article

IN VITRO ANTIOXIDANT AND ANTICANCER ACTIVITY OF *CARDIOSPERMUM HALICACABUM L.* AGAINST EAC CELL LINE

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ABSTRACT

Objective: To investigate the *in vitro* antioxidant and anticancer activity of chloroform and ethanol extracts of *Cardiospermum halicacabum* L. leaves.

Methods: Phytochemicals were analysed by using standard methods. *In vitro* antioxidant studies were carried out for the chloroform and ethanol extracts of the *Cardiospermum halicacabum* using various free radical models such a DPPH, Reducing power assay, nitric oxide scavenging, hydrogen peroxide (H_2O_2) scavenging, super oxide scavenging activity and ABTS. *In vitro* cytotoxic assay such as trypan blue dye exclusion and MTT assays were carried out against EAC cell line.

Results: Preliminary phytochemical analysis of the *Cardiospermum halicacabum L.* was carried out and it revealed the presence of alkaloids, coumarine, flavones, saponins, steroids, sugar, tannins and terpenoids. The results revealed that the chloroform extract has significant antioxidant potential than ethanol extract. The result revealed that the chloroform extracts of *Cardiospermum halicacabum L.* showed pronounced anticancer activity against Ehrlich Ascites Carcinoma (EAC) cell line than ethanol extract.

Conclusion: The result of the present study concluded that the chloroform extract of *Cardiospermum halicacabum L* have significant antioxidant and anticancer activity then the ethanolic extract. The potential antioxidant and anticancer activity of *Cardiospermum halicacabum L* might be due to the presence of phytochemicals.

Keywords: *Cardiospermum halicacabum L,* Ehrlich Ascites Carcinoma, Free radicals, Antioxidant.

INTRODUCTION

Cancer is a growing public problem whose estimated worldwide new incidence is about 6 million cases per year. It is the second major cause of deaths after cardiovascular diseases. Cancer is a general term applied of series of malignant diseases that may affect different parts of body. These diseases are characterized by a rapid and uncontrolled formation of abnormal cells, which may mass together to form a growth or tumor, or proliferate throughout the body, initiating abnormal growth at other sites. If the process is not arrested, it may progress until it causes the death of the organism. These cells are born due to imbalance in the body and by correcting this imbalance the cancer may be treated [1, 2]. Many things are known to increase the risk of cancer including tobacco use, dietary factors, certain infections, exposure to radiation, lack of physical activity, obesity and environmental pollutants. These factors can directly damage genes or combine with existing genetic faults within cells to cause cancerous mutations. Approximately 5–10% of cancers can be traced directly due to inherited genetic defects [3]. Chemicals such as tobacco smoke contain over fifty known carcinogens, including nitrosamines and polycyclic aromatic hydrocarbons. Alcohol is also a carcinogen [4]. Excessive free radicals produced during cellular metabolism can attack the deoxyribose DNA backbone and bases, which leads to cytotoxicity or mutation and finally it causes cancer. Free radicals also a factor which causing all types of cancer [5].

There is a necessity for research to search of new compounds with cytotoxic activity, as the treatment of cancer. The available anticancer drug is often unsatisfactory due to the problem, which cause cytotoxicity to the normal cells along with cancer cells. Plants are considered as the valuable sources of bioactive compounds with antioxidant activity, which produce certain substances that have effects on living animal cells. Some extracts rich in apigenin (found in parsley, bell pepper, etc.) causes cancer cells to undergo apoptosis (programmed cell death) in *in vitro* studies [6]. The aim of the present study is to evaluate the *in vitro* antioxidant and antitumor activity of *Cardiospermum halicacabum L.*

In recent years, there has been considerable emphasis on the identification of plant products with antioxidant property, as free radicals are considered to play a major role in most of the diseases including cancer. The medicinal value of the chosen plant *Cardiospermum halicacabum L* bark has been extensively worked out. However, its therapeutic efficacy in antioxidant and antitumor activity has not been evaluated. *Cardiospermum halicacabum L.* is the member of the family *Sapindaceae.* The major chemical constituents of *C. halicacabum* reported to contains (+)-pinitol, β-sitosterol, β-sitosterol-β-D-galactoside, apigenin-7-O-glucuronide, arachidic acid, chrysoeriol-7-O-glucuronide, linoleic acid, lutrolin-7-O-glucudonide, stearic acid [7]. *Cardiospermumhalicacabum L.* was used to treat various diseases such as skin diseases (rashes, itching, skin irritation, etc.), dandruff, rheumatoid arthritis, gastrointestinal diseases, respiratory tract diseases, urogenital diseases, etc.[8].

MATERIALS AND METHODS

Plant Collection

Plant source selected for the present study was *Cardiospermum halicacabum L.* The leaves of the selected plant were collected from in and around Thanjavur. The collected leaves were washed with tap water and shade dried under room temperature and grounded to fine powder using blender. The powder was preserved in an air tight bottle for further studies.

Preparation of plant extracts

Preparation of Chloroform Extract

250g of coarse powder of *Cardiospermum halicacabum L.* was soaked in chloroform for 48 hours. Filtered the solution and the filtrate was evaporated to the dryness. The residue was dissolved in chloroform and used for study [9].

Preparation of Ethanol Extract

250g of dried plant material was soaked in ethanol for 48 hours. Filtered the solution and the filtrate was evaporated to the dryness.

The residue was dissolved in ethanol and used for study [10]. Preliminary phytochemical screening of drug powder and various extracts were carried out as per the standard textual procedure [11].

***In vitro* Antioxidant activity**

Antioxidant activity measured by using DPPH radical scavenging assay method [12], Reducing Power assay [13], Hydrogen peroxide radical scavenging activity [14], Nitric oxide scavenging activity[15], Superoxide radical scavenging activity [16] and ABTS Radical scavenging activity [17]. Tests were carried out in triplicate for 3–5 separate experiments. The amount of extract needed to inhibit free radicals concentration by 50%, IC_{50}, was graphically estimated using a nonlinear regression algorithm.

Anticancer screening

Experimental animals [18]

Healthy adult Swiss Albino mice of both sexes, weighing 25-35g were obtained from Tamil Nadu Veterinary and Animal Sciences University, Chennai. The animals were allowed to acclimatize under laboratory conditions for a period of 5 days prior to the experiment. Animals were housed in standard polypropylene cages. Six animals were housed per cage, so as to provide them with sufficient space and to avoid unnecessary morbidity and mortality. Animals were maintained under standard condition of 12:12- hour's light/ dark cycle and at an ambient temperature at 23 ± 2°C, with 65 ± 5 % humidity. Animals were fed with standard rat chow pellet obtained from Sai Durga Foods and Feeds, Bangalore, India. All studies were conducted according to the ethical guidelines of CPCSEA after obtaining necessary clearance from the committee (Approval No: 790/03/ac/CPCSEA).

Maintenance of EAC Cells [19]

Ehrlich Ascites Carcinoma cell line was obtained from Amla Cancer Research Centre, Thrissur and was maintained by weekly intra-peritoneal inoculation of $1X10^6$cells/mouse.

***In Vitro* Cytotoxicity assays**

Trypan Blue [20]

Short- term *in-vitro* cytotoxicity was assessed using Ehrlich Ascites Carcinoma cell line by incubating different concentrations of chloroform and ethanol extracts of *Cardiospermum halicacabum L.* at 37°C for 3 hours. The tumor cells were aspirated from the peritoneal cavity of tumor bearing mice using an insulin syringe and transferred to a test tube containing isotonic saline. The cells were then washed in normal saline and the cell number was determined using a haemocytometer and adjusted to$1x10^6$ cells/ml. For the cytotoxity assay, different concentrations of the extract (50-1000 µg/ml) were added to each tube and the final volume was adjusted to 1ml with saline. Control tubes were maintained with the saline and tumor cells, but without the plant extract. All the tubes were incubated at 37ºC for 3 hours. After incubation 0.1ml of 0.2% tryphan blue dye in isotonic saline was added to a watch glass along with 0.1ml of test sample and the number of viable (unstained) and non-viable (stained) cells were counted using haemocytometer.

MTT Assay [21]

Increasing concentrations of chloroform and ethanol extracts of *Cardiospermum halicacabum L.* were added to the cells and incubated at 37ºC for 24 hrs in CO_2 incubator with 5% CO_2. The media was replaced with a fresh growth medium along with 20 µl of 3(-4, 5-dimethyl thiazol-2-yl) 2, 5 diphenyl tetrazolium bromide (MTT, sigma). Again it was incubated for 4 hrs at 37ºC. After incubation purple precipitate was clearly visible under the inverted microscope then the growth medium was removed and 200µl of 0.1% 0.1N acidic isopropyl alcohol was added to the cells to dissolve the formazon crystals. Then the covered plates were kept in the dark at 18-24ºC per overnight. The samples were then drawn every 2 hours and observed the reading at 570nm. If the reading was low the plates were returned for incubation. Each experiment was conducted in triplicate. The average was calculated and compared with the control test samples. The percentage growth inhibition was calculated using the following formula.

RESULTS AND DISCUSSION

Cancer results from a series of molecular events that fundamentally alter the normal properties of cells. In cancer cells the normal control systems that prevent cell overgrowth and the invasion of other tissues are disabled [22].Current cancer chemotherapy can damage or kill the rapid dividing healthy cells and causes serious side effects such as neutropesia, anemia, etc. In addition, the cost of chemotherapy drug is high. Natural compounds may reduce these problems. Currently, a few plant products are being used to treat cancer effectively [23].

In the present study attempt was made to develop a novel anticancer drug from a common plant source. From the literature, we found that plant belonging to the family *Sapindaceae* is well documented for their anticancer potential[24].Hence in the present work a commonly available plant belonging to the family *Sapindaceae*a nd botanically equated as *Cardiospermumhalicacabum L.* was selected. *Cardiospermumhalicacabum L.* was well known for its medicinal values. The preliminary phytochemical screening of various extracts of the *Cardiospermumhalicacabum L.* was analyzed. Chloroform extract contains alkaloids, coumarin, flavones, quinones, saponins, steroids and tannins. Ethanol extract showed the presence of alkaloids, coumarin, flavones, quinones, saponin, terpenoids, steroids and sugar.

The chloroform and ethanol extracts of the leaves of selected plant material was screened for its antioxidant potential using various free radical models such a DPPH, Reducing power assay, NO, H_2O_2, superoxide radical sacvenging and ABTS and anticancer activity against EAC cell line.

DPPH is a relatively stable free radical and the assay determines the stability of chloroform and ethanol extracts of *Cardiospermumhalicacabum L.*to reduce DPPH radical to the corresponding hydrazine by converting the unpaired electrons to paired ones. Antioxidants can act by converting the unpaired electrons to paired ones. Chloroform and ethanol extracts of plant was allowed to react with stable DPPH radicals is determined by decrease in its absorbance at 517nm, which is induced by an antioxidant present in the extracts after 10 minutes. The concentration of antioxidant needed to decrease the initial DPPH concentration by 50% is known as IC_{50} value. The IC_{50} value of the extracts was found to be 214µg/ml for chloroform and 295µg/ml for ethanol extract. The scavenging of DPPH radical by the extracts was compared with standard ascorbic acid (Table 1).

DPPH radical scavenging activity of methanol extract of leaves of the plant *Adiantum philippense* L. was found to increase with increasing concentration of the extract. This assay was based on the ability of1,1-diphenyl-2-picryl-hydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants, which denotes the plant has antioxidant potential [25].

Thymelaea hirstuta has been shown that the scavenging effect of DPPH sharply increased with increase in concentration and the results are compared with standard ascorbic acid. The antioxidant potential of *T. hirstuta* was determined by the effective scavenging of DPPH by the plant extracts [26]. DPPH has been used to evaluate the antioxidative activity of natural products in organic solvents. Hydrogen-donating abilityof the antioxidant is responsible for its free radical-scavenging activity. The DPPH method is based on the reduction of DPPH solution to DPPH-H in the presence of a hydrogen-donating antioxidant. The aqueous extract of *Ricinus communis* L. leaves and the isolated compounds showed a concentration dependent antiradical activity. The aqueous extract of the leaves was found to possess radical-scavenging activity. Ascorbic acid was used as positive control [27].

Fe (III) reduction is often used as an indicator of electron donating activity, which is an important mechanism of antioxidant activity. In the reducing power assay, the presence of antioxidant in the test drug converts the Fe^{3+} to Fe^{2+} by donating electron. Amount of Fe^{2+} complex formed can be measured at 700nm. Increasing absorbance indicated an increase in reductive ability. Table 2 shows the reducing power of the extracts also increased with increase in their

concentrations. The IC_{50} values for the chloroform and ethanol extracts are 325µg/ml and 400µg/ml respectively. Reducing power is one of the mechanisms for the possible antioxidant activity and may serve as significant indicator of potential antioxidants. Ethyl acetate fraction of *Hybanthus enneaspermus* showed good concentration dependent manner with a perfect reducing power, thus indicating a good electron donors and ability to terminate radical scavenging reaction [28]. The methanol extracts of seeds of the *Citrullus colocynthis* has a significant reducing power, which indicates the antioxidant potential of the plant. It reduces the Fe^{3+} to Fe^{2+} by donating an electron. The antioxidant potential increases with increase in the concentration of the extract. The results are compared with standard ascorbic acid [29].

Hydrogen peroxide scavenging activities of the extracts are given in Table 9 and are compared with the standard ascorbic acid. The chloroform and ethanolic extracts of *Cardiospermum halicacabum* shows their activities at different concentration. H_2O_2 is highly important because of its ability to penetrate biological membranes. H_2O_2 is not very reactive and it is a weak oxidizing agent, but it can sometimes be toxic to cell because it may give rise to hydroxyl radical in the cells. It can cross cell membranes rapidly, once inside the cell, H_2O_2 can probably react with Fe^{2+} and possibly Cu^{2+} ions to form hydroxyl radical and this may be the origin of many of its toxic effects. It can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups [30].

Table 1: DPPH radical scavenging assay of chloroform and ethanolic extracts of *Cardiospermum halicacabum L.* leaf

S. No.	Concentrations (µg/ml)	DPPH radical Scavenging activity (%)		
		Standard (Ascorbic acid)	Chloroform extract	Ethanol extract
1.	50	25.22±1.76	10.70±0.74	20.00±1.4
2.	100	30.14±2.10	25.20±1.76	28.50±1.99
3.	150	36.22±2.53	35.00±2.45	35.48±2.48
4.	200	44.06±3.08	42.80±2.99	43.24±3.02
5.	250	55.40±3.87	53.50±3.74	54.20±3.79

IC_{50} value of chloroform extract = 214 µg/ml, IC_{50} value of ethanol extract = 295 µg/ml

Table 2: Reducing power assay of chloroform and ethanolic extracts of *Cardiospermum halicacabum L.* leaf

S. No.	Concentrations (µg/ml)	Reducing power (%)		
		Standard (Ascorbic acid)	Chloroform extract	Ethanol extract
1.	100	22.04±1.54	20.00±1.4	20.00±1.4
2.	250	45.26±3.16	44.50±3.11	42.80±2.99
3.	500	65.98±4.61	62.60±4.38	55.50±3.88
4.	750	78.10±5.46	75.00±5.25	60.11±4.20
5.	1000	88.96±6.22	84.00±5.88	66.65±4.66

IC_{50} value of chloroform extract = 325 µg/ml, IC_{50} value of ethanol extract = 400 µg/ml

At minimal concentration (200µg/ml) of the chloroform and ethanol extracts showed 18.18% and 16.66% H_2O_2 scavenging activity, respectively. At maximal concentration the chloroform and ethanol extracts showed potent H_2O_2 scavenging activity 77.27% and 70.00% respectively. The IC_{50} values for the chloroform and ethanol extracts for scavenging of H_2O_2 were 510µg/ml and 580µg/ml (Table 3).

Leaf extract of *Lantana camara* showed good hydroxyl radical scavenging activities (45%-73%)at a concentration of 0.2-0.8 mg/ml in the reaction mixture. Leaf extract showing hydroxyl radical-scavenging activity was increased with increasing concentration of the extract sample. Leaf fraction of *L. camara* had higher activity but lower than that of ascorbic acid [31]. Hydrogen peroxide itself is not very harmful, but sometimes it may harmful to the cell due to the fact that it may give rise to hydroxyl radical present in the cells. Therefore, eradication of $H_2O_2$is the superior step for antioxidant defense in cell or food system. The various extracts of the *Hybanthus enneaspermus*(Linn.) plant have significant antioxidant potential and it scavenge H_2O_2 potentially [28]. In the present study, the extracts compete with oxygen to react with nitric acid and thus inhibit the generation of the anions. In the experiment, the NO was generated from sodium nitro prusside in aqueous solution at physiological pH was incubated with different concentration of plant extracts (100 - 1000µg/ml).

NO produced from sodium nitro prusside react with O_2 to formed nitrite which reacts with griess reagent forms chromophore which was read at 546 nm [32].

The decrease in the OD of test drug incubation directly correlated with inhibition nitric oxide production. From Table 4, the percentage of inhibition of nitrite by the extract of the plant drug was found to be dose dependent one, the IC_{50} values for the chloroform and ethanolic extracts was 475µg/ml and 450µg/ml respectively. The results were compared with standard ascorbic acid. This may be due to the presence of antioxidant principle present in the extracts, which inhibits the binding of oxygen with nitric oxide. Nitric oxide (NO) is an important chemical mediator generated by endothelial cells, macrophages, neurons, etc. and involved in the regulation of various physiological processes. Excess concentration of NO is associated with several diseases. Oxygen reacts with the excess nitric oxide to generate nitrite and peroxy nitrite anions, which act as free radicals[33]. The aqueous extract of *Lantana camara* leaves and the isolated compounds showed a concentration dependent antiradical activity. The aqueous extract of the leaves was found to possess NO radical-scavenging activity. The antioxidant activity of *L. camara*was increased with increase in plant extract concentration. *L. camara*act in a dose dependent manner. Ascorbic acid was used as positivecontrol[31].

Table 3: H_2O_2 radical scavenging assay chloroform and ethanolic extracts of *Cardiospermum halicacabum L.* leaf

S. No.	Concentrations (µg/ml)	H_2O_2 radical Scavenging activity (%)		
		Standard (Ascorbic acid)	Chloroform extract	Ethanol extract
1.	200	20.38±1.42	18.18±1.27	16.66±1.16
2.	400	39.32±2.75	36.36±2.54	33.33±2.33
3.	600	63.34±4.43	59.09±4.13	53.35±3.73
4.	800	75.30±5.27	72.72±5.09	63.31±4.43
5.	1000	78.42±5.48	77.27±5.40	70.00±4.90

IC_{50} value of chloroform extract = 510 µg/ml, IC_{50} value of ethanol extract = 580 µg/ml

Table 4: Nitric oxide scavenging assay chloroform and ethanolic extracts of *Cardiospermum halicacabum L.* leaf

S. No.	Concentrations (µg/ml)	Inhibition of NO production (%)		
		Standard (Ascorbic acid)	Chloroform extract	Ethanol extract
1.	100	38.32±2.68	36.33±2.54	15.70±1.09
2.	250	43.00±3.01	40.00±2.8	26.30±1.84
3.	500	55.16±3.86	53.32±3.73	52.68±3.68
4.	750	70.12±4.90	66.61±4.66	68.41±4.78
5.	1000	86.86±6.08	80.02±5.60	84.22±5.89

IC_{50} value of chloroform extract = 450 µg/ml, IC_{50} value of ethanol extract = 475 µg/ml

Superoxide radical is known to be very harmful tocellular components as a precursor of more reactive oxidative species, such as singlet oxygen and hydroxyl radicals. Furthermore, superoxide radical is considered to play an important role in the peroxidation of lipids [28]. Therefore, studying the scavenging effects of *Cardiospermum halicacabum L.* on superoxide radicals is one of the most important waysof clarifying the mechanism of antioxidant activity. The inhibition of O^{2-} was found to be concentration dependentand the percentage of inhibition was increased with the increased in concentration. The IC_{50}value of the chloroform and ethanolic extracts was found to be 380µg/ml and 460µg/ml, respectively (Table 5). These results indicated that the *Cardiospermum halicacabum L.* had a notable effect in scavenging superoxide radicals. Inhibition of superoxide generation by *Cardiospermum halicacabum L.* may be due to the presence of phytochemicals such as flavonoids, alkaloids and phenol.

Superoxide anion plays an important role in plant tissues and it involved in the formation of other cell-damaging free radicals. The methanol extract of *Dimocar puslongan*Lour.has potent antioxidant capability was detected by the scavenging potential of the superoxide anion. The plant has high phenolics. It is known that the hydroxyl group of the phenolics contributes to superoxide anion scavenging activity by their electron donation [22]. The chloroform extract of the *Ricinus communis* L. has a potent superoxide anion scavenging ability which confirmed that the plant has antioxidant property. Naturally medicinal plants have the antioxidant potential. The superoxide anion scavenging potential of the plant increased with increase in the plant extract concentration. The antioxidant potential of the *Ricinus communis* L. to scavenge superoxide anion may due to the presence of alkaloids and flavonoids[27].

The ABTS assay is based on the inhibition of the absorbance of radical cation ($ABTS^{+}$), which has a characteristic wavelength at 734nm by antioxidants. The principle behind the technique involves the reaction between ABTS and potassium per sulphate to produce the ABTS radical cation ($ABTS^{+}$) which is a blue green chromogen. In the presence of antioxidant reductant, the coloured radical is converted back to colourless ABTS. The order of ABTS radical scavenging activity of the extract was almost similar to that observed for DPPH. The IC_{50}value of the chloroform and ethanol extracts was found to be 530µg/ml and 570µg/ml respectively (Table 6). The antioxidant potential of the aqueous extract of the *Thymelaea hirsuta L.* was determined by using the ABTS assay and it has showed a potent antioxidant property. The ABTS radical was significantly scavenged by the plant extract. ABTS react with potassium per sulphate to produce $ABTS^{+}$,which was scavenged by the plant extract [26].

Table 5: Superoxide radical scavenging assay of chloroform and ethanolic extracts of *Cardiospermum halicacabum L.* leaf

S. No.	Concentrations (µg/ml)	Superoxide radical Scavenging activity (%)		
		Standard (Ascorbic acid)	Chloroform extract	Ethanol extract
1.	100	12.18±0.85	10.00±0.7	6.25±0.43
2.	200	19.22±1.34	16.66±1.16	15.62±1.09
3.	300	35.32±2.47	33.33±2.33	18.75±1.31
4.	400	55.34±3.87	53.33±3.73	40.00±2.8
5.	500	72.24±5.05	70.00±4.90	56.25±3.93

IC_{50} value of chloroform extract = 380 µg/ml, IC_{50} value of ethanol extract = 460 µg/ml

Table 6: ABTS radical scavenging activity of chloroform and ethanolic extracts of *Cardiospermum halicacabum L.* leaf

S. No.	Concentrations (µg/ml)	ABTS radical Scavenging activity (%)		
		Standard (Ascorbic acid)	Chloroform extract	Ethanol extract
1.	100	10.38±0.72	7.14±0.49	6.89±0.48
2.	200	22.46±1.57	10.71±0.74	20.83±1.45
3.	300	27.46±1.92	25.00±1.75	24.14±168
4.	400	39.66±2.77	32.14±2.24	37.93±2.65
5.	500	48.28±3.37	46.42±3.24	44.82±3.13
6.	600	60.20±4.21	57.14±3.99	51.72±3.62

IC_{50} value of chloroform extract = 530 µg/ml, IC_{50} value of ethanol extract = 570 µg/ml

The ABTS cation is scavenged by the plant extract of *Dimocarpus longan* Lour.has significant antioxidant potential. The ABTS assay principle resembles the DPPH assay. The mode of action of both assay are similar. The antioxidant potential increases with increase in concentration of the plant extract [22]. ABTS has been used to evaluate the antioxidative activity ofnatural products in organic solvents. Hydrogen-donating abilityof the antioxidant is responsible for its free radical-scavenging activity. The ABTS method is based on the reduction of ABTS in the presence of a hydrogen-donating antioxidant. The aqueous extract of *Ricinus communis*L. leaves and the isolated compounds showed a concentration dependent antiradical activity. The aqueous extract of the leaves was found to possess radical-scavenging activity. Ascorbic acid was used as positivecontrol

[27]. The Ehrlich tumor was initially described as a spontaneous murine mammary adenocarcinoma. It is a rapidly growing carcinoma with very aggressive behavior and is able to grow in almost all strains of mice [34]. Hence EAC cell is used in the present study to screen the anticancer potential of the selected plant drug. The chloroform and ethanol extracts of the leaves of *Cardiospermum halicacabum L.* was tested against EAC cell line. Different concentration of plant (chloroform and ethanol) extracts was inoculated with selected cell line and the cytotoxicity was assessed using trypan blue dye exclusive method. The test based on the principle that the dead cell accepts dye and stain with blue colour. The plant drug may disturb the membrane integrity and caused the cell death, which is one of the hall marks of apoptosis. The chloroform and ethanol extracts showed 73.71% and 60.19% of cytotoxicity (1000 µg/ml) against EAC cell line (Table 7, 8). The alcoholic extract of *Premna herbacea* has showed a significant antitumor activity against MCF-7 and EAC cell line was detected by using trypan blue dye method. The plant extract encounters the cancer cells by inducing the apoptosis [35]. *In vitro* cytotoxicity study was carried out by the chloroform and ethanolic extracts of *Cardiospermum halicacabum L.* against EAC cell line employing MTT assay method. The reduction of tetrazolium salts is now widely accepted as a reliable way to examine the cell proliferation. The yellow tetrazolium MTT (3- (4, 5 dimethyl thiazolium -2) - 2, 5 diphenyltetrazolium bromide) is reduced by metabolic active cells by the action of mitochondrial dehydrogenase enzymes, to generate reducing equivalents such as NADH and NADPH. The resulting intracellular purple formazon can be solubilized and quantified by spectrophotometric method [36].

Table 7: Cytotoxic effect of chloroform extract of *Cardiospermum halicacabum L.* leaf on EAC cell line (Trypan Blue Method)

S. No.	CONCENTRATIONS (µg/ml)	VIABLE CELLS (%)	DEAD CELLS (%)
1.	Control	96.15±6.73	3.84±0.26
2.	100	85.00±5.95	15.00±1.05
3.	250	76.00±5.32	24.00±1.68
4.	500	64.00±4.48	36.00±2.52
5.	750	47.31±3.31	52.69±3.68
6.	1000	26.28±1.83	73.71±5.15

Dead cell= Stained with Trypan blue dye, Viable cell= Not stained with Trypan blue dye

Table 8: Cytotoxic effect of ethanolic extract of *Cardiospermum halicacabum L.* leaf on EAC cell line (Trypan Blue Method)

S. No.	Concentration (µg/ml)	Viable cells (%)	Dead cells (%)
1.	Control	97.00±6.79	3.00±0.21
2.	100	85.50±5.98	14.50±1.01
3.	250	75.44±5.28	24.56±1.71
4.	500	67.00±4.69	33.00±2.31
5.	750	58.31±4.08	41.69±2.91
6.	1000	39.81±2.78	60.19±4.21

Dead cell= Stained with Trypan blue dye, Viable cell= Not stained with Trypan blue dye

Table 9: Cytotoxic effect of chloroform extract of *Cardiospermum halicacabum L.* leaf against EAC cell line (MTT assay)

S. No.	Concentration (µg/ml)	OD 1	OD 2	OD 3	Average	%
1.	Control	0.377±0.02	0.414±0.028	0.304±0.021	0.365±0.025	-
2.	50	0.307±0.02	0.327±0.022	0.341±0.023	0.325±0.022	10.95±0.766
3.	75	0.283±0.01	0.276±0.019	0.259±0.018	0.272±0.019	25.47±1.78
4.	100	0.244±0.01	0.232±0.016	0.211±0.014	0.229±0.016	37.26±2.60
5.	125	0.198±0.01	0.194±0.013	0.191±0.013	0.194±0.013	46.75±3.27
6.	150	0.183±0.01	0.174±0.012	0.162±0.011	0.173±0.012	52.60±3.68
7.	200	0.125±0.008	0.136±0.009	0.125±0.008	0.127±0.008	65.11±4.55

IC_{50} value of chloroform extract = 140 µg/ml

Table 10: Cytotoxic effect of ethanolic extract of *Cardiospermum halicacabum L.* leaf against EAC cell line (MTT assay)

S. No.	CONCENTRATIONS (µg/ml)	OD 1	OD 2	OD 3	AVERAGE	%
1.	Control	0.466±0.032	0.453±0.031	0.461±0.0322	0.460±0.032	-
2.	50	0.499±0.034	0.323±0.022	0.397±0.027	0.406±0.028	11.73±0.821
3.	75	0.370±0.025	0.366±0.025	0.348±0.024	0.361±0.025	21.52±1.50
4.	100	0.295±0.020	0.311±0.021	0.318±0.022	0.308±0.021	33.04±2.312
5.	125	0.283±0.019	0.255±0.017	0.241±0.016	0.259±0.018	43.69±3.058
6.	150	0.245±0.017	0.223±0.015	0.218±0.015	0.228±0.015	50.43±3.530
7.	200	0.191±0.013	0.185±0.012	0.184±0.012	0.186±0.013	59.56±4.169

IC_{50} value of ethanol extract = 150 µg/ml

From the result, it was observed that the chloroform and ethanolic extracts of plant under study has potent cytotoxicity and maximum cytotoxicity 65.11% and 59.56%was found at the concentration of 200µg/ml and the IC_{50} values were found to be 140µg/ml and 150µg/ml respectively (Table 9 & 10). The plant drug might have disturbs the mitochondrial assembly which resulted in the increased cytotoxicity of EAC cell line. *In vitro* cytotoxicity of brucine, a natural plant alkaloid was analyzed against the EAC cell line and human cancer cell line by using MTT assay method.

The brucine shows a significant cytotoxicity against the EAC cell line and human cancer cell line. The brucine may disturbs the mitochondrial membrane and leads to cancer cell death. *In vitro* cytotoxicity of ethanolic extractof *Cordiaver benacea leaves* was analyzed against the EAC cell line by using the MTT assay method. It destructs the mitochondrial membrane and induced the apoptosis of cancer cells [37]. The results of the present study concluded that the preliminary phytochemical screening of Chloroform extract contains alkaloids, coumarin, flavones, quinones, saponins, steroids and tannins. Ethanol extract showed the presence of alkaloids, coumarin, flavones, quinones,

saponin, terpenoids, steroids and sugar. *In vitro* antioxidant studies were carried out for the chloroform and ethanol extracts of the test drug using various free radical models such a DPPH, Reducing power assay, NO, H_2O_2, SOD and ABTS. The results revealed that the chloroform extract has significant antioxidant potential than ethanol extract. *In vitro* cytotoxic assay such as trypan blue dye exclusion and MTT assays were carried out against EAC cell line. The result revealed that the chloroform extracts of *Cardiospermum halicacabum L.* showed pronounced anticancer activity against Ehrlich Ascites Carcinoma (EAC) cell line than ethanol extract.

CONFLICT OF INTERESTS

Declared None

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