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Biological Evaluation of *Cissus vitiginea* Leaves Ethanol Extract with Anticancer Activities against MCF-7 and Vero Cell Lines

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Abstract: The present study investigated the phytoconstituents and anticancer potential followed by apoptotic studies of *Cissus vitiginea* leaves ethanol extracts on MCF-7 and Vero cells. Qualitative phytochemical analysis revealed the presence of carbohydrate, saponins, flavonoids, alkaloids, anthocyanin and betacyanin, quinones, glycosides, cardiac glycosides, terpenoids, triterpenoids, phenols, coumarins, acids, protein and steroids. On the other hand, tannins were absent in the ethanol extract. A dose-dependent anti-proliferative assay revealed that the 50% cell viability was observed at the concentration of 125 µg/ml with the ethanol extract. The maximum cell growth inhibition was observed at the concentration 1000 µg/ml (24.64 %). From the obtained data the ethanol extract were able to reduce the viability of MCF-7 cell line in direct dose dependent manner and thereafter, ethanol leaf extract was taken for further studies to assess its biocompatibility with Vero cells. Treatment of Vero cells with 24 h at different concentration of ethanol extract did not cause any changes in the Vero cells. From the results, it is clear that ethanol extract is biocompatible and can be used as a drug for treating various diseases including cancer.

Keywords: *Cissus vitiginea*, Ethanol extract, Flavonoids, MCF-7, GC-MS, Cytomorphological, Phytochemicals, Vero cells

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Introduction

Cancer is a broad group of diseases involving unregulated cell growth. In cancer, cells divide and grow uncontrollably, forming malignant tumours and invade nearby parts of the body. The cancer

may also spread to more distant parts of the body through the lymphatic system or the blood stream (Anand *et al.*, 2008). 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 (Joensuu, 2008). 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 derivatives which have been shown to be useful for the treatment or prevention of cancer in humans (Cragg and Newman, 2005; Shoeb, 2006).

Plant and plant products are being used as a source of medicine since long. According to World Health Organization (WHO) more than 80% of the world's population, mostly in poor and less developed countries depend on traditional plant-based medicines for their primary healthcare needs (Ekor, 2014). Medicinal plants are the nature's gift to human being to make disease free healthy life. It plays a vital role to preserve our health. India is one of the most medico-culturally diverse countries in the world where the medicinal plant sector is part of a time-honored tradition that is respected even today. Here, the main traditional systems of medicine include Ayurveda, Unani and Siddha (Alves and Rosa, 2007). The earliest mention of the use of plants in medicine is found in the Rigveda, which was written between 4500 and 1600 BC. During British period due to Western culture our Traditional art of natural healing disappeared. Now it is reappearing due to realization of its importance in curing diseases without any side effect. Owing to the global trend towards improved quality of life; there is considerable evidence of an increase in demand for medicinal plant (Petrovska, 2012).

Cissus vitiginea (L) is a woody foetid straggler when young and become a liana at mature stage. The aerial parts are densely covered with grey hairs (pubescent), stem swollen at the nodes and the tendrils are simple, stout and grow up to the length of 30-50 cm (Gnanasundaram and Balakrishnan, 2018). Leaves are simple, 3-5

angled, broadly cordate at the base, lobed, pubescent, margins dentate and acuminate and ranges from 2-4 inch in length. Petioles are long, stipules triangular and pubescent (Balasundram *et al.*, 2006). Stem bark is blackish to reddish. Flowers are pale yellow colored and arranged in dichotomous cymes. Calyx is cup-shaped, triangular-ovate and recurved. Stamens are 4, filaments slender and oblong anthers. Ovary two celled and each cell consists of 2 ovules (Manokari and Shekhawat, 2019). Fruits are berry, pendulous, ovoid, 1/3 inch in length, bluish black when ripe and single seeded but occasionally two seeds were reported. Flowering and fruiting observed during May-December. It prefers to grow in dry and moist deciduous forests, and also in the plains (Thakar *et al.*, 2022).

A high concentration of *Cissus vitiginea* extract plant had induced apoptosis and preoxidant and reduced secretion of IL-6 enzymes (Parimala and Selvan, 2017; Wu *et al.*, 2020; Nocedo-Mena *et al.*, 2021). We experimented to confirm the phytoconstituents and cytotoxicity of *Cissus vitiginea* leaves ethanol extract on MCF-7 and Vero cells. Aljarba *et al.* (2021) reported that isolated alkaloid and sterol compounds of different medicinal plants induced synergistic anticancer effect on young Swiss albino mice. Reactive oxygen species (ROS) produced by mitochondria not only takes part in signalling of stress in normal cells but also contributes to the initiation of nuclear or mitochondrial DNA mutations that promote neoplastic transformation. Damaging of DNA generates activation of arrest cell growth, and the p53 gene stimulates apoptosis, as per the level of the ratio of oxidative stress. Excessive production of ROS in cells leads to degeneration of mitochondrial membrane potential (Almutairi *et al.*, 2021). To the best of our knowledge, there exists no studies that have been reported on the cytotoxic nature of ethanol extract of the *Cissus vitiginea* on MCF-7 and Vero cells.

Materials and Methods

Plant material and Preparation of extract:

The leaves of the *Cissus vitiginea* were collected

from in and around the areas of Vellore, Tamil Nadu, India during the month of January 2022. The collected plants were identified and authenticated *Cissus vitiginea* by Dr. S. Soosairaj, Department of Botany, St. Joseph's College, Tiruchirapalli, Tamil Nadu, India. The leaves of plants were cut into small pieces and shade dried at room temperature for 15 days. The powdered leaves were used for the preparation of extract. The collected plant materials were washed, sliced and completely dried in a hot-air oven at 37°C. The dried materials was ground to make a fine powder and used for extraction. Three hundred grams (300 g) of the powdered leaves were extracted with ethanol (70%) using Soxhlet Apparatus for 48 h. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in a refrigerator until used. The extract contained both polar and non-polar phytochemicals.

Preliminary phytochemicals screening:

Chemical tests were carried out on the ethanolic extract using standard procedures to identify the preliminary phytochemical screening following the method of Harborne (1973).

GC-MS spectral analysis:

GC-MS spectral analysis of ethanolic extract of leaves of the *Cissus vitiginea* was done using Clarus 680 GC fused with silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250 µm df) and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min. The injector temperature was set at 260°C during the chromatographic run. 1 µl of extract sample was injected into the instrument and the oven temperature was as follows: 60 °C (2 min); followed by 300 °C at the rate of 10 °C min⁻¹; and 300 °C, where it was held for 6 min. The mass detector conditions were: transfer line temperature 240 °C; ion source temperature 240 °C; and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec. The fragments from 40 to 600 Da. The spectrums of

the components were compared with the database of spectrum of known components stored in the GC-MS NIST (2008) library.

Anticancer activity of MCF-7 and Vero Cell line:

Human Breast Cancer Cell line (MCF-7):

Human Breast Cancer Cell line (MCF-7) and Vero used for the present study was procured from National Centre for Cell Science (NCCS), Pune, India.

Preparation of Growth Medium:

Ten grams of Dulbecco's Modified Eagle's Medium (DMEM) was dissolved in 990 ml of sterilized double distilled water. To this solution, 1.5 g of sodium bicarbonate and 10.0 ml of gentamycin cocktail were added and mixed thoroughly. Later this medium was filtered using membrane filter (0.22 µm), dispensed into sterilized container and stored at 4°C. Fetal Bovine Serum (FBS) (10%) was added to this medium and used for cell culture.

Cell Line and Passage:

The cells were grown in T-75 culture flask containing DMEM supplemented with 10% FBS and the flask was placed at 37°C in humidified incubator with 5% CO₂. When the cells reached 70-80% confluent, the spent medium was discarded and the monolayer was rinsed with Phosphate Buffered Saline (PBS). Trypsin- EDTA solution was added and placed in incubator for 2 min. After incubation, 5.0 ml of growth medium was added to the flask and mixed gently. Then, it was transferred into a 15 ml falcon tube and centrifuged at 1000 rpm for 5 min. The supernatant was carefully aspirated and the pellet was gently re-suspended in 2.0 ml of growth medium. The cells were diluted with appropriate volume of growth medium and the aliquot was transferred to a new culture flask at the density of 2×10³/cm² and kept back to controlled environment for large scale production.

Standardization of Crude Extracts:

Standardization of crude extracts against MCF-7

cell line was done by MTT assay.

MTT Assay:

MTT assay method was followed to assess the viability of MCF-7 cells as described by Mosmann (1983). MCF-7 cells (1×10^4 cells/ml) were plated in 96 well plates with DMEM containing 10% FBS. The cells were incubated for 24 h under 5% CO₂ and 95% O₂ at 37°C. The medium was removed, washed with PBS and fresh serum free medium was added and kept in an incubator for 1 h. After starvation, the cells were treated with crude extracts at different concentrations such as 7.8 to 1000 µg/ml and incubated for 24 h. After incubation, 10.0 µl of 5.0 mg/ml MTT solution was added to each well and incubated for 4 h. After incubation, supernatant was aspirated and 100 µl of DMSO was added to solubilize the crystals. A microplate reader was used to measure the OD at 570 nm for each well. Percentage of cell viability was calculated as follow:

$$\text{Cell viability (\%)} = \frac{\text{OD of Test}}{\text{OD of Control}} \times 100$$

Graphs are plotted using the % of Cell Viability at Y-axis and concentration of the sample in X-axis. Cell control and sample control is included in each assay to compare the full cell viability assessments.

Cytomorphological Studies:

Cytomorphological changes of ethanol extract treated MCF-7 and Vero cell line were assessed. Cancer cells (1×10^6 cells/ml) were plated in 100 mm dishes and incubated for 24 h under controlled environment. Then, the spent medium was removed, followed by addition of fresh medium with or without methanol extract at 24 h. After incubation, the cells were visualized under inverted microscope at 10 X magnification.

Results and Discussion

The phytochemical characteristics of *Cissus vitifolia* leaves ethanol extract revealed the presence of carbohydrate, saponins, flavonoids, alkaloids, anthocyanin and betacyanin, quinones,

glycosides, cardiac glycosides, terpenoids, triterpenoids, phenols, coumarins, acids, protein and steroids. On the other hand, tannins were absent in the ethanol extract (Table 1).

Although researchers know that this trait is common in many plants, it is still difficult to determine the precise role of each secondary metabolite. Secondary metabolites are used in anti-feeding activity, toxicity or acting as precursors to physical defense systems. A steroid is a type of organic compound that contains a characteristic arrangement of four cycloalkane rings that are joined to each other. Examples of steroids include the dietary lipid cholesterol, bile acids, the sex hormones estradiol and testosterone and the anti-inflammatory drug dexamethasone. Steroidogenesis is the biological process by which steroids are generated from cholesterol and transformed into other steroids (Hanukoglu, 1992). Terpenes are released by trees more actively in warmer weather, acting as a natural form of cloud seeding. The clouds reflect sunlight, allowing the forest to regulate its temperature (John and Koperuncholan, 2012). A glucoside is a glycoside that is derived from glucose. Glucosides are common in plants, but rare in animals. These compounds give a permanent froth when shaken with water (Ono, 2017). They also cause hemolysis of red blood cells. Saponin glycosides are found in liquorice. Their medicinal value is due to their expectorant, and corticoid and anti-inflammatory effects (Wu *et al.*, 2022).

Alkaloids act on a diversity of metabolic systems in humans and other animals, they almost uniformly invoke a bitter taste (Pandurangi *et al.*, 2022). Flavonoids are synthesized by the phenylpropanoid metabolic pathway where the amino acid phenylalanine is used to produce 4-coumaryl-CoA, and this is then combined with malonyl-CoA to produce chalcones which are backbones of Flavonoids (Yusoff *et al.*, 2022). Coumarin is also used as a gain medium in some dye lasers as a sensitizer in older photovoltaic technologies (Mohammed *et al.*, 2022).

Table 1: Qualitative phytochemical analysis of *Cissus vitifolia* leaves ethanol extract

S. No.	Secondary metabolites	Ethanol
1	Carbohydrate	+++
2	Tannins	-
3	Saponins	++
4	Flavonoids	+++
5	Alkaloids	+++
6	Anthocyanin & Betacyanin	+++
7	Quinones	+
8	Glycosides	+++
9	Cardiac glycosides	++
10	Terpenoids	++
11	Triterpenoids	++
12	Phenols	++
13	Coumarins	++
14	Acids	+
15	Protein	+
16	Steroids	+++

+++ strong presence; ++ positive; + mildly present; - absent

Stigmasterol and Campesterol are a group of phytosterols. Stigmasterol is an unsaturated plant sterol occurring in the plant fats or oils of soybean, calabar bean and rape seed, and in a number of medicinal herbs. Edible oils contain higher amount of stigmasterol than vegetables (Jun-Hua *et al.*, 2008). It acts as an intermediate in the biosynthesis of androgens, estrogens, and corticoids. It is also used as the precursor of Vitamin D₃ (Kametani and Furuyama, 1987). It is also useful in prevention of certain cancers including ovarian, prostate, breast and colon cancers. It also possesses potent antioxidant, hypoglycemic and thyroid inhibiting properties (Panda *et al.*, 2009). Stigmasterol is precursor of anabolic steroid boldenone. Boldenone undecylenate is commonly used in veterinary medicine to induce growth in cattle, but it is also one of the most commonly abused anabolic steroids in sports (Ros *et al.*, 2007).

Squalene is a hydrocarbon, triterpene, natural and vital part of the synthesis of all plant and animal sterols, including cholesterol, steroid hormones, and vitamin D in the human body. All plants and animals produce squalene as a

biochemical intermediate, including humans. Squalene is one of the most common lipids produced by human skin cells (Smith, 2000). Squalene is used in cosmetics, and more recently as an immunologic adjuvant in vaccines. It is a chemopreventive substance that protects people from cancer (Owen *et al.*, 2004). Thirteen compounds were identified in *Cissus vitifolia* leaf by Gas Chromatogram- Mass spectrometry (GC-MS) analysis (Table 2, Fig. 1). GC MS studies of ethanol extract indicated that the prevailing compounds were 2,3-Anhydro-D-Galactosan, Hydroxylamine, O-Decyl-, Tetradecane, 1-Chloro-, Ledol and 2,6,10,14-Hexadecatetraen-1-OL, 3,7,11,15-Tetramethyl-, Acetate, (E,E,E)-. The presence of various bioactive compounds justified the use of the whole plant for various ailments by traditional practitioners. This study explores the usefulness of the leaf of the plant *Cissus vitifolia* which has a commendable sense of purpose and can be advised as a plant of phytopharmaceutical importance. Compounds belonging to the respective groups have been reported to impart various medicinal characteristics to the plants. Alkaloid exhibited promising anti-diarrheal, anti-inflammatory, anti-cancer, anti-diabetic activities

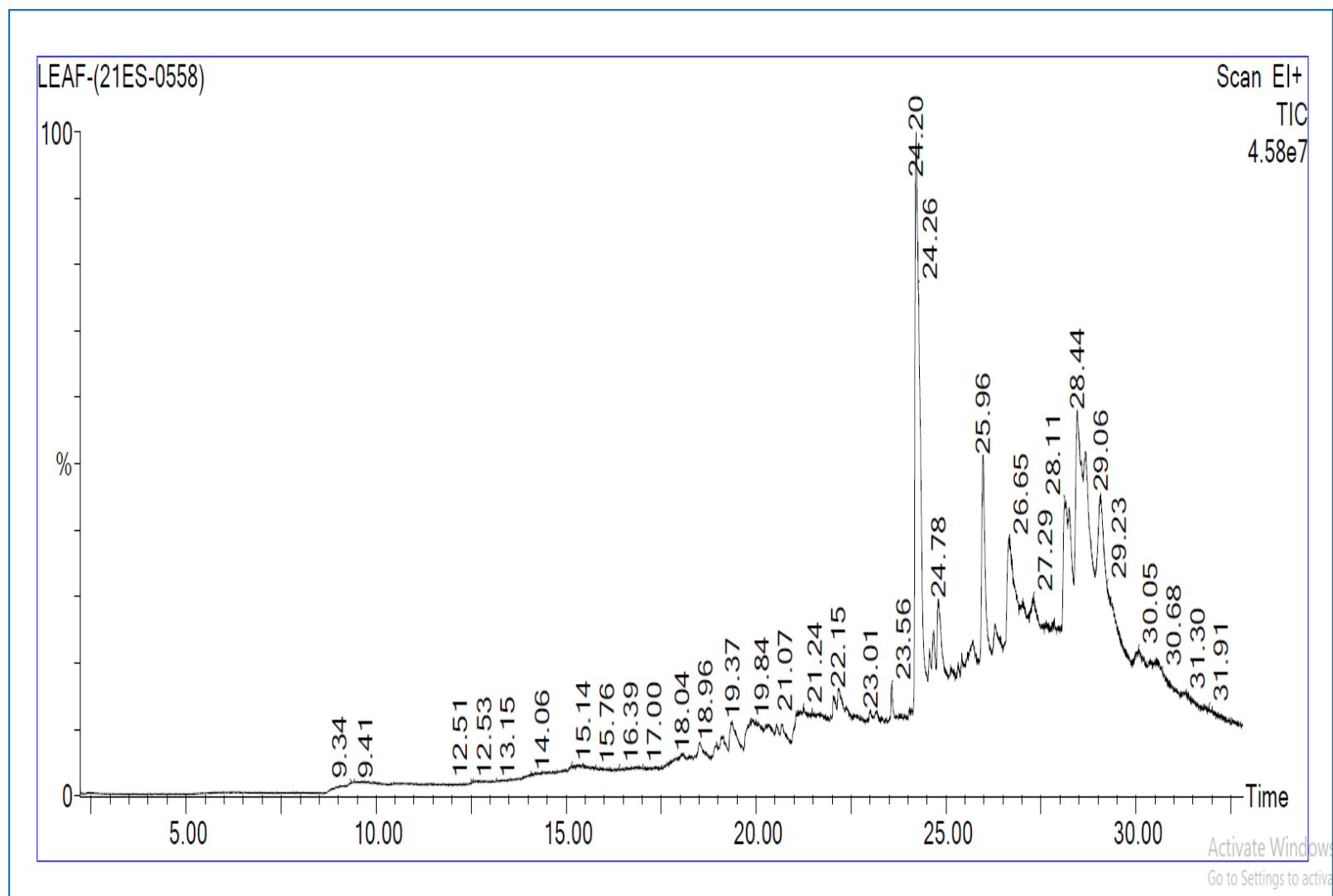


Fig. 1: GC-MS chromatogram of *Cissus vitiginea* leaves ethanol extract.

Table 2: Analysis of bioactive compounds in *Cissus vitifera* leaves ethanol extract by GC MS

S. No.	RT	Scan	Height	Peak Area	Area %	Norm %	MW	Compound name	Formula	CAS	Biological activity
1	19.365	3432	2,102,429	344,565.6	1.620	6.47	228	3-ACETOXYDODECANE	C ₁₄ H ₂₈ O ₂	60826-26-8	Antifouling agent (König and Wright, 1997), homogeneous catalysis (Bochmann and Thomas, 1984)
2	19.880	3535	1,736,579	530,286.0	2.494	9.96	144	2,3-ANHYDRO-D-GALACTOSAN	C ₆ H ₈ O ₄	900129-98-9	Antibacterial and anticancer activity (Das <i>et al.</i> , 2020), antioxidant (Mohansrinivasan <i>et al.</i> , 2017; Puraikalan, 2018; Rameshkumar <i>et al.</i> , 2018)
3	24.207	4400	39,460,180	5,323,467.5	25.035	100.00	208	6,11-DIMETHYL-2,6,10-DODECATRIEN-1-OL	C ₁₄ H ₂₄ O	900196-53-3	Antioxidant and anti-inflammatory activity (Das <i>et al.</i> , 2020), antibacterial and antioxidant activities (Niamah and Alali, 2016), anti-nutritional properties (Abubakar <i>et al.</i> , 2015) and anticancer activity using MDA-MB-231 triple-negative breast cancer cells (Thi-Kim Nguyen <i>et al.</i> , 2019)
4	24.782	4515	4,912,868	511,949.1	2.408	9.62	170	UNDECANAL	C ₁₁ H ₂₂ O	112-44-7	Sex attractant pheromone trap for wax moth (Dickens <i>et al.</i> , 1986), antimicrobial activity (Matasyoh <i>et al.</i> , 2009) and larvicidal activity (Senthilkumar and Venkatesalu, 2010)
5	25.973	4753	13,860,099	1,301,938.6	6.123	24.46	173	HYDROXYLAMINE, O-DECYL-	C ₁₀ H ₂₃ ON	29812-79-1	Antioxidant activity (Okla <i>et al.</i> , 2014; Soleha <i>et al.</i> , 2020), antibacterial activity (Techaoei <i>et al.</i> , 2020),
6	26.663	4891	7,834,267	1,666,768.5	7.838	31.31	165	2,4-DIMETHYL-7-OXO-4,7-DIHYDRO-	C ₆ H ₇ ON ₅	61402-43-5	Antioxidant and antidiabetic

								TRIAZOLO(3,2-C)TRIAZINE			potential (Uddandrao <i>et al.</i> , 2020)
7	27.018	4962	2,900,382	537,059.2	2.526	10.09	328	SPIRO[ANDROST-5-ENE-17,1'-CYCLOBUTAN]-2'-ONE, 3-HYDROXY-, (3.BETA.,17.BE	C22H32O2	60534-16-9	Antibacterial activity (Riyaz <i>et al.</i> , 2021)
8	27.293	5017	2,662,734	410,834.4	1.932	7.72	232	TETRADECANE, 1-CHLORO-	C14H29Cl	2425-54-9	Antibacterial property (Rai <i>et al.</i> , 2021), antidiabetic agent (Aleykutty and Akhila, 2012) and antioxidant activity (Suryanti <i>et al.</i> , 2020)
9	28.114	5181	9,269,561	1,010,894.3	4.754	18.99	388	COPROSTAN-16.BETA.-OL	C27H48O	900252-96-1	Used as a supplement to anti-snake venom (Nayak <i>et al.</i> , 2020), anti-inflammatory and hepatoprotective effect (Razak <i>et al.</i> , 2020)
10	28.229	5204	8,691,732	1,022,457.4	4.808	19.21	332	BISNORALLOCHOLANIC ACID	C22H36O2	900251-94-7	Cardioprotective effect (Vaithiswari <i>et al.</i> , 2019), antioxidant, anti-inflammatory, anti-microbial activity and anti-cancer activity (Susanna <i>et al.</i> , 2022)
11	28.439	5246	15,626,010	2,856,821.0	13.435	53.66	222	LEDOL	C15H26O	577-27-5	Antimicrobial agent (Bombarda <i>et al.</i> , 2001) and anti-inflammatory activity (Baananou <i>et al.</i> , 2015)
12	28.649	5288	12,911,601	2,696,881.5	12.683	50.66	332	8A(2H)-PHENANTHRENOL, 7-ETHENYLDODECAHYDRO-1,1,4A,7-TETRAMETHYL-, A	C22H36O2	41756-14-3	Antibacterial activity (Nabi <i>et al.</i> , 2022)
13	29.059	5370	10,402,482	3,050,065.2	14.344	57.29	332	2,6,10,14-HEXADECATETRAEN-1-OL, 3,7,11,15-TETRAMETHYL-, ACETATE, (E,E,E)-	C22H36O2	61691-98-3	anti-diabetic, anti-cancer and anti-tuberculosis (Suhitha <i>et al.</i> , 2015), cytotoxic activity (Kumar <i>et al.</i> , 2016; Shen <i>et al.</i> , 2018) and antioxidant activity (San and Kyaw, 2019)

and cure urinary disorders (Jain *et al.*, 2012). The present study results confirmed the presence of these phytochemicals in ethanol leaf extract of *Cissus vitiginea* hence, justified usage of this plant in traditional systems of medicine and confirm its therapeutic abilities.

The identified phytocomponents are used for antioxidant, antimicrobial and anticancer activities. Biochemical compound identification of the plant constituents was conducted depending upon their retention time (RT), molecular formula, peak area, area %, molecular formula and molecular weight. GC-MS spectroscopy analysis showed the existence of various compounds with different chemical constituents. Thus, improving the methods for qualitative and quantitative determination of medicinal plants is very important for quality assessment in the medicinal plant industry. In addition, the phytochemical analysis gives a good monitoring method of the seasonal changes of the active constituents and during cultivations and harvesting which assists in collecting the largest amounts of the active constituents. The phytochemical investigation concluded that the stronger extraction capacity of methanol could have been produced number of active constituents responsible for many biological activities. So that those might be utilized for the development of traditional medicines and further investigation needs to elute novel active compounds from the medicinal plants which may be created a new way to treat many incurable diseases including cancer. We report the presence of some of the important components resolved by GC-MS analysis and their biological activities. Thus, this type of GC-MS analysis is the first step towards understanding the nature of active principles in this medicinal plant and this type of study will be helpful for further detailed study.

The cytotoxic activity of *Cissus vitiginea* leaves ethanol extract on MCF-7 cell line was determined by MTT assay based on the detection of mitochondrial dehydrogenase activity in living cells. The Vero cell line was exposed to different concentration (7.8 to 1000 µg/ml) of *Cissus*

vitiginea leaves methanol extract. The inhibitory effect was observed after 24 h of incubation. Results revealed that at higher concentrations there is significant cell mortality. Table 3 and Figure 2 show the changes in the percentage of inhibition in treated MCF-7 cells. 50% cell viability was observed at the concentration of 125 µg/ml of sample with the ethanol extract. The maximum cell growth inhibition was observed at the concentration 1000 µg/ml (24.64 %). From the obtained data the ethanol extract was able to reduce the viability of MCF-7 cell line in direct dose dependent manner. Figure 3 shows the cytomorphological changes in the cell line at various concentrations of ethanol extract. From the results we could infer that as the concentration of ethanol increases the cell damage also increase leading to cell viability.

Cissus vitiginea leaves exhibits its ability to emerge as a potent medicine and has a very promising potential for its use in the treatment of human diseases, especially cancer. The importance of herbal plant based research has been worldwide acclaimed over the last few years. Advantages of herbal plants offer several advantages including improved tumor targeted efficacy and safety. The extraction process found to be cost effective which can make the formulation as preferred choice. In future, pre-clinical and clinical investigations are highly essential to obtain mechanistic characteristic for translating anticancer herbal extract as effective and safe drug to treat cancers. The present study found that ethanol extract increase the expression level of tissue inhibitors of metalloproteinase (TIMPs) by several folds and at the same time lowers the expression of MMPs by several folds. The higher dose i.e. 1000 µg/ml (24.64 %) showed more efficacy in lowering the expression level of MMPs and increasing the expression level of TIMPs as compared to dose of 125 µg/ml in MCF-7 cell line.

The cytotoxicity mechanisms include metabolite activation of promutagen, acts as blocking agents and results in formation of

Table 3: Percentage of cell viability on MCF-7 cell line induced *Cissus vitiginea* leaves ethanol extract

S. No.	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell viability (%)
1	1000	Neat	0.274	24.64
2	500	1:1	0.375	33.72
3	250	1:2	0.456	41.00
4	125	1:4	0.535	48.11
5	62.5	1:8	0.626	56.29
6	31.2	1:16	0.727	65.37
7	15.6	1:32	0.841	75.62
8	7.8	1:64	0.942	84.71
9	Cell control	-	1.112	100

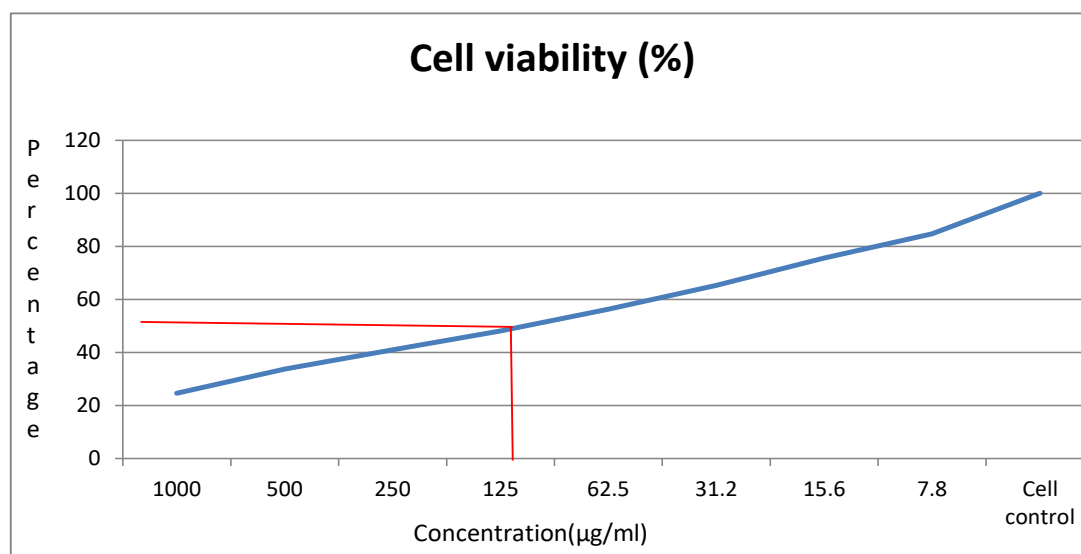


Fig. 2: Cytotoxic effect of *Cissus vitiginea* leaves ethanol extract against and MCF-7 cell line.

adducts with the help of mutagens, free radical scavenging, and suppression of tumour cell invasiveness and finally inhibition of matrix metalloproteinase 2/-9 activity (Kwan *et al.*, 2016). In our study, cell death of MCF-7 cells may be due to various reasons like receptor binding inhibitors, protein binding and DNA replication interaction. After confirming that ethanol extracts show anticancer potential against MCF-7 cells, their cytotoxic potential was also tested against

non-cancerous cell line (Vero) which represents normal cells. The results showed that ethanol extract have no cytotoxic potential against normal cells at 24 h time point (Table 4, Figs. 4, 5) indicating that these extracts are having a good anticancer potential and should be explored for further evaluation.

The cytomorphological changes of MCF-7 cells were photographed in 10X magnification. The

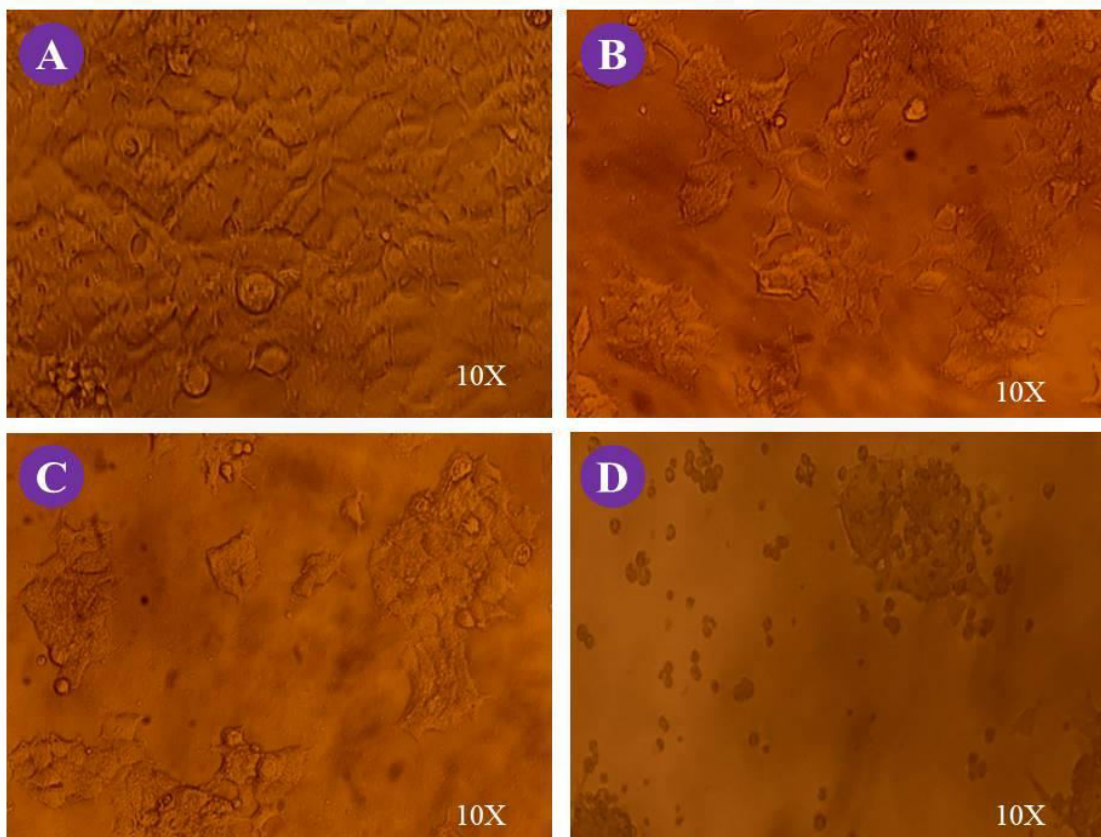


Fig. 3: Cytomorphological changes of *Cissus vitiginea* leaves ethanol extract against MCF-7 cell line. (A) Control MCF-7 cells, (B) 7.8 µg/ml of ethanol extract treated cells, (C) 125 µg/ml of ethanol extract treated cells and (D) 1000 µg/ml of ethanol extract treated cells.

Table 4: Percentage of cell viability on Vero cell line induced *Cissus vitiginea* leaves ethanol extract

S. No.	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell Viability (%)
1	1000	Neat	1.203	54.11
2	500	1:1	1.346	60.54
3	250	1:2	1.468	66.03
4	125	1:4	1.601	72.01
5	62.5	1:8	1.715	77.14
6	31.2	1:16	1.846	83.04
7	15.6	1:32	1.985	89.29
8	7.8	1:64	2.133	95.95
9	Cell control	-	2.223	100

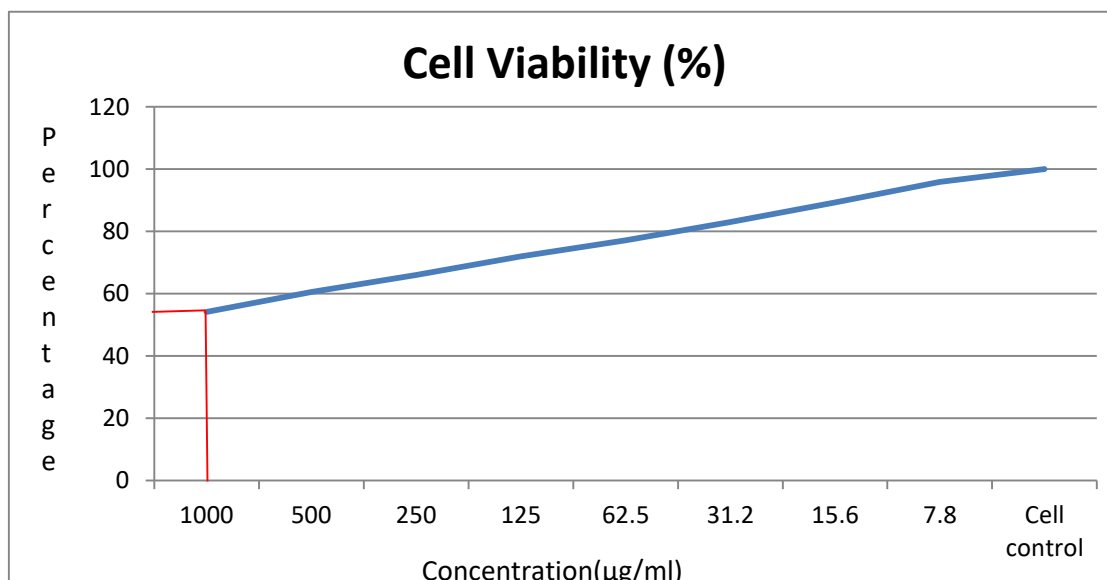


Fig. 4: Cytotoxic effect of *Cissus vitiginea* leaves ethanol extract against and Vero cell line.

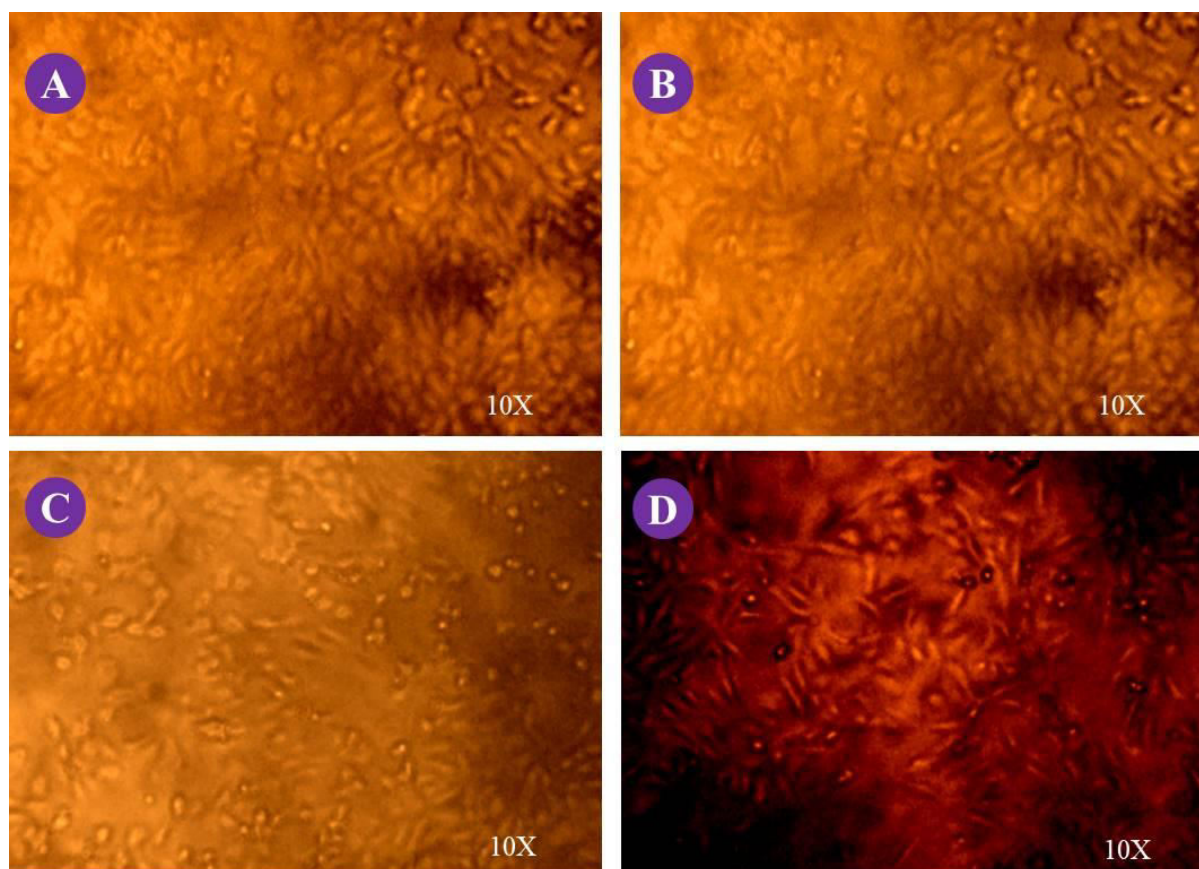


Fig. 5: Cytomorphological changes of *Cissus vitiginea* leaves ethanol extract against and Vero cell line. (A) Control MCF-7 cells, (B) 7.8 µg/ml of ethanol extract treated cells, (C) 125 µg/ml of ethanol extract treated cells and (D) 1000 µg/ml of ethanol extract treated cells.

untreated MCF-7 cells revealed irregular clumping and polygonal cell morphology. On contrary, treatment with different concentration of *Cissus vitiginea* leaves ethanol extract for 24 h caused contraction of polygonal MCF-7 cells to spherical shape. The cell shrinkage was dosage and time-dependent. The rate of cell shrinkage was high in ethanol leaf extract treated cells (Figs. 3 B, C, D) and thereafter, ethanol leaf extract was taken for further studies to assess its biocompatibility with Vero cells. Treatment of Vero cells for 24 h at different concentration of ethanol extract did not cause any changes in the Vero cells (Fig. 5). From the results, it is clear that ethanol extract is biocompatible and can be used as a drug for treating various diseases including cancer. These conclusions are hopeful since it is well known that human breast cancer is one of the deadly cancers among women and the ethanol could suppress and arrest its development during cell viability itself. Likewise, ethanol extract also inhibited cell migration of MCF-7 cells. Cell migration is the key characteristic of cancer progression and metastasis as opined by Hamidi and Ivaska (2018), and suppression of cell migration may establish key in inhibition of metastasis *in vitro*, thus supporting the results of our study.

Conclusion

The present study demonstrated that *Cissus vitiginea* leaves ethanol extract, a novel natural polyphenolic extract from *Cissus vitiginea*, exhibited potential anti-proliferative effects on MCF-7 cells *in vitro*, and apoptosis induction was involved in its potential mechanisms. Moreover, intrinsic mitochondrial pathway were activated by the polyphenolic compounds present in the ethanol extract, thus inducing apoptosis, and the elevation of the Caspase-8, Caspase-10, FAS, FADD, BAD, BAK and Bcl-2 which might serve as a crucial factor for the threshold of this process. All of our findings provide more of a basis for Caspase-8, Caspase-10, FAS, FADD, BAD, BAK and Bcl-2 activity which has to be explored through wet lab studies to prove the efficacy of ethanol extract as

an anticancer agent in combating breast cancer. In future, this study should focus on validation of the drug by research experiments and by human clinical trials, so that they can be used as a natural drug that can be used on par with chemical drugs for the treatment of human breast cancer.

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