



E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(4): 1521-1526
Received: 05-05-2017
Accepted: 06-06-2017

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Anticancer and Antioxidant activity of *Cissus pallida* and *Cissus vitegenia*

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Abstract

Objective: To evaluate anticancer activity of methanolic extract of *Cissus pallida* and *Cissus vitegenia* on MCF 7, HT 29, HeLa, A549 and DLA cells. Also to assess the antioxidant nature of the plant extracts in superoxide and nitrous oxide assay.

Methods: Methanolic extract of both the plants were screened against MCF 7 (Breast cancer cells), HT 29 (Colon cancer cells), HeLa (Cervical carcinoma cells), A549 (Lung adenocarcinoma cells) in *in vitro* and Daltons Ascites Lymphoma in *in vivo* model. The effect of the extracts was assessed by percentage inhibition of the cells, changes in the hematological & liver enzymatic levels and antioxidant action of superoxide dismutase and nitrous oxide.

Results: The extract produced cytotoxicity effect on dose dependent manner comparable with that of the standard drugs used. It also reduced the superoxide desmutase and nitrous oxide free radicals tested *in vitro*. Significant ($P < 0.001$) changes were observed in the haematological parameters and the liver enzymatic levels.

Conclusions: The results obtained were significant in all the cell lines studied and the extract possess antioxidant action shown by the free radical scavenging effect and the protection of cells and prevent the DNA damage were might be due to the phenolic & flavonoids present in the extract.

Keywords: *Cissus pallida*, *Cissus vitegenia*, methanolic extract, MCF 7, HT 29, HeLa, A549 and DLA cells, Superoxide scavenging, nitrous oxide reduction

1. Introduction

Cancer is a broad group of diseases involving unregulated cell growth. In cancer cells divide and grow uncontrollably, forming malignant tumours and invade near by parts of the body. The cancer may also spread to more distant parts of the body through the lymphatic system or the blood stream [1]. Chemotherapy is the treatment of cancer with one or more cytotoxic antineoplastic drugs as a part of standardized regimen. Chemotherapy may be given with a curative intent or it may aim to prolong life or to palliate symptoms. It is often used in conjunction with other cancer treatments such as radiation therapy or surgery [2]. Traditional chemotherapeutic agents act by killing cells that divide rapidly, one of the main properties of most cancer cells. Plant sources of anticancer agents are plants, the derivatives of which have been shown to be useful for the treatment or prevention of cancer in humans [3, 4].

2. Materials and Methods

2.1 Chemicals

5-Flourouracil, Tamoxifen and Doxorubicin were obtained from Dabur Pharmaceutical Limited (New Delhi, India). Trypan blue, Nitro blue tetrazolium, Griess reagent and other chemicals were obtained from HiMedia, Hyderabad, India. Petroleum ether, methanol and other solvents, chemicals were obtained from Vijaya Chemical Suppliers, Hyderabad, India.

2.2 Plant material and Extraction

The plants *Cissus pallida* stem & roots and *Cissus vitegenia* aerial parts were collected from Nallamalla forest of Kurnool district. They were identified and authenticated by Dr. Madhav Chetty, Professor, Department of Botany, S.V. University, Thirupathi, Andhra Pradesh, India. The air dried plant material was coarsely powdered and extracted using methanol by Soxhlet apparatus. The solvent free extract was used for the preliminary phytochemical studies, *in vitro* cytotoxicity, *in vivo* anticancer and *in vitro* antioxidant studies.

2.3 Preliminary phytochemical analysis

The extract was analysed for the presence of various phytochemicals by standard tests [5]. Flavonoids, alkaloids and phenolics were estimated from the plant extracts.

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2.4 Estimation of Total Phenolic content

Total phenolic content for methanolic extracts of CP and CV were determined by using Folin-Ciocalteu reagent. The color formed by extract is due to the polyphenol content and measured with UV-1601(Shimadzu) spectrophotometer at 760nm and results are expressed as gm/100g. For the estimation of total phenolic content 2ml of the sample solution (methanolic extracts (of CV and CP) was prepared, 10 ml of water and 2 ml of Folin-phenol reagent were mixed and volume of solution was made upto 25ml by adding sodium carbonate solution. Resultant solution was kept for 30 mins for incubation in dark. Then, the absorbance of solutions was measured using UV-spectrophotometer at 760nm [6].

2.5 Estimation of Total Flavonoids

Total flavonoids of methanolic extracts of samples were determined by using the method Liu *et al.* In brief the extract was diluted with 80% aqueous ethanol (0.9ml), 0.5ml of extract was added to test tube containing 0.1ml of 10% aluminium nitrate, 0.1ml 1M aqueous potassium acetate and 4.3 ml of 80% alcohol. The reaction tubes were set aside for 40 mins at room temperature. At the end, optical density of each sample was determined at 415nm using a UV-spectrophotometer. Total flavonoids content was calculated by interpolation on a standard curve established with a reference standard, quercetin. Quercetin and Folin – Ciocalteu reagent were obtained from sigma Aldrich, Germany [7].

2.6 Estimation of Total Alkaloids and Glycosides by Gravimetry

To remove the interfering impurities like tannins, oils and soluble colouring matter, purification of the extract is done by the use of immiscible solvents or by chemical methods. In this method purification is done by the former method. The crude extract is repeatedly extracted with aqueous and organic solvents. Original organic solvent extract containing the basic alkaloid is shaken with dilute acid, thus transferring the alkaloid to aqueous layer due to the formation of more polar acid salt. The aqueous layer is then made basic with ammonia and again extracted into an immiscible organic solvent as a free base. This process is repeated until the alkaloid is sufficiently pure for the assay. In the gravimetric method the total alkaloidal extract is subjected to evaporation to dryness in a tared container, the increase in the weight of the container representing the weight of total alkaloid [8].

2.7 *In vitro* antioxidant activity

2.7.1 Superoxide scavenging activity

Superoxide radical (O_2^-) was generated from the photoreduction of riboflavin and was deduced by nitro blue tetrazolium dye (NBT) reduction method. Measurement of superoxide anion scavenging activity was performed based on the method described by Winterbourne *et al* (1975) [9]. The assay mixture contained sample with 0.1ml of Nitro blue tetrazolium (1.5 mM NBT) solution, 0.2 ml of EDTA (0.1M EDTA), 0.05 ml riboflavin (0.12 mM) and 2.55 ml of phosphate buffer (0.067 M phosphate buffer). The control tubes were also set up where in DMSO was added instead of sample. The reaction mixture was illuminated for 30 min and the absorbance at 560 nm was measured against the control samples. Ascorbic acid was used as the reference compound. All the tests were performed in triplicate and the results averaged. The percentage inhibition was calculated by comparing the results of control and test samples.

2.7.2 Nitric oxide scavenging activity

Sodium nitroprusside (10 mg) in phosphate buffer saline was mixed with different volume levels of test sample (100, 120, 140, 160, 180 and 200 μ l) made 200 μ l of each dose level by dilution with methanol. Incubate the solution at room temperature for 150 minutes. The same reaction mixture without the extract but equivalent amount of methanol served as control. After the incubation period 5ml of Griess reagent was added. The absorbance was taken in UV visible spectrophotometer at 546 nm. Ascorbic acid was used as positive control. The % reduction was calculated [10].

2.8 Tumour cells and Inoculation

MCF – 7 (Human Breast Cancer cells), HT-29 (Colon cancer cells), HeLa (Human cervical cancer cells), A 549 (Human lung adenocarcinoma cells) and DLA cells were supplied by IICT, Hyderabad, India. The cells were maintained *in vivo* in Swiss albino mice by intraperitoneally transplantation. Tumour cells aspirated from peritoneal cavity of mice were washed with normal saline and used for further studies.

2.9 *In vitro* Cytotoxic activity on human cancer cell lines

Stock cells of all the cell lines were prepared according to the following procedure and used for the study. Trypsinization: Media from the flask containing cell lines was aspirated. 5-10 mL phosphate buffer saline (PBS) was used to rinse cell monolayer. Rinsing solution was aspirated. 2 mL of 0.1% trypsin EDTA was spread evenly over cell monolayer incubated for 2-5 min for trypsin action on cell. Flask was tapped gently to dislodge the cell. Cells were resuspended in 8 mL of medium containing serum to stop the action of the trypsin. Cell suspension was transferred to 15 mL centrifuge tube. Centrifugation of the cells: Tube was centrifuged at 1000 rpm for 5 min. Pellet was resuspended with 5-10 mL of media. Cell counting: Counting the viable cells was done. Plating of cells: 100 μ L cell suspensions were aseptically poured into the plate. Incubation: For 3 h in carbondioxide incubator. Drug addition: Preparation of sample solution: Compounds were dissolved in DMSO & further diluted with water so that DMSO concentration should not cross 1% of total solution. Four different concentrations were prepared (7.08125, 15.645, 31.25, 62.5, and 125 μ g/mL for MCF-7 and A-549, 31.25, 62.5, 125, 250, 500 μ g/mL for HT-29 & HeLa in DMSO). Incubation: For 24 h at 37°C in a humidified atmosphere of 5% CO₂. MTT addition: MTT was added and incubated for 24 hrs. Finally 80 μ L of lysate buffer (15% sodium dodecyl sulfate dissolved in a mixture of DMF and water in 1:1 ratio) was also added. Reading: ELISA reader- at an absorbance of 492 nm. Calculation: Percentage viability of cells was calculated from readings. Solvent DMSO treated cells acted as control % Growth inhibition=100- [Mean OD of individual Test Group/ Mean OD of control cells] \times 100 [11].

2.10 Experimental animals

Health adult male Swiss albino mice weighing (26 \pm 2) gram were obtained from National Institute of Nutrition, Tarnaka, Hyderabad, India. The animals were grouped and housed in propylene cages and maintained at standard laboratory conditions (25 \pm 2°C) with 12h dark/light cycle. The animals were fed with standard animal diet and water *ad libitum*. The experimental protocols received ethical clearance from Institutional Animal Ethical Committee (IAEC) and CPCSEA.

2.11 Acute Toxicity study

The acute toxicity studies were performed to study the acute toxic effects according to OECD guideline 423 Acute oral Class method to determine the minimum lethal dose of the drug extracts. Swiss albino mice of either sex weighing between 18-25gm were used for the study. The methanolic extracts of *C.pallida* and *C.vitegenia* were administered orally to different groups of overnight fasted mice at the dose 30, 100, 300, 1000 and 2000mg/kg body weight. After the administration of the extracts, animals were observed continuously for the first 8hrs for any toxic manifestation. Thereafter observations were made at regular intervals for 24hrs. Further the animals were under investigation upto a period of one week [12].

2.12 Anti-tumour study

Anti-tumour activity of methanolic extract of *Cissus pallida* and *Cissus vitegenia* was evaluated by the procedure of Senthil Kumar *et al.* The animals were divided into 7 groups containing 6 animals in each group. Group I served as control, group II served as DLA control (serial intraperitoneal (i.p) transplantation of 1×10^6 tumor cells (0.25ml in phosphate buffered saline, pH 7.4) per animal), group III are treated with oral administration of *C.pallida* at 200mg/kg and group IV 400mg/kg and group VI *C.vitegenia* 200 & group VII 400mg/kg body weight for 14 days and group VIII animals are treated with standard drug (5-Fluorouracil) at 20mg/kg body weight. On 15th day these animals were sacrificed after an overnight fast by decapitation. Blood was collected in conventional way and used for the estimation of Red blood cell count (RBC) and White blood cell count (WBC) [14], Haemoglobin (Hb), WBC differential counts [15]. The remaining blood was centrifuged and serum was used for the estimation of liver function marker enzymes like AST and ALT [15], ACP and LDH [16] and ALP [17].

2.13 Statistical analysis

All the values were expressed as Mean \pm SEM. The data were statistically analysed by one way ANOVA, followed by Dunnett's t-test P values <0.05 was considered as significant.

3. Results

3.1 Preliminary phytochemical analysis

The preliminary phytochemical analysis showed the presence of saponins, flavonoids, alkaloids, carbohydrates, phenols, tannins etc.

3.2 Estimation of total phenolic, flavonoid, alkaloid and alkaloidal content

Total phenol content was found to be 44.9 and 60.31 μ g/mg for *Cissus pallida* and *Cissus vitegenia* whereas the flavonoidal content was found to be 8.56 and 8.73 μ g/mg for CP and CV respectively. Standards used for phenolic estimation and flavonoidal estimation were Gallic acid and Quercetin respectively. The alkaloidal and glycoside content was found to be 0.4% w/w & 0.8 %w/w and 8.5% w/w & 15.6% w/w for *Cissus pallida* and *Cissus vitegenia* respectively. The results were shown in the Table – 1 & 2.

3.3 Antioxidant assay

The extract produced dose dependent antioxidant action in the superoxide method and the nitric oxide method. Ascorbic acid was used as standard in both the methods and the percentage inhibition and IC₅₀ values were calculated. The results were shown in the Table – 3 & 4.

3.4 In vitro cytotoxicity activity

In the *in vitro* cytotoxicity studies, the cells were treated with various concentrations of the extract (25, 50, 100 and 200 μ g/ml). The percentage cytotoxicity progressively increased in a concentration dependent manner. The IC₅₀ values were calculated for the all the cell lines studied and the results were given in Table 5, 6, 7 & 8 respectively.

3.5 Acute toxicity study

Methanolic extract treated animals didn't show any toxic symptoms or mortality at the dose of 2000mg/kg per oral. Hence, the extract was safe at the tested dose levels. Thus, 1/10th 200mg/kg and 1/5th 400mg/kg doses were selected for the *in vivo* study.

3.6 Effect of extract on haematological parameters and Liver enzymatic levels

The haematological parameters of DAL mice were found to be significantly changed from normal group. The WBC, neutrophils, eosinophils were found to be increased and with a reduction in the RBC, Hb and lymphocytes. All the values were brought to the normal levels after treated with the extract and the standard drug 5-FU. The decrease in the levels of Hb, RBC, lymphocytes and increase in the levels of WBC, neutrophils in DAL in animals were reverted to normal on the extract administration. Treatment with the extract showed the protective action on the haemopoietic system. The results were shown in Table – 9.

Liver enzymes AST, ALT, ACP, ALP and LDH showed significant increase in the serum of DLA group animals when compared to the normal group. All the enzymatic levels were restored to the normal levels in group IV, VI and VII animals. Elevation of ALT may attributed due to hepatocytes damage and also pathological changes in bile flow. The extract and the standard drug treated animals showed normal levels of the enzymatic levels by the therapeutic efficacy of the extract and the standard drug 5-FU. The results were shown in Table – 10.

Table 1: Results showing the total phenolic and flavonoidal content of the extracts

S.No	Extract	Total phenolic content(μ g/mg)	Total flavonoidal content(μ g/mg)
1	CP	44.9 \pm 0.4204	8.56 \pm 0.3535
2	CV	60.31 \pm 0.3914	8.73 \pm 0.2728

Table 2: Results showing gravimetric analysis of alkaloids in CP and CV

Phytochemical	<i>Cissus pallida</i>	<i>Cissus vitegenia</i>
Alkaloidal content	0.4% w/w	0.8% w/w
Glycosidal content	8.5% w/w	15.6% w/w

Table 3: Results showing the percentage inhibition of Superoxide free radical scavenging activity

Test substance	Dose μ g/ml	Percentage inhibition	IC ₅₀ μ g/ml
<i>Cissus pallida</i> Methanolic extract	10	19.6	15.4
	15	30.3	
	20	75.4	
<i>Cissus vitigenia</i> Methanolic extract	10	13.9	15.1
	15	48.2	
	20	66.5	
Standard Ascorbic acid	1	20.13	3.57
	2.5	37.15	
	5	64.67	

Table 4: Results showing the percentage inhibition of Nitric oxide free radical scavenging activity

Test substance	Dose $\mu\text{g/ml}$	Percentage inhibition	IC ₅₀ $\mu\text{g/ml}$
<i>Cissus pallida</i> Methanolic extract	10	28.7	15.5
	15	42.4	
	20	70.9	
<i>Cissus vitigenia</i> Methanolic extract	10	13.6	15.7
	15	27.4	
	20	53.2	
Standard Ascorbic acid	1	20.13	3.57
	2.5	37.15	
	5	78.67	

Table 5: *In vitro* Anti-cancer activity of extracts against MCF-7 cell line

Dose ($\mu\text{g/ml}$)	% inhibition		
	<i>Cissus pallida</i>	<i>Cissus vitigenia</i>	Tamoxifen
25	3.04	2.53	10.9
50	12.05	8.79	32.9
100	26.26	16.23	62.4
200	33.74	20.13	78.10
IC ₅₀	100	120	85

Table 6: *In vitro* Anti-cancer activity of extracts against A549 cell line

Dose ($\mu\text{g/ml}$)	% inhibition		
	<i>Cissus pallida</i>	<i>Cissus vitigenia</i>	Doxorubicin
25	2.17	2.70	11.29
50	4.20	4.01	22.22
100	8.96	8.14	45.49
200	17.85	16.36	88.27
IC ₅₀ ($\mu\text{g/ml}$)	110	125	112.5

Table 7: *In vitro* Anti-cancer activity of extracts against HT-29 cell line

Dose ($\mu\text{g/ml}$)	% inhibition		
	<i>Cissus pallida</i>	<i>Cissus vitigenia</i>	Doxorubicin
25	2.27	2.70	11.24
50	5.12	5.02	22.23
100	10.29	10.04	45.51
200	20.65	20.28	89.26
IC ₅₀ ($\mu\text{g/ml}$)	100	110	112.5

Table 8: *In vitro* Anti-cancer activity of extracts against HeLa cell line

Dose ($\mu\text{g/ml}$)	% inhibition		
	<i>Cissus pallida</i>	<i>Cissus vitigenia</i>	Doxorubicin
25	2.27	3.70	33.24
50	4.12	19.02	52.23
100	7.29	20.04	72.51
200	13.65	23.28	89.26
IC ₅₀	100	110	112.5

Table 9: Effect of *C. Pallida* and *C. vitigenia* Methanolic Extrcat on Haematological Parameters

Parameters	Control	DAL Control	<i>C. pallida</i> 200mg/kg	<i>C. pallida</i> 400mg/kg	<i>C. vitigenia</i> 200mg/kg	<i>C. vitigenia</i> 400mg/kg	5Fluorouracil 20mg/kg
WBC (T/mm ³)	8.61±0.02	20.26±0.14*	14.12±0.02	15.09±0.03*	13.10±0.05	14.08±0.04*	10.54±0.19*
RBC(m/mm ³)	10.13±0.19	5.45±0.43*	9.80±0.4	10.89±0.8*	8.75±0.9	9.25±0.08*	11.83±0.30*
Hemoglobin (g/dl)	13.56±0.45	7.60±0.43*	11.05±0.42	12.10±0.50*	10.11±0.40	11.08±0.06*	12.87±0.29*
Lymphocytes (%)	63.12±0.50	23.05±0.09*	31.75±0.32	32.80±0.40*	30.68±0.30	31.60±0.25*	60.40±0.43*
Neutrophils (%)	30.52±0.94	74.65±0.49*	61.40±0.66	62.45±0.68*	60.38±0.57	61.35±0.62*	35.53±1.12*
Eosionophils (%)	6.50±0.52	7.63±0.27*	6.09±0.25	7.12±0.29*	5.08±0.29	6.10±0.32*	6.23±0.14*

n=6, *P<0.05, All values are expressed in mean ± SEM analysed by Oneway ANOVA followed by Dunnett's t-test

Table 10: Effect of *C. Pallida* and *C. vitigenia* Methanolic Extrcat on Haematological Parameters

Group	AST	ALT	ACP	ALP	LDH
Control	80.07±0.24	33.05±0.30	47.10±0.23	77.60±1.19	92.95±1.02
DLA	110.02±0.13	74.08±0.20	85.30±0.42	83.59±1.60	124.20±0.75
<i>C. pallida</i> (200mg/kg)	72.10±0.34	60.09±0.65	53.20±0.18	63.18±1.45	98.23±0.55
<i>C. pallida</i> (400mg/kg)	74.15±0.39*	66.21±0.69*	54.24±0.20*	64.41±1.48*	96.28±0.57*
<i>C. vitigenia</i> (200mg/kg)	75.18±0.41	38.05±0.70	56.28±0.24	68.43±1.52	93.80±0.55
<i>C. vitigenia</i> (400mg/kg)	76.22±0.45*	40.08±0.73*	58.32±0.27*	69.48±1.58*	94.84±0.58*
5-FU (20mg/kg)	84.08±0.20*	42.10±0.75*	48.68±0.55*	73.95±1.37*	95.87±0.59*

n=6, *P<0.05, All values are expressed in mean ± SEM analysed by Oneway ANOVA followed by Dunnett's t-test

3.7 Discussion

The effect of methanolic extract of *Cissus pallida* and *Cissus vitigenia* has been studied on various cancer cell lines. The results show that the extract has potent cytotoxic effect in

both *in vitro* and *in vivo* models. Treated with the extract showed concentration dependent inhibition of the cell growth of human cancer cell lines studies. The cytotoxic effect was more pronounced upon all the cell lines analysed. The

phytochemicals present in the methanolic extract may particularly attribute to this anticancer action. Different cytotoxic effect of the extract against different types of cancer cell lines shows it can be use against various cancers might show positive results.

The most common problems presented during cancer chemotherapy major problems are being from myelosuppression and anemia. Anemia is found frequently in cancer patients. This is mainly due to reduction in RBC or haemoglobin production and this may occur either due to iron deficiency or to haemolytic or other myelopathic conditions [18]. Treatment with the methanolic extracts brought back the haemoglobin contents, RBC and WBC levels very near to the normal animals. This shows that the extract have a protective effect on the haemopoietic system.

Abnormal proliferation, a characteristic feature of cancer in inversely proportional to superoxide oxidation and the disease itself imparts an excess oxidative stress on the host. This can be viewed by the rapid elevation of SOD and NO levels and impaired antioxidant levels in the animals. SOD, NO and Catalase is considered as primary antioxidant enzymes, since they are involved in the direct elimination of the reactive oxygen species. Methanolic extract effectively reduces the oxidative stress in the DAL induced animals and restored the activities of enzymatic antioxidants.

The reactive oxygen species may induce oxidative damage of biomolecules like lipids, proteins carbohydrates and DNA [19-21] which leads to ageing, diabetes, cancer, cardiovascular diseases and neurodegenerative diseases [22-24]. Most of the phenolic compounds present in food are potent anti-oxidants [25, 26]. Natural anti-oxidants present in food materials are useful in preventing cardiovascular diseases, Parkinson's disease, cancer and Alzheimer's disease [27]. So the methanolic extracts of *Cissus pallida* and *Cissus vitegenia* are screened for their *in vitro* antioxidant activity and they showed good antioxidant potentials. The extracts were also tested for the total phenolic and flavonoidal content and were found to contain considerable amount of phenols and flavonoids.

Flavonoids are able to prevent the oxidative damage caused by free radicals. With certain mechanisms like direct ROS scavenging, anti-oxidant enzyme activation, metal chelation, alpha-tocopheryl radical reduction and oxidases inhibition and also showing pro-oxidant properties with mechanisms as direct oxidant functions, oxidation of free radicals by flavonoids phenoxyl radicals and oxidation by peroxidases.

The extracts contain considerable amounts of phenolic compounds the anti-oxidant, activity may be due phenolic content. In earlier reports its reported that, antioxidant activity of phenolic compounds are generally due to redox properties, hydrogen donor capacity and singlet oxygen quenching properties of phenolic compounds.

Herbal extracts possess antioxidant principles showed cytotoxicity towards tumour cells and anti-tumour activity in experimental animal models. Cytotoxicity actions of these antioxidants are either through induction of apoptosis or by inhibition of angiogenesis. The phytocompounds present in the extract like flavonoids, alkaloids, phenolics explains the mechanism of action for the anticancer and antioxidant property. Flavonoids have been found to possess anti-mutagenic and anti-malignant effect. Moreover, they have a Chemopreventive role in cancer through their effects on signal transduction in cell proliferation and inhibition neovascularisation. They may also prevent the angiogenesis formation and metastasis of tumour cells. They arrest cell

cycle and apoptosis. The presence of phenolics is more attributed to the antioxidant levels and they involve in the direct free radical scavenging mechanism.

Triterpenes, sterols, flavonoids alkaloids, glycosides and polyphenol are widely distributed in the *Cissus* family [28]. It has been shown to mediate a wide variety of cancer cells. Hence both the plant extracts were screened for anti-cancer potentials using *in-vitro* cell lines. In our study, we deeply believe that the cross killing occurred due to cytotoxic activity against the Colon cancer HT-29 cell lines. The results of the present study demonstrated the potent cytotoxic activity of the methanolic extracts of *Cissus pallida* and *Cissus vitegenia*. The phytochemical constituents such as flavonoids and terpenoids are the major components which are responsible for the potential cytotoxic activity. Further research also need for proving with other cancer models.

In conclusion, the methanolic extract of *Cissus pallida* and *Cissus vitegenia* was effective in free radical scavenging effect and *in vitro* and *in vivo* anticancer effect.

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