Research Article

In vitro Assessment of the Antioxidant and Cytotoxic Activities of *Cissus quadrangularis* Using HeLa Cells

Gnaneshwar Reddy Kontham[1], Gopal Viraj Koundinya Vutukuru[1], Kalyani Chepuri[2,*], Pranitha Chittepu[2], Harikrishna Kathuroju[2], Geethikalal Vadakavila[2], Uma Addepally[2], Sesha Srinivas Vutukuru[2]

[1]Department of Biotechnology, School of Technology, GITAM University, Vishakhapatnam, Andhra Pradesh, INDIA.
[2]Centre for Biotechnology, University College of Engineering Science and Technology, Jawaharlal Nehru Technological University, Hyderabad, Telangana, INDIA.

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ABSTRACT

Aim: Cervical cancer is a significant global health concern, and usually treated by Cisplatin in combination with radiation therapy, often associated with side effects. *Cissus quadrangularis* has antioxidant and immune-boosting effects. In view of its therapeutic potential in treating cervical carcinoma, we evaluated the antioxidant and anticancer activities of acetone and methanolic extracts of *Cissus quadrangularis*. **Materials and Methods:** The phytochemical profiling of the plant extract was done by GC-MS, antioxidant activity was assessed by FRAP, DPPH, and ABTS assays, and MTT assay was used to study the cytotoxicity of plant extract in HeLa cells. **Results:** Phytochemical profile comprised pentadecanoic acid, 9,12,15 octadecatrienoic acid, 9,12 octadecanoic acid, methyl stearate, α-amyrin, α-methyl sorboside, β-amyrin, methyl tetradeconate, heptadecanoic acid, stigamasta, diglycerol, which are testified to have antioxidant and anticancer activities. The FRAP, DPPH and ABTS assays demonstrated a strong antioxidant activity in acetone and methanol as the concentration of the extract increased. In the anticancer assays, both acetone and methanol extracts exhibited dose-dependent cytotoxic effects on the HeLa cell lines, with notable inhibition of cell proliferation. In acetone extract, cell viability was 96.21 % in 0.10 mg/mL and 44.67% in 1.60 mg/mL, whereas in methanol extract, cell viability was 89.32% in 0.10 mg/mL and 32.12% in 1.60 mg/mL. The IC_{50} values of acetone and methanolic extracts were 1.33 and 0.97 mg/mL, respectively. **Conclusion:** The results advocate a prospective role of *C. quadrangularis* in cervical carcinoma therapy.

Keywords: Anti-cancer properties, Antioxidant, *Cissus quadrangularis*, HeLa cells, *in vitro* studies.

Correspondence:
Dr. Kalyani Chepuri, M.Tech., Ph.D.
Assistant Professor,
Centre for Biotechnology,
University College of Engineering Science and Technology,
Jawaharlal Nehru Technological University,
Hyderabad-500085,
Telangana, INDIA.

Email: kalyanichepuri@jntuh.ac.in

INTRODUCTION

Drug development from medicinal plants offers an effective, economic, and benign alternative to conventional procedures like animal cell cultures or microbial fermentation.[1] Phytochemicals present in medicinal plants are diverse with a rich range of aromatic compounds like phenols or their derivatives.[2] Cancer is an intricate disease caused by multiple factors that include physico-chemical, ecological, metabolic, genetic and epigenetics. It poses substantial public health distress in both advanced and emerging countries.[2] Cancer is initiated by the generation of Reactive Oxygen Species (ROS) and their impaired scavenging by the body's biodefense mechanisms. The ROS form adducts with DNA leading to cellular damage, mutations, and eventually cancer.[3,4] Globally, malignancy is the second important reason of death. Between diverse cancers, cervical malignancy is one of the most common type

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of cancers to affect women. Current treatment choices include surgery, radiotherapy and chemotherapy, often allied with acute adverse effects that cause morbidity. On the other hand, bioactive compounds from *Cissus quadrangularis* were shown to be safe and efficacious in combating breast cancer and its metastatic activity, with no established side effects.[5]

Cissus quadrangularis has emerged as a plant of significant interest due to its strong antioxidant, antimicrobial, anti-inflammatory and anticancer activities. It was reported to be used in traditional medicine for its putative benefits in bone health and weight management.[2] Previous research elsewhere identified a wide range of phytochemicals in *C. quadrangularis* including polyphenols, flavonoids, and triterpenes, which are essentially accountable for its antioxidant activity.[6] Furthermore, *C. quadrangularis* has demonstrated potential anticancer activity against lung,[2] breast,[5] Glioblastoma cells[7] cancer cells, through multiple mechanisms. They include apoptosis, cell-cycle arrest, and reduced tumour progression. These results underscore *C. quadrangularis* as a promising candidate for complementary cancer therapy. A recent review[8] highlighted the anticancer potential of *C. quadrangularis* against many cancers.

To extract the phytochemicals from medicinal plants, many polar solvents are used. Of these, acetone and methanol are particularly effective for extracting bioactive compounds from plant or microbial sources, making them versatile solvents for both lipophilic and hydrophilic components. However, studies on the relative efficiency of these solvents in extracting phytochemicals from *C. quadrangularis* and their cytotoxic effects are sparse. Therefore, we analyzed the phytochemical profile in acetone and methanolic extracts of *C. quadrangularis* by GC-MS. Antioxidant activity of the above extracts was evaluated by Ferric Reducing Antioxidant Power (FRAP), 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-Azino-Bis (3-ethylbenzothiazoline-6-Sulfonic acid (ABTS) assays using Nanodrop. The cytotoxic effect of acetone and methanolic extracts of *C. quadrangularis* was assessed in human cervical cancer cells (HeLa cells) by MTT assay. This research focused on the evaluating the relative efficacy of plant extracts in acetone and methanol solvents to explore their therapeutic potential in cervical cancer treatment.

MATERIALS AND METHODS

Preparation of *Cissus quadrangularis* extract

Cissus quadrangularis powder was procured from a medical shop in Hyderabad. 50 g of the powder was taken separately in two 250 mL conical flasks. Subsequently, 150 mL of acetone and 150 mL of methanol were added (1:3 ratio), respectively, into each of the flasks. The contents were sufficiently mixed and flasks were placed in an orbital shaker (Hicool make) for 72 hr. From the filtered extract, 10 mL was taken into 15 mL screw capped test tubes, for the identification of phytochemicals.

Preparation of Sample for phytochemical analysis

To identify bioactive compounds, phytochemical analysis of *C. quadrangularis* extracts was done by using sample (1 mL), and diluted with methanol (9 mL) of for each of the following constituents.

Saponins

The presence of saponins in the sample were detected by mixing 1 mL of the sample with 2 mL of water in a test tube and the suspension was vigorously shaken for 15 min. Foam layer on top of the mixture confirms the presence of saponins.

Phenols

The phenolic compounds were tested by taking 1 mL of the extract plus 2 mL of distilled water and adding 10% ferric chloride drops in a test tube. The presence of phenolic compounds was specified by a blue-green or black colour.

Tannins

To 1 mL of the sample 2 mL of 2% ferric chloride were added. Formation of a blue-green or blue-black colour was observed in the resulting mixture.

Terpenoids

Terpenoids were tested by mixing 1 mL of the extract with 2 mL of chloroform followed by 2 mL of concentrated sulphuric acid. Reddish brown coloration at the boundary determines their presence.

Flavonoids

Flavonoids were analysed by mixing 2 mL of the extract with 1 mL of 2N sodium hydroxide, observed by yellow colour.

Test for Glycosides

The extract was mixed with 2 mL of glacial acetic acid comprising 2 drops of 2% $FeCl_3$. The mix was transferred into one more test tube holding 2 mL of concentrated sulphuric acid. Formation of a brown ring at the interface showed their presence.

Alkaloids

Few drops of Mayer's reagent were added to 1 mL plant extract wherein yellowish or white precipitate suggests the presence of alkaloids.

Preparation of Stock solutions for Antioxidant and Cytotoxicity assays

The residual extract was taken into porcelain dish and dried under shade for 5 days. The dried contents were scraped and transferred into the Eppendorf tubes (2 mL). Stock solution of 100 mg/mL concentration was prepared in methanol by dissolving the above plant extracts.

Antioxidant assays

The plant extract's antioxidant activity was established by preparing working aliquots in geometric ratio i.e., 0.1 mg/mL, 0.20 mg/mL, 0.40 mg/mL, 0.80 mg/mL, 1.60 mg/mL, 3.20 mg/mL and 6.40 mg/mL from the stock solution (5 mg/mL concentration). To assess the antioxidant activity, FRAP assay,[9] DPPH assay[10] ABTS assay[11] were performed

GC-MS Profiling Protocol for *Cissus quadrangularis* Extracts

The *C. quadrangularis* extract (acetone and methanol solvents) was taken to Department of Analytical and Structural Chemistry, CSIR-Indian Institute of Chemical Technology, Hyderabad for GC-MS analysis (Agilent Technologies 7890B GC system with 5977A Mass Selective Detector (MSD)). The extract from both solvents was concentrated in a rotary evaporator to remove solvent. Next, it was reconstituted it in hexane. To remove particulate matter, the reconstituted sample was sieved over a 0.45 µm filter. At that juncture, the sample (1 µL) was introduced into HP-5 capillary column by injection (30 m×0.25 mm i.d., 0.25 µm thickness). The conditions used were: carrier gas- helium (He) at a constant flow rate of 1.0 mL/min, injection operation temperature was maintained at 200ºC, while column oven temperature was programmed as 50-250ºC at a rate of 10ºC/min injection mode. The MSD conditions used are: ionization voltage of 70 eV; ion source temperature of 250ºC; interface temperature of 250ºC; and mass range of 50-600 mass units. To classify the mass spectrum of the unidentified phytochemicals, the National Institute Standard and Technology (NIST) database comprising more than 62,000 spectrum patterns of the known compounds was employed.

Cytotoxicity assays

Procurement and culture of cell lines

HeLa cells (cervical cancer) were procured from NCCS (National Centre for Cell Science), Pune, India. The cells were cultured under aseptic conditions in T_{25} flasks containing RPMI 1640 growth medium augmented with serum. The flasks were incubated at 37ºC in 95% humidity augmented by 5% CO_2. Later they were passaged after every 3-4 days depending on the confluence attained. All these cell culture experiments were conducted at Animal Cell Culture laboratory, Centre for Biotechnology, University College of Engineering Science and Technology, JNTUH, Hyderabad.

Cell Seeding

After discarding the spent medium, Phosphate-Buffered Saline (PBS) was used to splash the adherent cells a couple of times. After trypsinization the cells were incubated briefly for detachment. To attain a single cell suspension, 4 mL of fresh medium were added. Small aliquot was taken from this to check the cell viability using a Neubauer hemocytometer (Germany).

Evaluation of cytotoxic effect of *C. quadrangularis* in HeLa cells by MTT Assay

C. quadrangularis extract was prepared (100 mg/mL) by dissolving the dried powder in Dimethyl Sulphoxide (DMSO), and used as a stock solution. From this, 10, 20 and 40 mg/mL concentrations were finally prepared and used for MTT assay.

Cell treatment with *C. quadrangularis* extracts

After 24 hr, expended medium was thrown away while the cells were exposed to different concentrations like 0.10, 0.20, 0.40, 0.80 and 1.60 mg/mL by adding 5, 10, 20, 40 and 80 µL aliquots of the plant extract. The remaining volume of 500 µL was made up with fresh growth medium. A control was maintained by adding 500 µL of growth medium. After further incubation for 24 hr, morphological changes in treated cells were visualised under inverted microscope (Olympus).

MTT assay

The cytotoxic effect of plant extract was assessed through MTT assay (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide assay).[12] A 500 µL of the cell suspension (2 x 10^4 cells) was seeded into each of the 12-well plate with 500 µL fresh medium. The cell adherence and growth were allowed by incubating the plate at 37ºC with 5% CO_2 for 24 hr. From the 12-well plate, spent medium was removed from all wells and then splashed with 500 µL of Phosphate-Buffered Saline (PBS). Later, 500 µL of MTT reagent (0.1 mg/mL) were added to all wells and placed in CO_2 incubator at 37ºC with 5% CO_2 for 1-4 hr. Next, the wells were observed for purple coloured formazan crystals, which indicated viable cells.

The MTT reagent was subsequently discarded from all wells and 500 µL of Dimethyl Sulfoxide (DMSO) were added for dissolving the formazan crystals.

Complete dissolution was ensured by shaking the plate gently side-to-side and front-to-back. Aliquots from each well were then reloaded to a 96-well plate in triplicate, and the optical density (OD) was read at 540 nm in an ELISA reader. The following formula was used to calculate the percentage of cell viability:

$$\text{Percent Cell Viability} = \frac{\text{OD of untreated cells}}{\text{OD of treated cells}} \times 100$$

To calculate the inhibitory concentration (IC_{50}) values, graphs were plotted by taking the % of cell viability on the Y-axis and concentration of plant extract on the X-axis.

RESULTS

Phytochemical analysis

In our present study, phytochemical analyses of *C. quadrangularis* extracts were carried out to identify various bioactive compounds in both acetone and methanol. Table 1 suggests that *C. quadrangularis* has most of the vital phytochemicals with proven therapeutic effects in cancer treatment like phenols, tannins, terpenoids, flavonoids, glycosides and alkaloids, while saponins are absent in both extracts.

From Table 1, it is clear that *C. quadrangularis* has vital phytochemicals that have potential pharmacological effects to treat cancer. Therefore, we assessed their anti-oxidant and cytotoxic activity using HeLa cells, to explore alternative treatment strategies for cervical cancer.

The GC-MS profile of the methanolic extracts of *C. quadrangularis* was presented in Table 2. In our study, methanol extract of *C. quadrangularis* revealed phytochemicals like pentadecanoic acid (100%), 9,12,15 Octadecatrienoic acid (76.75%), 9,12 Octadecanoic acid (58.91%), Methyl stearate (51.34%), α-amyrin (40.07%),

Table 1: Qualitative analysis of phytochemicals in *C. quadrangularis* extract.

Plant extract	Phytochemical test		Result	
	Phytochemical	Method	Acetone	Methanol
C. quadrangularis	Saponins	Frothing test	-	-
	Phenols	Ferric chloride test	+	+
	Tannins	Ferric chloride test	+	+
	Terpenoids	Salkowski test	+	+
	Flavonoids	NaOH test	+	+
	Glycosides	Keller Killiani test	+	+
	Alkaloids	Mayer's test	+	+

Table 2: GC-MS profile showing the phytochemicals of *C. quadrangularis* in methanol extract.

S.No	Compound	Retention Time	Area %	Pharmacological activity
1	Pentanoic acid	2.648	5.32	Histone Deacetylase (HDAC)-inhibiting function in liver cancer.[13]
2	Silane	6.730	5.99	Anti-cervical cancer activity.[14]
3	Cyclotetrasilaxane	7.027	5.57	Anti-cervical cancer activity.[14]
4	Diglycerol	7.354	8.88	Anticancer activity.[15]
5	Dodecanoic acid	14.530	6.43	Anticancer activity.[16]
6	Alpha methyl sorboside	15.891	35.56	Anticancer activity.
7	Methyl tetradeconate	16.844	12.04	Anticancer activity against HeLa cells.[17]
8	Pentadecanoic acid	18.962	100	induce JAK2/STAT3 signalling, arrest of cell cycle in sub-G1 phase, caspase-induced apoptosis in MCF-7/SC.[18]
9	Heptadecanoic acid	19.914	9.13	Anticancer activity.[18]
10	9,12 Octadecanoic acid	20.595	58.91	Anticancer activity.[19]
11	9,12,15 Octadecatrienoic acid	20.652	76.75	Anticancer activity.[20]
12	Methyl stearate	20.861	51.34	Anticancer activity.[19]
13	Beta amyrin	22.388	32.54	Anticancer activity against HeLa cells.[21]
14	Alpha amyrin	22.459	40.07	Anticancer activity against HeLa cells.[22]
15	Eicosanoic acid	22.794	24.46	Anticancer activity.[23]
16	Stigamasta	23.745	9.02	Apoptosis in HepG2 cells.[24]

α-methyl sorboside (35.56%), β-amyrin (32.54%), methyl tetradeconate (12.04%), heptadecanoic acid (9.13%), stigamasta (9.02%), diglycerol (8.88%), dodecanoic acid (6.43%), silane (5.99%), cyclotetrasilaxane (5.57%), pentanoic acid (5.32%) were present. A perusal of the literature indicates their antioxidant and anticancer potential in cancer therapy.[13-24]

Antioxidant assays

FRAP assay

The dose-dependent ferric reducing antioxidant potential of *C. quadrangularis* was shown in Table 3a. In acetone extract, it was ranging from 0.516 in 0.10 mg/mL to 2.73 in 1.60 mg/mL, while in methanolic extract it ranged from 0.450 in 0.10 mg/mL to 3.920 in 1.60 mg/mL. In this study, FRAP activity was relatively more effective in methanol solvent compared to acetone. *C. quadrangularis* steadily demonstrated a dose-dependent relationship between the concentration of plant extracts and their ferric reducing power, underscoring its antioxidant capacity.

DPPH assay

In this study, *C. quadrangularis* extract in acetone demonstrated DPPH scavenging activity in the range of 5.53% at 0.40 mg/mL to a maximum 71.39% scavenging activity at 6.40 mg/mL concentration. On the other hand, in methanol solvent, the plant extract also scavenged 70.7% at 6.40 mg/mL concentration (Table 3a). These results when compared to ascorbic acid standard (Table 3b) indicate that both acetone and methanolic extracts have enough potential to scavenge DPPH radical effectively.

ABTS assay

In the present study, *C. quadrangularis* extract in acetone demonstrated ABTS scavenging activity in the range of 3.86% at 0.10 mg/mL to a maximum 80.7% scavenging activity at 6.40 mg/mL concentration (Table 3a). In methanol solvent, the plant extract showed a minimum of 2.42% ABTS scavenging activity at 0.10 mg/mL while it scavenged 81.2% at 6.40 mg/mL. The methanol extract was almost on par with acetone extract in scavenging ABTS. These results demonstrated that *C. quadrangularis* extracts in both the solvents demonstrated a dose-dependent upsurge in scavenging activity at high concentrations.

Table 3b: Antioxidant scavenging potential of the L-ascorbic acid standard

Concentration µg/mL	FRAP value*	% of DPPH scavenged*	% of ABTS scavenged*
100	19.53±0.21	19.53±0.21	24.09±0.26
200	41.20±0.33	41.20±0.33	47.72±0.13
400	66.12±0.14	66.12±0.14	75.47±0.42
800	82.43±0.71	82.43±0.71	89.83±0.31
1600	90.97±0.32	90.97±0.32	96.07±0.54

(*Values are expressed as the mean±standard deviation (n=3)).

Cytotoxic effect of *C. quadrangularis* extracts

C. quadrangularis extracts in both acetone and methanol demonstrated potential cytotoxic effects, capably reducing cell viability in all the tested concentrations. The cell viability in acetone extract was maximum 0.10 mg/mL (96.2%) and a marked decrease in viability (44.67%) was observed at 1.60 mg/mL. Similar trend

Table 3a: Antioxidant scavenging potential of *C. quadrangularis* extracts.

Conc of extract µg/mL	FRAP value*		% of DPPH scavenged*		% of ABTS scavenged*	
	Acetone	Methanol	Acetone	Methanol	Acetone	Methanol
100	0.516±0.23	0.450±0.31	-	-	3.86±0.12	2.42±0.21
200	0.647±0.54	0.629±0.47	-	-	7.67±0.23	4.1±0.37
400	0.797±062	0.760±0.74	5.53±0.33	1.9±0.10	20.8±0.34	22.7±0.63
800	1.365±0.84	1.650±0.69	16.17±0.54	21.3±0.23	39.3±0.69	48.8±0.87
1600	2.730±0.89	3.920±0.72	20.55±0.89	33.3±0.42	47.7±0.78	64.1±0.74
3200	-	-	46.50±0.41	29.5±0.63	71.8±0.97	75.8±0.56
6400	-	-	71.39±0.36	70.7±0.45	80.7±0.84	81.2±0.89
Average	1.211	1.482				

*Values are expressed as the mean±standard deviation (n=3))-No antioxidant activity.

Table 4: IC_{50} values of *C. quadrangularis* extracts compared to L-ascorbic acid (Control).

DPPH*			ABTS*		
L-Ascorbic acid	Acetone mg/mL	Methanol mg/mL	L-Ascorbic acid mg/mL	Acetone mg/mL	Methanol mg/mL
0.378± 0.23	4.14± 1.96	3.97± 2.34	0.215	2.26	1.39

(*Values are expressed as the mean±standard deviation (n=3)).

was also observed in the methanol extract which exhibited 89.3% cell viability at 0.10 mg/mL which subsequently decreased to 32.12 % at 1.60 mg/mL (Figure 1a and b). A noticeable drop in cell viability of HeLa cells was observed as the extract concentration increased from 0.10 to 1.60 mg/mL, demonstrating cytotoxic effect of the plant. The IC_{50} value in acetone and methanol extracts was1.33 mg/mL and 0.970 mg/mL, respectively (Table 5). These values represent the minimum concentrations essential to achieve 50% cell mortality, underlining the comparable cytotoxic efficiency of the two solvent extracts against HeLa cells. The results suggest that methanol extract induced more cytotoxic effects compared to acetone. This could be attributed to the moderate polarity of acetone compared to methanol. In contrast, methanol being a highly polar solvent, effectively extracted polar compounds like phenols, flavonoids, and alkaloids. Its strong polarity could have facilitated efficient solubilization of these compounds from *C. quadrangularis*.

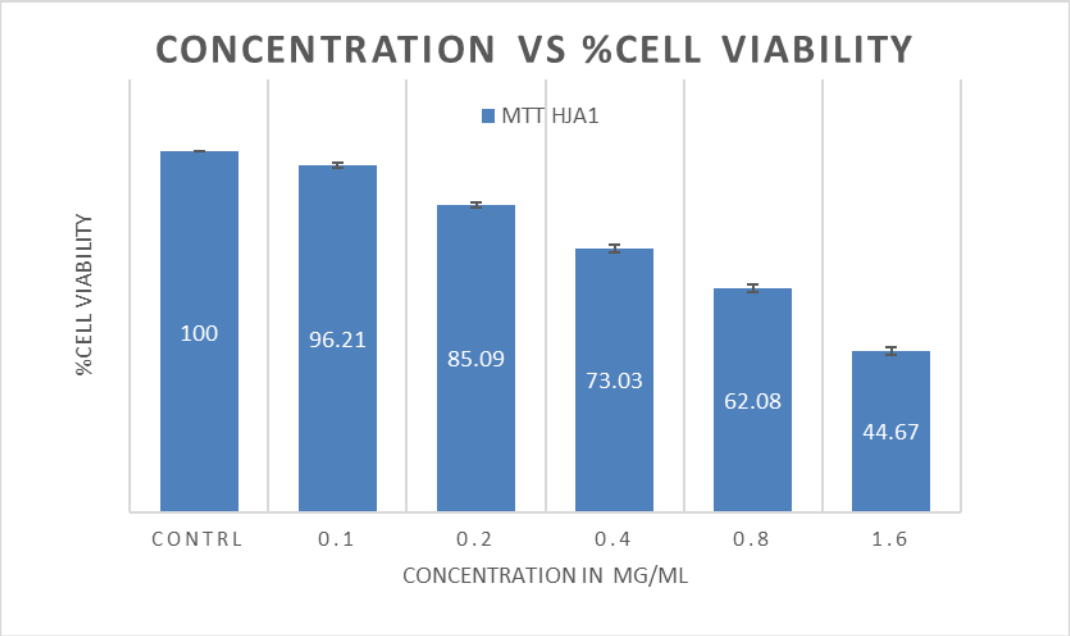

Figure 1 a: Cell viability in HeLa cells exposed to acetone extract of *C. quadrangularis.*

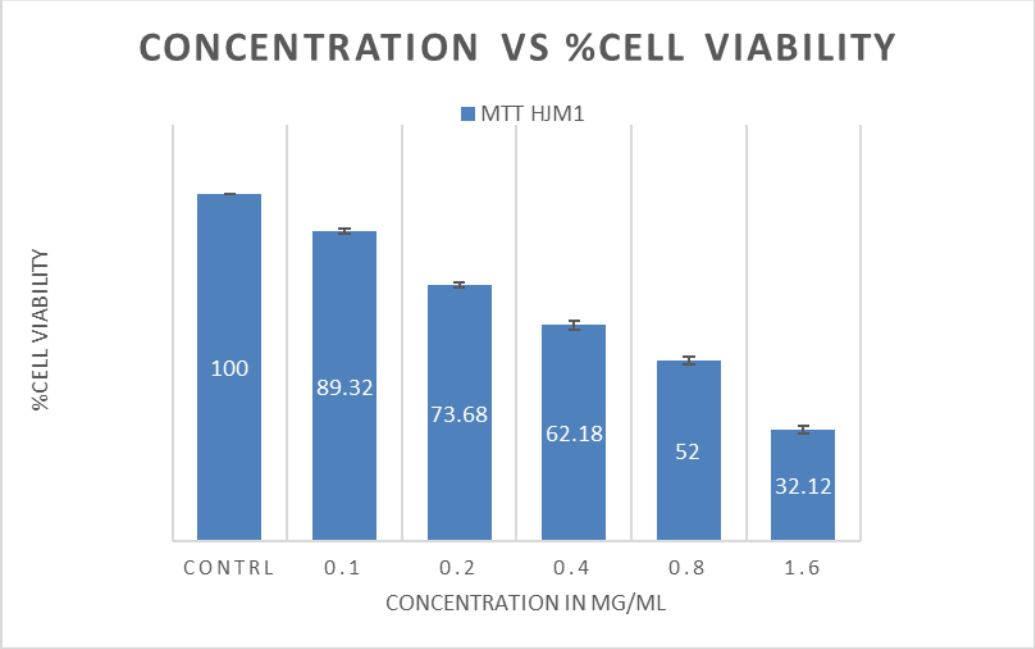

Figure 1 b: Cell viability in HeLa cells exposed to methanol extract of *C. quadrangularis.*

Table 5: Cell viability at different concentrations of *C. quadrangularis* extracts compared to Cisplatin.

Sl. No.	*C. quadrangularis* extract	Concentration (mg/mL)	%Cell viability	IC_{50} (mg/mL)
1	Acetone	0.10	96.21±0.83	
		0.20	85.08±0.77	
		0.40	73.03±1.15	1.33
		0.80	62.08±1.06	
		1.60	44.67±1.01	
2	Methanol	0.10	89.32±0.87	
		0.20	73.68±0.78	
		0.40	62.18±1.2	0.97
		0.80	52.00±1.03	
		1.60	32.12±0.98	
3	Cisplatin	5	70.08±0.35	
		10	61.45±0.42	
		20	50.87±0.78	0.23
		40	25.37±0.39	
		80	18.13±0.43	

(*Values are expressed as the mean±standard deviation (n=3)).

DISCUSSION

This study investigated the phytochemical profile of *C. quadrangularis* since it is crucial in the study of natural products wherein it helps to identify compounds that have potential therapeutic effects. Previous research reported that phenolic compounds could possibly regulate various caner related signalling pathways. They are known to moderate key signalling pathways like the PI3K/Akt and MAPK pathways which are crucial for cell survival and proliferation. Phenolic compounds while up-regulating the pro-apoptotic proteins like Bax, simultaneously downregulate anti-apoptotic proteins such as Bcl-2, thereby inducing apoptosis in cancer cells.[25] Phenolic compounds are reported to disrupt the tumour vasculature and thereby inhibit cancer proliferation and induce cancer cell death.[26,28] Tannins essentially chelate metal ions and exhibit antioxidant activity and prevent the generation of reactive oxygen species. Tannins have the ability to bind and precipitate proteins. The anticancer effects of tannins could be due to inhibition of key enzymes involved in cancer progression.[25,29] Terpenoids scavenge free radicals effectively, and induce anti-cancer effects by controlling various signalling pathways in carcinogenesis.[27] Other phytochemicals like flavonoids, glycosides and alkaloids, were also reported to exert similar mechanisms cited above in cancer therapy.

High percentage of pentadecanoic acid was observed in our study (Table 2). Bioactive compounds like pentadecanoic acid are reported to supress Interleukin-6 (IL-6), induce signalling of JAK2/STAT3 pathway, seizure of cell cycle at sub-G1 phase. Moreover, it was reported to promote apoptosis which is caspase-dependent in human breast carcinoma MCF-7/ Stem-like Cells (SC).[18] This study also reported that heptadecanoic acid (C17:0) wield anti-cancer properties in Non-Small Cell Lung Carcinoma (NSCLC) cells, highlighting the effectiveness of heptadecanoic acid in aiming human lung cancer cells. Therefore, it is possible that high percentage of pentadecanoic acid (9.13%) observed in the present study could also kick-start similar mechanisms in HeLa cells, and could have contributed to the cytotoxicity of *C. quadrangularis* extracts. These findings suggest that eating pentadecanoic acid-rich food could be beneficial all through cervical malignancy treatment.

As per a recent study,[14] 9,12 Octadecanoic acid, Cyclotetrasilaxane and Methyl stearate extracted from *Origanum vulgare* were reported to possess significant anti cervical cancer potential. In our investigation, GC-MS profile of the *C. quadrangularis* methanolic extract contained 9,12 Octadecanoic acid (58.91%), methyl stearate (51.34%), cyclotetrasilaxane (5.57%). The cytotoxic effects of the *C. quadrangularis* extract can be due to the significant occurrence of the above cited compounds with established anti-cervical cancer properties.

Current researches[22] established that α-amyrin from *Callistemon citrinus* stem bark exhibited moderate cytotoxic effects against cervical cancer cells. In our study, we found that α-amyrin (51.34%) was abundant in the *C. quadrangularis* which could have contributed

for the cytotoxic effect observed 0.970 mg/mL. A recent study,[25] evaluated the anti-inflammatory and anticancer action of three prospective phytochemicals-sitosterol, β-amyrin, and epiafzelechin. They evaluated the effects of the compounds both individually and in various therapeutic amalgamations against colon cancer to establish the important mechanisms that diminish cancer-causing effect of nickel in rat. The authors concluded that β-sitosterol, β-amyrin, and epiafzelechin possess anticancer property with an ability to target the cancerous biomarkers in colon cancer. β-amyrin activates p38 mitogen-activated protein kinase and Jun N-terminal kinase through transcriptional factor, GADD45β. This eventually triggers caspase-9 and caspase-3 and drives the HeLa cells to apoptosis. Even our results show the significant presence of β-amyrin (32.54%), and the cytotoxicity observed in cervical cancer cells highlight the anticancer potential of this compound.

A recent study[23] evaluated the effectiveness and anticancer prospective of the methanol extract of *Monotheca buxifolia* leaves in human breast cancer cells aiming WNT/β-catenin signalling pathway. The GC-MS investigation of the plant extract shown the occurrence of phytochemicals like 9,12,15 Octadecatrienoic acid, 9,12 Octadecanoic acid, heptadecanoic acid, stigmasterol and eicosanoid acid which contributed for the anticancer activity of the extract. In the present investigation, *C. quadrangularis* contained significant percentage of above cytotoxic compounds, which might contribute to the anticancer action of the plant extract. For instance, the presence of stigmasterol in our study (Table 2) could have contributed to the antioxidant and cytotoxic activity of the methanolic extract of *C. quadrangularis*. Recent research suggested that stigmasterol of phyto and algal origin is a favourable phyto-constituent to develop anticancer drugs. Stigmasterol outstandingly interrupted angiogenesis in human cholangiocarcinoma by tumour necrosis factor-α (TNF-α) and vascular endothelial growth factor receptor-2 (VEGFR-2) suggesting their down-regulation. The combination of stigmasterol and sorafenib was shown to promote caspase-3 activity and down-regulation of anti-apoptotic protein Bcl-2 in breast cancer.[26] The findings of these studies underscore the anticancer potential of stigmasterol against various types of cancers.

Table 4 shows that the acetone extract of *C. quadrangularis* resulted in IC_{50} value of 4.14 mg/mL for DPPH radical scavenging. This is notably higher compared to L-ascorbic acid (0.378 mg/mL), a well-known antioxidant used as reference. In contrast, methanolic extract scavenged up to 71% at 6.40 mg/mL. The higher IC_{50} value for the acetone extract specifies that a higher concentration is required to achieve 50% DPPH scavenging activity compared to L-ascorbic acid. The results of the present study indicate that *C. quadrangularis* exhibited significant antioxidant activity in both solvents, while it is moderately more effective in methanol. This suggests that both extracts contain equal amounts of active antioxidants. The dose-dependent nature of antioxidant activity could be attributed to presence of potent phytochemicals observed in this investigation (Tables 1 and 2). In our study, the IC_{50} value of *C. quadrangularis* at which 50% of ABTS is scavenged is 2.25 mg/mL in acetone and 1.40 mg/mL in methanol compared to L-ascorbic acid (0.215 mg/mL). Overall, the higher IC_{50} values for the acetone and methanol extracts of *C. quadrangularis* elucidate that higher concentrations of both extracts are required to achieve 50% ABTS scavenging activity compared to L-ascorbic acid.

The data from Tables 1 and 2 advocate that both extracts of *C. quadrangularis* contain phytochemicals with good anti-proliferative effects on cervical cancer cells. Previous studies have emphasized the potential of flavonoids, terpenoids, and other phenolic compounds in *C. quadrangularis* to its anticancer activity.[2] The findings of our study supported by earlier reports accentuate the therapeutic potential of *C. quadrangularis* in cervical cancer treatment. Although the results of our study are promising, further research is essential to isolate the specific compounds responsible for the cytotoxicity Moreover, exploring their effect on mechanistic aspects like apoptosis, cell-cycle arrest could provide better understanding into how these compounds trigger cell death in cervical cancer cells. Once these concepts are established, the phytochemicals can be used as adjuvants in cancer therapy.

CONCLUSION

This study convincingly demonstrated the antioxidant and anticancer properties of *C. quadrangularis*. The FRAP assay results indicate that the ferric reducing antioxidant potential of *C. quadrangularis* was relatively more effective in acetone solvent compared to methanolic solvent. The DPPH scavenging activity was more or less equal and effective in both the extracts. *C. quadrangularis* extract in both acetone and methanol extracts showed same ability to scavenge ABTS. This study emphasized the promising anticancer properties of *C. quadrangularis* against HeLa cells. The results established that there is linear correlation between the concentration and cell survival, indicating enhanced

mortality of HeLa cancer cells when the concentration of *C. quadrangularis* extracts enhanced from 0.10 to 1.60 mg/mL. The significant cytotoxic effects shown could be primarily through mechanisms like apoptosis induction and cell cycle arrest. Additionally, the plant's phytochemicals and their possible role as adjuvants with conventional chemotherapeutic agents in cancer therapy warrants further research.

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AUTHORS CONTRIBUTION

Dr. Uma Addepally developed the concept, defined the intellectual content and reviewed the manuscript. Dr. Kalyani Chepuri conceptualized the study objectives, hypothesis and designed the experimental work. Gnaneshwar Reddy Kontham, Gopal Viraj Koundinya Vutukuru contributed in the literature search and experimental work. Pranitha Chittepu, Harikrishna Kathuroju, Geethikalal Vadakavila supervised the experimental studies. Dr. Sesha Srinivas Vutukuru prepared and edited the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no competing financial/personal interests that could have influenced the work reported in this paper.

ABBREVIATIONS

FRAP: Ferric Reducing Antioxidant Power; **DPPH:** 2,2-Diphenyl-1-picrylhydrazyl; **ABTS:** 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid; **MTT:** 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide.

SUMMARY

In this study, the phytochemical composition, antioxidant properties, and potential cytotoxic effect of acetone and methanolic extracts of *Cissus quadrangularis* was investigated. The study focused on the relative efficacy of plant extracts acetone and methanol solvents to explore their therapeutic potential in cervical cancer treatment.

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