Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 8, 2014

Original Article

REACTIVE OXYGEN AND NITROGEN SPECIES SCAVENGING AND ANTICANCER POTENTIAL OF *CISSUS QUADRANGULARIS* L. AGAINST EAC CELL LINE

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Received: 12 Jun 2014 Revised and Accepted: 19 Jul 2014

ABSTRACT

Objective: To investigate the *in vitro* antioxidant and anticancer activity of chloroform and ethanol extracts of *Cissus quadranqularis 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 *Cissus quadranqularis* L using various free radical models such a DPPH, Reducing power assay, Nitric oxide, Hydrogen peroxide, Superoxide scavenging and ABTS. *In vitro* cytotoxic assay such as trypan blue dye exclusion and MTT assays were carried out both ethanol and chloroform extract against EAC cell line.

Results: The preliminary phytochemical screening of Chloroform extract contains alkaloids, sugar, quinines, steroids and tannins. Ethanol extract showed the presence of alkaloids, coumarin, quinines, steroids and sugar. The result revealed that the ethanolic extracts have significant antioxidant potential than chloroform extract. The result revealed that the ethanol extracts of *Cissus quadranqularis L.* showed pronounced activity against the tested cell line.

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

Keywords: *Cissus quadranqularis L.,* Antioxidant, Phytochemicals, EAC cell line, Cancer.

INTRODUCTION

Cancer can be defined as a disease in which a group of abnormal cells grow uncontrolled by disregarding the normal rules of cell division. Normal cells are constantly subject to signals that dictate whether the cells should divide, differentiate into another cells are die. Cancer cells develop a degree of autonomy from these signals, resulting in uncontrolled growth and proliferation. If this proliferation is allowed to continue and spread, a process called metastasis. [1]. Two types of tumors exist, benign and malignant. Benign tumor is generally slow growing expansive masses that compress rather than invade surrounding tissue. As such they generally pose little threat, except when growing in a confined space like the skull, and can occurring in the large intestine, and these should be removed before malignancy develops [2].

Cancer is caused by both external factors (tobacco, chemicals, radiation and infectious organisms) and internal factors (inherited mutations, hormones, immune conditions and mutations that occur from metabolism [3]. The first FDA approved chemopreventive agent was tamoxifen, for reducing the risk of breast cancer. The serious side effects of the FDA approved chemopreventive drugs is an issue of particular concern when considering long-term administration of a drug to healthy people who may or may not develop cancer. This clearly indicates the need for agents, which are safe and efficacious in preventing cancer. Diet derived natural products will be potential candidates for this purpose [4].

Several classes of anticancer drugs have been developed and many of them are of natural origin. Natural products have been the mainstay of cancer chemotherapy for the past 30 years. However, most of the currently used anticancer drugs cause undesirable side effects due to lack of tumor specificity and multidrug resistance. Therefore the search for potent, safe and selective anticancer compounds is crucial for new drug development in cancer research. Natural products, due to their structural diversity, provide excellent templates for the construction of novel compounds [5]. Sixty percent of currently used anticancer agents are derived in one way or another from natural sources. In light of the continuing need for effective anticancer agents, and the association of fruit and vegetable consumption with reduced cancer risk, edible plants are increasingly considered as sources of anticancer drugs [6]. Keeping this in view, the present study is investigating the Free radical scavenging and anticancer potential of *Cissus quadrangularis* L. (Family: Vitaceae) stem. The plant has been documented in Ayurveda for the treatment of osteoarthritis, rheumatoid arthritis, helminthiasis, anorexia, dyspepsia, colic, flatulence, skin diseases, leprosy, hemorrhage, epilepsy, convulsion, haemoptysis, tumors, chronic ulcers, swellings. The stout fleshy quadrangular stem is traditionally used for treatment of gastritis constipation, eye diseases, piles anemia and osteoporosis [7].

MATERIALS AND METHODS

Plant Collection

Plant source selected for the present study was *Cissus quadrangularis* L. The leaves of the selected plant were collected from in and around Thirunageswaram. 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 *Cissus quadrangularis* 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 [8].

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 [9].

Preliminary Phytochemical Screening of Various Extracts

Preliminary phytochemical screening of drug powder and various extracts were carried out as per the standard textual procedure [10].

***In vitro* antioxidant activity**

Antioxidant activity measured by using DPPH radical scavenging assay method of Gyamfi *et al.*, [11], Reducing Power assay [12], Hydrogen peroxide radical scavenging activity [13], Nitric oxide scavenging activity [14], Superoxide radical scavenging activity [15] and ABTS Radical scavenging activity [16]. 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 [17]

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 SaiDurga 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 [18]

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 [19]

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 1 ml with saline. Control tubes were maintained with the saline and tumor cells, but without the plant extract. All the tubes were incubated at 37^0C for 3 hours. After incubation 0.1 ml of 0.2% tryphan blue dye in isotonic saline was added to a watch glass along with 0.1 ml of test sample and the number of viable (unstained) and non-viable (stained) cells were counted using haemocytometer.

MTT Assay [20]

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 37°C.

After incubation purple precipitate was clearly visible under the inverted microscope then the growth medium was removed and 200 µl 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 570 nm. 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

Phytochemical analysis shows the presence of many medicinally important secondary metabolite types of phytoconstituents like alkaloids,, coumarin,, flavones, saponins, triterpenes, which indicates that the plant possesses high profile values and can be used to treat various kinds of diseases. The qualitative phytochemical investigation gave valuable information about the different phytoconstituents present in the extracts, which helps the future investigators regarding the selection of the particular extract for further investigation of isolating the active principle [21].

The preliminary phytochemical screening of various extracts of the *cissus quadrangularis L.* was analyzed. Chloroform extract contains alkaloids, sugar, quinines, steroids and tannins. Ethyl acetate extract contains sugar, steroids and tannins. Ethanol extract showed the presence of alkaloids, coumarin, quinines, steroids and sugar. Water extract contains alkaloids sugar, coumarin, flavones, quinines, saponins, and tannins.

The phytochemical constituents different extracts of *Anacardium occiddentale* and *Psidium guajava* was studied. Aqueous and methanol extracts of leaf, bark, and root cashew and guava were analysed quantitatively for tannin, total polyphenol, oxalate, saponin and alkaloids. Highest concentrations of the bioactive principles were detected in ethanolic extracts of the plants except in the case of saponin where hot water extract produced the bioactive principle [22].

Phenolic compounds are considered to be the most important antioxidants of plant materials. They constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators. Antioxidant activity of phenolic compounds is based on their ability to donate hydrogen atoms to free radicals [23]. The phenolic compounds are known to act as antioxidants not only because of their ability to donate hydrogen or electrons but also because they are stable radical intermediates. Interest in phenolics is increasing in the food industry because of their ability to retard oxidative degradation of lipids, thereby improving the quality and nutritional value of foods [24]. In some plants the phenolic content and antioxidant activities do not show positive correlation [25, 26].

Flavonoids are important secondary metabolite of plant modulating lipid peroxidation involved in atherogenesis, thrombosis and carcinogenesis. It has been confirmed that pharmacological effect of flavonoids is correlating with their antioxidant activities [27].

Flavonoids as one of the most diverse and widespread group of natural compounds are probably the most important natural phenolics. Flavonoids are very important constituents of plants because of the scavenging ability conferred by their hydroxyl groups. The flavonoids may contribute directly to anti-oxidative action. It is known that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans when up to 1 g daily is consumed from a diet rich in fruits and vegetables [28]. Flavonoid compounds from plants are known to be good natural antioxidants. These compounds possess a broad spectrum of chemical and biological activities including radical scavenging properties [29]. Antioxidant activity depends upon the number and positions of hydroxyl groups, other substituents and glycosylation of flavonoid molecules [30].

The Table 1 represent that chloroform and ethanol extracts of the test drug *Cissus quadrangularis* L**.** scavenge the free radicals. In the chloroform extract at the low concentration (100µg/ml), it has 10.70% activity at where as high concentration (500µg/ml) of the extract showed the maximum activity. In the ethanol extract at the low concentration (100µg/ml), it has 8.69% activity at where as high concentration (500µg/ml) of the extract showed the maximum activity. The result was comparable to that of standard ascorbic acid. The IC_{50} value of the extracts was found to be 300µg/ml and 470 µg/ml.

DPPH assay has become quite popular in natural antioxidant studies. One of the reasons is that this method is simple and highly sensitive. DPPH is one of the few stable and commercially available organic nitrogen radicals [31]. The antioxidant effect is proportional to the disappearance of DPPH in test samples. Various methods of monitoring the amount of DPPH in the antioxidant test system have

been reported: electron spin resonance spectroscopy (ESR)/plant powders, NMR/catechins and UV spectrophotometry/polyphenols [32]. However, monitoring DPPH with a UV spectrophotometer has become the most widely and commonly used method recently because of its simplicity and accuracy. DPPH shows a strong absorption maximum at 517 nm (purple). Generally, DPPH radical scavenging activity of the plant extracts is concentration dependent and a lower IC_{50} value reflects better protective action.

The Table 2 denotes that the reducing potential of the chloroform and ethanol extract of *Cissus quadrangularis* L**.** At the low concentration (100µg/ml) which showed 20% & 20% of inhibition. At the high concentration (1000µg/ml) which showed 80.95% & 80% inhibition. The IC_{50} value of the extract was found to be 250µg/ml and 400µg/ml. FRAP assay is commonly used for the routine analysis of single antioxidant and total antioxidant activity of plant extracts [33, 34]. Antioxidative activity has been proposed to be related to its reducing power.

The FRAP assay measures directly the ability of antioxidants to reduce a ferric tripyridyltriazine complex (Fe^{3+}-TPTZ) to the ferrous complex (Fe^{2+}-TPTZ) [35]. When Fe^{3+}-TPTZ complex is reduced to Fe^{2+} form by an antioxidant under acidic conditions, an intense blue color with absorption maximum at 593 nm. Therefore, the reducing ability can be evaluated by monitoring the formation of a Fe^{2+}-TPTZ complex with a spectrophotometer [32].

Table 1 DPPH radical scavenging assay of *Cissus quadrangularis* L. stem

S. No.	Concentration (µg/ml)	DPPH radical Scavenging activity (%)		
		Standard (Ascorbic acid)	Chloroform extract	Ethanol extract
1.	100	25.22 ± 1.77	10.70 ± 0.75	8.69 ± 0.59
2.	200	30.14 ± 2.11	25.20 ± 1.76	13.04 ± 0.91
3.	300	36.22 ± 2.54	35.00 ± 2.45	15.94 ± 1.12
4.	400	44.06 ± 3.08	42.80 ±3.00	42.02 ± 2.94
5.	500	55.40 ±3.88	53.50 ± 3.75	53.62 ± 3.75

Values were expresses as Mean ± SD for triplicates, IC_{50} Value of chloroform extract =300µg/ml; IC_{50} Value of ethanol extract =470 µg/ml

Table 2: Reducing power assay of *Cissus quadrangularis* L. stem

S. No.	Concentration (µg/ml)	Reducing power ability (%)		
		Standard (Ascorbic acid)	Chloroform extract	Ethanol extract
1.	200	22.04 ± 1.54	20.00 ± 1.40	20.00 ± 1.40
2.	400	45.26 ± 3.17	50.00 ± 3.50	50.00 ± 3.50
3.	600	65.98 ± 4.62	71.42 ± 5.00	71.42 ± 5.00
4.	800	78.1 ± 5.47	76.47 ± 5.35	80.00 ± 5.60
5.	1000	88.96 ± 6.23	80.00 ± 5.6	80.95 ± 5.67

Values were expresses as Mean ± SD for triplicates, IC_{50} Value of chloroform extract =250µg/ml; IC_{50} Value of ethanol extract =400 µg/ml

The Table 3 mentioned the hydrogen peroxide scavenging activity of the chloroform and ethanol extract of *Cissus quadrangularis* L**.** stem at the Low concentration (200µg/ml) showed 20.13% & 20.15% of scavenging activity. At the high concentration (1000µg/ml) showed 49.35% & 47.15% scavenging activity. The IC_{50} value of the extracts was found to be 400µg/ml & 450µg/ml. The hydroxyl radical scavenging assay allows assessment of abilities of extracts to exert pro-oxidant action, scavenge hydroxyl radicals generated by Fenton systems, and assessment of abilities to chelate metal iron [36]. Diplock [37] has suggested that the scavenging activity for hydroxyl radicals was not due to directly scavenging but due to inhibition of hydroxyl radical generation by chelating ions such as Fe^{2+} and Cu. The Table 4 represents the inhibition of NO production of the plant drug under the study. The chloroform and ethanol extract showed inhibitory activity and it was found that the activity was increased with increase in concentration and it was compared with ascorbic acid. The IC_{50} value of the extracts was found to be 200µg/ml and 250µg/ml. Nitric oxide (NO) is observed that.

Table 3: H_2O_2 Radical scavenging activity of *Cissus quadrangularis* L. stem

S. No.	Concentration (µg/ml)	H_2O_2 radical Scavenging activity (%)		
		Standard (Ascorbic acid)	Chloroform extract	Ethanol extract
1.	200	20.38 ± 1.43	20.13 ± 1.41	20.15 ± 1.41
2.	400	39.32 ± 2.75	25.35 ± 1.77	35.35 ± 2.47
3.	600	63.34 ± 4.43	28.45 ± 1.99	40.15 ± 2.81
4.	800	75.31 ± 5.27	30.24 ± 2.12	45.24 ± 3.17
5.	1000	78.49 ± 5.49	49.35 ± 3.45	47.50 ± 3.33

Values were expresses as Mean ± SD for triplicates, IC_{50} Value of chloroform extract =400µg/ml; IC_{50} Value of ethanol extract =450 µg/ml

Table 4: Nitric oxide scavenging activity of *Cissus quadrangularis* L. stem

S. No.	Concentration (µg/ml)	Inhibition of NO production (%)		
		Standard (Ascorbic acid)	Chloroform extract	Ethanol extract
1.	200	38.32 ± 2.68	11.12 ± 0.78	37.50 ± 2.63
2.	400	43.0 ± 3.01	55.55 ± 3.89	36.63 ± 2.56
3.	600	55.16 ± 3.86	66.60 ± 4.67	63.63 ± 4.45
4.	800	70.12 ± 4.91	77.77 ± 5.44	72.70 ± 5.09
5.	1000	86.2 ± 6.03	88.80 ± 6.22	81.78 ± 5.72

Values were expresses as Mean ± SD for triplicates, IC_{50} Value of chloroform extract =200µg/ml; IC_{50} Value of ethanol extract =250 µg/ml

scavenging by the extract is concentration dependent. It is also observed that all the extracts are likely to have the nitric oxide scavenging activity. The plant/ plant products may have the property to counteract the effect of NO formation and in turn may be of considerable interest in preventing the ill effects of excessive NO generation in the human body. Further, the scavenging activity may also help to arrest the chain of reactions initiated by excess generation of NO that are detrimental to the human health. Nitric oxide is also implicated for other disorders like inflammation, cancer and other pathological conditions. NO plays a crucial role in the pathogenesis of inflammation where it is secreted as a mediator, this may explain the use of *H. rosa* extract for the treatment of inflammatory diseases [38].

Table 5: Superoxide radical scavenging activity of *Cissus quadrangularis* L. stem

S. No.	Concentration (µg/ml)	SOD radical Scavenging activity (%)		
		Standard (Ascorbic acid)	Chloroform extract	Ethanol extract
1.	200	12.18 ± 0.85	20.00 ± 1.40	12.50 ± 0.86
2.	400	19.22 ± 1.35	26.66 ± 1.87	18.75 ± 1.31
3.	600	35.32 ± 2.47	33.33 ± 2.33	33.33 ± 2.33
4.	800	55.34 ± 3.87	40.00 ± 2.80	66.66 ± 4.63
5.	1000	72.24 ± 5.06	60.00 ± 4.20	86.66 ± 6.07

Values were expresses as Mean ± SD for triplicatesIC_{50} Value of chloroform extract =450µg/ml; IC_{50} Value of ethanol extract =350 µg/ml

Table 6: ABTS Radical scavenging activity of *Cissus quadrangularis* L. stem

S. No.	Concentration (µg/ml)	ABTS radical Scavenging activity (%)		
		Standard (Ascorbic acid)	Chloroform extract	Ethanol extract
1.	200	10.38 ± 0.73	3.50 ± 0.25	10.71 ± 0.75
2.	400	22.46 ± 1.57	17.85 ± 1.25	14.28 ± 1.00
3.	600	27.46 ± 1.92	32.14 ± 2.25	28.57 ± 2.00
4.	800	39.66 ± 2.79	35.14 ± 2.46	35.71± 2.50
5.	1000	60.20 ± 4.21	39.28 ± 2.75	42.85 ± 3.00

Values were expresses as Mean ± SD for triplicates, IC_{50} Value of chloroform extract =450µg/ml; IC_{50} Value of ethanol extract =350 µg/ml

Superoxide anions play important role in the formation of ROS such as hydrogen peroxide, hydroxyl radical and singlet oxygen which induce oxidative damage in lipids, proteins and DNA [39]. Superoxide anion radical (O_2 is a weak oxidant but it gives rise to the generation of powerful and dangerous hydroxyl radicals as well as singlet oxygen, both free radicals contribute to oxidative stress [40]. In PMS-NADH-NBT system, superoxide anion derived from dissolved oxygen by PMS-NADH coupling reaction reduces NBT. Antioxidants are able to inhibit the blue NBT formation.

The decrease of absorbance at 560 nm with antioxidants thus indicates the consumption of superoxide anion radical in the reaction (Table 5). Superoxide anion plays an important role in plant tissues and is involved in the formation of other cell-damaging free radicals. All extract exhibited excellent superoxide anion scavenging activity, which can be correlated with high content of flavonoids like hibiscetin, cyanidine, cyclopropenoides in extracts. Study suggested that the flavonoids may be involved in the dismutation of super-oxide anion radical. The IC_{50} values for the chloroform and ethanol extracts were 450µg/ml and 350µg/ml respectively.

The ABTS assay is based on the inhibition of the absorbance of radical cation, ABTS+, which has a characteristic wavelength at 734 nm, by antioxidants (Table 6). The principle behind the technique involves the reaction between ABTS and potassium per sulphate to produce the ABTS radical cation (ABTS+) which is a bluegreen 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 [41].

The ABTS method is one of the most often used method for the determination of total antioxidant capacity. It is based on neutralization of radical cation formed by a single-electron oxidation of a synthetic ABTS chromophore to a strongly absorbing ABTS•+ radical according to the reaction ABTS-e- → ABTS• +. A stable ABTS radical cation, which has blue-green chromophore absorption, was produced by oxidation of ABTS with potassium persulfate prior to the addition of antioxidants. The antioxidant activity of the natural products, including carotenoids, phenolic compounds, and some plasma antioxidants, is determined by the decolorization of the ABTS, by measuring the absorbance at 734 nm [42].

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. Hence EAC cell is used in the present study to evaluate the anticancer potential malignant tumor which appeared originally as lymphocytes in a mouse. It grows in both solid and ascites forms.

The chloroform and ethanolic extract of stem of *Cissus quadrangularis* was tested against EAC cell lines. Different concentration of plant extract was inoculated with selected cell line and the cytotoxicity was assessed using trypan blue dye exclusive method and MTT method. The test based on the principle that the dead cell accepts dye and stain with blue color. The plant drug may disturb the membrane integrity and caused the cell death, which is one of the hall marks of apoptosis. The chloroform extract showed 80.60% and ethanol extract showed 85.40% of cytotoxicity (1000 µl) against EAC cell line (Table 7 and 8) [43].

In depth *in vitro* cytotoxicity study was carried out same chloroform and ethanolic extract of *Cissus quadrangularis* against EAC cell line employing MTT assay method. The reduction of tetrazolium salts is now widely accepted as a reliable way to examine cell proliferation. The yellow tetrazolium MTT (3-(4,5 dimethyl thiazolium -2)-2,5 di phenyl tetrazolium 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 intra cellular purple formazan can be solubilized and quantified by spectrophotometric method. [44].

From the result chloroform extract showed 60.65% and ethanol extract showed 56.50% of cytotoxicity (200 µl) against EAC cell line. The drug under study was revaluated for its cytotoxic potential against EAC cell lines using Trypan blue method and the data of the results obtained were encouraging (Table 9 and 10).

The extract must be having considerable membrane damage potential and might have activated the apoptotic pathway inside the cancer cells as reported by earlier workers in the same family [45].

Table 7: Cytotoxic effect of chloroform extract against EAC cell line (MTT assay)

S. No.	Concentration (µg/ml)	Viable cells (%)	Dead cells (%)
1.	Control	97.00 ± 6.79	3.00 ± 0.21
2.	100	88.32 ± 6.18	11.70 ± 0.82
3.	250	85.83 ± 6.01	14.16 ± 1.00
4.	500	30.76 ± 2.15	68.57 ± 4.80
5.	750	24.07 ± 1.68	75.90 ± 5.31
6.	1000	17.47 ± 1.22	85.40 ± 6.00

Values were expresses as Mean ± SD for triplicates, IC_{50} Value of chloroform extract =450µg/ml

Table 8: Cytotoxic effect of ethanol extract against EAC cell line (MTT ASSAY)

S. No.	Concentration (µg/ml)	% of cytotoxic
1.	Control	-
2.	50	5.92 ± 0.41
3.	75	20.24 ±1.42
4.	100	28.80 ± 2.02
5.	125	39.75 ± 2.78
6.	150	49.13 ± 3.44
7.	200	56.50 ± 4.00

Values were expresses as Mean ± SD for triplicates, IC_{50} Value of ethanol extract =450 µg/ml

Table 9: Cytotoxic effect of chloroform extract on EAC cells (Trypan blue method)

S. No.	Concentration (µg/ml)	Viable cells (%)	Dead cells (%)
1.	Control	96.15 ± 6.73	3.80 ± 0.27
2.	100	93.25 ± 6.53	6.74 ± 0.47
3.	250	84.28 ± 5.90	15.71 ± 1.10
4.	500	59.44 ± 4.16	40.50 ± 2.84
5.	750	26.97 ± 1.89	73.02 ± 5.11
6.	1000	19.34 ± 1.35	80.60 ± 5.64

Values were expresses as Mean ± SD for triplicates
Dead cell= Stained with Trypan blue dye
Viable cell= Not stained with Trypan blue dye

Table 10: Cytotoxic effect of ethanolic extract on EAC cells (Trypan Blue Method)

S. No.	Concentration (µg/ml)	Viable cells (%)	Dead cells (%)
1.	Control	97.00 ± 6.79	3.00 ± 0.21
2.	100	88.32 ± 6.18	11.70 ± 0.82
3.	250	85.83 ± 6.00	14.16 ± 1.00
4.	500	30.76 ± 2.15	68.57 ± 4.80
5.	750	24.07 ± 1.68	75.90 ± 5.31
6.	1000	17.47 ± 1.22	85.40 ± 5.98

Values were expresses as Mean ± SD for triplicates
Dead cell= Stained with Trypan blue dye
Viable cell= Not stained with Trypan blue dye

CONCLUSION

The results of the present study demonstrate that *In vitro* antioxidant of chloroform and ethanol extracts of *Cissus quadranqularis L.* using various free radical models such a DPPH, Reducing power assay, Nitric oxide, Hydrogen peroxide, Superoxide and ABTS. The result revealed that the ethanol extracts have significant antioxidant potential. *In vitro* cytotoxic assay such as trypan blue dye exclusion and MTT assays were carried out both ethanol and chloroform extract against EAC cell line. The result revealed that the ethanol extracts of *Cissus quadranqularis L.* showed pronounced activity against the tested cell line. Thus the result revealed that the ethanolic extracts of *Cissus quadranqularis L.* has significant antioxidant and anticancer activity then the chloroform extract.

CONFLICT OF INTERESTS

Declared None

ACKNOWLEDGEMENT

The authors are grateful to Dr. S. Velavan, Director, Harman Institute of Science Education and Research (www. harmanresearchcentre. com), Thanjavur, Tamil Nadu for providing necessary support.

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