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Original Article

# *In vitro* Evaluation of the Antioxidant and Anticancer Activities of Acetone and Methanolic Extracts of *Asparagus racemosus* in Human Cervical Cancer Cell Lines (HeLa Cells)

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## ABSTRACT

**Background:** Cervical cancer is the fourth most common cancer among women across the globe. Typically, it is treated by Cisplatin in combination with radiation therapy, which has side effects. We evaluated the phytochemical composition, antioxidant capacity, and anticancer potential of acetone and methanolic extracts of *Asparagus racemosus* in HeLa cells for exploring alternate therapy of cervical cancer. **Materials and Methods:** Phytochemical analysis was done to identify key bioactive compounds, along with GC-MS profile in both acetone and methanolic extracts. *In vitro* evaluation of their antioxidant activity was done by FRAP (Ferric Reducing Antioxidant Power), DPPH (2,2-Diphenyl-1-picrylhydrazyl), and ABTS (2,2'-Azino-bis (3-ethylbenzothiazoline-6 -sulfonic acid) assays. Cytotoxicity studies using HeLa cells were assessed by MTT assay. **Results and Discussion:** The FRAP assay indicated that acetone extracts exhibited greater antioxidant potential (0.407 to 2.950 in 100 to 1600 µg/mL) compared to methanol extracts (0.363 to 1.867 in 100 to 1600 µg/mL). The DPPH scavenging activity ranged from 4.46% to 59% in acetone extracts, while in methanol extracts it showed only 22.7% at 6400 µg/mL. ABTS scavenging activity was high in acetone (14.30% to 87.9%) compared to methanol (5.52% to 87.40%). In MTT assay, a dose-dependent cytotoxicity was observed against HeLa cells in both acetone and methanol extracts ($IC_{50}$ 771.44 µg/mL and 768.44 µg/mL), respectively. The cell survival in acetone ranged from 87.1% at 100 µg/mL to 18.9% at 1600 µg/mL, while in methanol it was 89.3% to 15.4% at the same concentrations. These findings suggest that phytochemicals of *A. racemosus* can be potentially exploited as complementary therapeutic agents in cervical cancer treatment and warrants further research.

**Keywords:** Anti-cancer properties, Anti-oxidant, *Asparagus racemosus*, He La cells, *In vitro* studies.

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## INTRODUCTION

Phytochemicals from medicinally important plants have long been renowned as a vital source of therapeutic agents, and include alkaloids, tannins, carotenoids, proteins, chlorophyll, phytosterols, glycosides, phenols, flavonoids, and diterpenes, besides some minerals, vitamins and other crucial nutrients. These compounds are suitable to develop novel therapeutics due to their potent pharmacological activities.[1] Recent advances in natural product research highlight a deeper understanding of the pharmacological effects of medicinal plants, emphasizing their potential in pharmacotherapeutic studies. This growing interest underscores the value of medicinal plants and natural product extracts as alternative therapies for a range of diseases.[2]

Human exposure to environmental toxicants and dietary xenobiotics generates Reactive Oxygen Species (ROS) and contribute to oxidative stress under various pathophysiological conditions.[3] Free radicals are highly reactive, and their accumulation aggravate pathological conditions, resulting in mutations which increases cancer risk.[4] In this context, antioxidants derived from natural sources have received significant attention in view of their potential to alleviate oxidative damage.

Scientific evidence indicates that antioxidants from various medicinal plants with established anticancer, anti-mutagenic, antitumor, antibacterial, antiviral, and anti-inflammatory properties are safe and efficient.[5] *Asparagus racemosus*, commonly known for its use in traditional Indian medicine systems like Ayurveda, Unani, and Siddha, has been highlighted in both the

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Indian and British Pharmacopoeias. This plant is traditionally employed for its antioxidant and anti-stress effects. Its extracts were also used in ulcer treatment and wound healing.[6-8] Extensive phytochemical analyses using analytical techniques such as GC-MS[9] have identified key constituents like saponins, flavonoids and phenolic acids in *A. racemosus* that contribute to the plant's medicinal properties.

The antioxidant properties of *A. racemosus* are well-documented, showcasing its efficacy in reducing oxidative stress through various mechanisms.[10] Enhanced antioxidant capacity was reported in root extracts of the plant.[11] Methanolic extracts demonstrated high free radical scavenging activity with substantial phenolic and flavonoid content.[12] A recent review of the of the *A. racemosus* reported significant anticancer, antidiabetic anti-inflammatory, antimicrobial, antioxidant and antiviral effects in animal and human studies underscoring its anticancer potential.[13]

Therefore, we analyzed the phytochemical profile of *A. racemosus* and evaluated its antioxidant and anticancer activities of acetone and methanolic extracts through *in vitro* assays, and the corresponding results are presented in this paper. We attempted to elucidate the therapeutic prospects of *A. racemosus* in scavenging free radicals and inhibiting cancer cell proliferation particularly in human cervical cancer cell lines (HeLa cells) by cytotoxicity studies (MTT assay). The outcome of this study helps to explore the potential application of *A. racemosus* extracts in developing alternative treatment strategies for oxidative stress-related disorders and cancer.

## MATERIALS AND METHODS

### Preparation of Plant Extracts

*Asparagus racemosus* powder was obtained from a local Ayurveda medical shop. Fifty grams of the dried powder was mixed with 150 mL of methanol (1:3 ratios) in a 250 mL conical flask. The mixture was incubated on an orbital shaker (Hicool make) for 72 hr. Subsequently, the extract was filtered, and 10 mL aliquots were transferred into 15 mL screw-capped test tubes for phytochemical analysis. A similar extraction process was performed with another 50 g of powder using acetone as the solvent.

### Preparation of Stock Solutions for Antioxidant and Anticancer Assays

The remaining extract was dried in a porcelain dish for 5 days in the shade. The dried material was scraped and transferred into Eppendorf tubes (2 mL). A stock solution (100 mg/mL) was prepared in methanol, diluted initially to 20 mg/mL, and subsequently to 5 mg/mL concentrations. Working aliquots of 100, 200, 400, 800, 1600, 3200, and 6400 µg/mL were prepared from this stock to evaluate antioxidant activity. For anticancer assays, a 100 mg/mL stock solution was prepared in Dimethyl Sulfoxide (DMSO). From this, 10, 20 and 40 mg/mL concentrations were prepared for the MTT assay.

### Phytochemical Analysis

Phytochemical analysis of *A. racemosus* extracts was carried out to identify bioactive compounds using the protocols given hereunder. Saponins were analyzed by shaking 3 mL of the extract vigorously, and formation of stable foam indicates the presence of saponins. For phenols, 1 mL of the extract was taken and 2 mL distilled water and a few drops of 10% ferric chloride were added. The presence of phenols is indicated by blue-green or black color. Tannins were analyzed by mixing 1 mL extract with 2 mL of 2% ferric chloride, indicated by the formation of blue-green or black color. For terpenoids, 1 mL extract was mixed with 2 mL of chloroform followed by 2 mL of concentrated sulphuric acid.

The presence of terpenoids is indicated by reddish brown color at the interface. Flavonoids were analyzed by mixing 2 mL extract with 1 mL of 2 N sodium hydroxide, indicated by yellow color. For glycosides, 2 mL glacial acetic acid containing 2 drops of 2% $FeCl_3$ was mixed with the extract. The mixture was added into another test tube containing 2 mL of concentrated sulphuric acid. The formation of a brown ring at the interface indicates their presence. To test alkaloids, a few drops of Mayer's reagent were added to 1 mL of the extract. Yellowish or white color precipitate indicated the presence of alkaloids.

### GC-MS Profiling Protocol for *Asparagus racemosus* Extracts

The phytochemicals in *A. racemosus* extract (acetone and methanol solvents) was taken to Department of Analytical and Structural Chemistry, CSIR-Indian Institute of Chemical Technology, Hyderabad for GC-MS profiling (Agilent Technologies 7890B GC system with 5977A Mass Selective Detector (MSD)). The extract from each solvent was concentrated using a rotary evaporator to remove the solvent. Next, it was reconstituted it in hexane. To remove particulate matter, the reconstituted sample was filtered through a 0.45 µm filter. Then, 1 µL of the sample was injected into HP-5 capillary column (30 m×0.25 mm i.d., 0.25 µm film thickness). The conditions used were: Helium (He) as carrier gas with a constant flow rate of 1.0 mL/min, injection operation temperature was maintained at 200°C, while column oven temperature was programmed as 50-250°C at a rate of 10°C/min injection mode. The MSD conditions were used: ionization voltage of 70 eV; ion source temperature of 250°C; interface temperature of 250°C; and mass range of 50-600 mass units. To identify the mass spectrum of the unknown phytochemicals, the database of National Institute Standard and Technology (NIST) with over 62,000 spectrum patterns of the known compounds was used for the interpretation on mass spectrum of GC-MS.[9]

### Antioxidant Assays

In this study, the FRAP (Ferric Reducing Antioxidant Power) Assay[14] was performed to assess the antioxidant potential of acetone and methanolic extracts of *A. racemosus*. Briefly, the

methanolic plant extract (0.5 mL) from each of the working concentrations used (100, 200, 400, 800, 1600 µg/mL) was added to 2.5 mL of phosphate buffer (pH 7.4) and 2.5 mL of aqueous potassium ferricyanide solution (1% w/v) in 5 test tubes. Control was also maintained with 0.5 mL of methanol and the above reagents without plant extract. Ascorbic acid was used as standard for comparison. The test tubes were placed at 50±2°C in water bath for 20 min and later they were cooled in running tap water and 2.5 mL of 10% (w/v) trichloroacetic acid was added. The tubes were centrifuged at 3000 rpm for 5 min (REMI R 4 C), and 2.5 mL of upper layer of the solution was mixed with 2.5 mL of distilled water. To these tubes, 0.5 mL of freshly prepared ferric chloride solution (0.1% w/v) was added and mixed thoroughly. After 15 min of initial mixing, the extracts from each concentration were added into 96 well plates in triplicates along with control (ascorbic acid). The absorbance was measured at 593 nm in a Nanodrop (Multiskan).

DPPH (2,2-Diphenyl-1-picrylhydrazyl) Assay[15] measures the ability of antioxidants to scavenge free radicals. The principle behind this method is the reduction reaction of ferric tripyridyltraizine complex to its colored form known as ferrous tripyridyltraizine complex in the presence of antioxidants. Briefly, 0.1 mM DPPH solution was prepared by dissolving 3.9 mg of DPPH in 100 mL of methanol, and the solution was kept in dark for 30 min. A volume of 100 µL of plant extract (acetone and methanol solvents) from each concentration used i.e. 100, 200, 400, 800, 1600, 3200 and 6400 µg/mL was mixed with 3.9 mL of freshly prepared DPPH solution. Control was also maintained with 100 µL of methanol and the above reagents without plant extract. Ascorbic acid was used as standard for comparison. After vigorous shaking, the test tubes were wrapped with aluminum foil and allowed to stand at room temperature in dark for 45 min. Subsequently, the extracts from each concentration were added into 96 well plates in triplicates along with control. The absorbance was measured at 517 nm in a Nanodrop (Multiskan).

DPPH percentage inhibition (%)=Abs (control)/Abs(sample)/ Abs(control)X100

To evaluate the antioxidant potential of *A. racemosus* extracts, ABTS assay[16] was performed. ABTS radical cations were generated by reacting ABTS (7 mM) with potassium persulfate (2.45 mM) at 1:1 (v/v ratio) for 16 hr in room temperature in the dark (incubation). The ABTS solution was then diluted with PBS (pH 7.4) to obtain an absorbance of 0.70±0.02 at 734 nm. The diluted solution was referred to as ABTS working solution which is freshly prepared for each assay. Ninety microliters (90 µL) of ABTS working solution were added to 96 well microplates and absorbance was measured at 734 nm followed by 10 µL of standard and different concentrations of the plant extract (100, 200, 400, 800, 1600, 3200 and 6400 µg/mL). The 96 well plates were placed in dark for 6 min for incubation and the absorbance was measured at 734 nm in Nanodrop (Multiskan). Ascorbic acid was used as standard reference.

## Anti-Cancer Activity in HeLa Cells

HeLa cells (cervical cancer) were procured from National Centre for Cell Science, Pune, India. The cells were grown under aseptic conditions in in $T_{25}$ flasks containing RPMI 1640 growth medium augmented with serum. The flasks were incubated at 37°C in 95% humidified atmosphere enriched by 5% $CO_2$. Later they were sub-cultured once in 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

The spent medium from $T_{25}$ flask was discarded, and the adherent cells were washed twice with phosphate-buffered saline. After trypsinization, the flask was incubated until cells were detached. Fresh medium (4 mL) was added to attain a single cell suspension. The cells were then counted for viability using a hemocytometer. For MTT assay, 500 µL of the cell suspension (2 x $10^4$ cells) was seeded into each well of a 12-well plate containing 500 µL of fresh medium. Cell adherence and growth was allowed by incubating the plate at 37°C with 5% $CO_2$ for 24 hr.

## Cell Treatment

After 24 hr, the spent medium was discarded, and cells were treated with varying concentrations of the plant extract: 100 µg/mL (5 µL extract+495 µL growth medium), 200 µg/mL (10 µL extract+490 µL growth medium), 400 µg/mL (20 µL extract+480 µL growth medium), 800 µg/mL (40 µL extract+460 µL growth medium), and 1600 µg/mL (80 µL extract+420 µL growth medium). A control was maintained by adding 500 µL of growth medium. The cells were incubated for an additional 24 hr, after which the morphological changes were observed using an inverted microscope (Olympus).

## Evaluation of Cytotoxicity by MTT Assay

The MTT assay[17] was performed to evaluate the cytotoxicity of plant extracts. The spent medium was removed from all wells of a 12-well plate, which were then washed with 500 µL of Phosphate-Buffered Saline (PBS). Subsequently, 500 µL of 0.1 mg/mL MTT reagent was added to each well, and the plate was incubated for 1 to 4 hr in a $CO_2$ incubator at 37°C with 5% $CO_2$. After incubation, the wells were checked for the formation of purple formazan crystals, which indicated viable cells. Subsequently, the MTT reagent was discarded from all wells, and 500 µL of Dimethyl Sulfoxide (DMSO) was added to dissolve the formazan crystals. To ensure complete dissolution, the plate was shaken gently side-to-side and front-to-back. Aliquots from each well were then transferred to a 96-well plate in triplicate, and the

Optical Density (OD) was measured at 540 nm using an ELISA reader.

### The percentage of cell viability was calculated using the formula:

$$\text{Percent Cell Viability} = \frac{\text{OD of untreated cells}}{\text{OD of treated cells}} \times 10$$

The Inhibitory Concentration ($IC_{50}$) values were determined by plotting the percentage of cell viability on the Y-axis against the concentration of the plant extract on the X-axis, from which the $IC_{50}$ concentrations were interpolated. This approach provides a quantitative assessment of the cytotoxic effects of the plant extracts on HeLa cells.

## RESULTS

### Phytochemical analysis

In the present investigation, phytochemical analyses of *A. racemosus* extracts were carried out to identify various bioactive chemical constituents. Table 1 shows that *A. racemosus* has almost all-important phytochemicals like phenols, tannins, terpenoids, flavonoids, glycosides and alkaloids in acetone and methanolic extracts with potential therapeutic effects. The GC profile of the phytochemicals in the acetone and methanolic extracts of *A. racemosus* was presented in Table 2. In acetone extract, compounds like diacetone alcohol (21.93%), oleic acid (10.19%), (2S*, Benzyloxy) methyl) propyl)-2,3-dihydro-4H-pyran-4-one (pyran derivative) (7.34%), palmitic acid (6.40%), n-Octadecane (5.44%), Di-n-decylsulfone (5.12%) and smilagenin were present which are reported to possess antioxidant and anticancer properties. Other compounds like 1, 1, 1, 3, 5, 5, 5-heptamethyltrisiloxane, 4-butoxybutanol, cyclopentane carboxylic acid, 3-tridecyl ester,

**Table 1: Qualitative analysis of phytochemicals in *A. racemosus* extract in acetone and methanol.**

| Plant extract | Phytochemical test | | Result | |
|---|---|---|---|---|
| | Phytochemical | Method | Acetone | Methanol |
| *A. racemosus* | 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 phytochemical constituents of *A. racemosus* in acetone.**

| Sl. No. | Compound | RT | Area % | Pharmacological activity |
|---|---|---|---|---|
| 1 | Diacetone alcohol | 4.526 | 21.93 | Anti-proliferative and antimicrobial activity.[18] |
| 2 | Oleic acid | 21.159 | 10.19 | Anticancer activity.[19] |
| 3 | (2S*,1'S*)-2(1' ((Benzyloxy) methyl) propyl)-2,3-dihydro-4H-pyran-4-one | 27.266 | 7.336 | Pyran derivatives show anticancer, anti-inflammatory, antioxidant, neuroprotective, and antimicrobial effects.[20] |
| 4 | Palmitic acid | 19.454 | 6.404 | Promising anti-tumor agent with demonstrated efficacy against gastric cancer, liver cancer, cervical cancer, breast cancer, and colorectal cancer cell cycle arrest and promote apoptosis.[21] |
| 5 | n-Octadecane | 27.179 | 5.435 | Used in cosmetics and personal care products as an emollient and a thickening agent. |
| 6 | Di-n-decylsulfone | 27.024 | 5.176 | Antimicrobial and anticancer.[22] |
| 7 | 9,12-Octadecadienoicacid(Z, )- | 21.114 | 5.151 | Antitumor and antioxidant properties.[23] |
| 10 | Octadecanoic acid | 21.338 | 2.210 | Antitumor properties.[24] |
| 15 | Smilagenin | 34.369 | 1.607 | Steroidal saponin with anticancer activity.[25] |

RT= Retention Time.

hydromethylsiloxane, trans-stigmasta-5,22-dien-3. beta-ol etc. were present in low amounts with no established pharmacological activity, and hence not presented.

On the other hand, the methanolic extract of *A. racemosus* showed the presence of 5 hydroxy methyl furfural (12.83%) followed by 3-Deoxy-d-mannoic lactone (6.69%), heptyl caprylate (6.36%), dihydroxy acetone (6.25%), 6,6-Dideutero-Nonen-1-Ol-3 (6.08%) xanthosine (5.96%). Many other compounds like 1,4-Tetrabutyleneglycol monobutyl ether, 2-Deoxyribose, ethyltrimethylolmethane, 1-PROPANOL-O-D, Thiophen-2-methylamine, N-(2-fluorophenyl), maltol, cytidine, 2-Deoxy-D-ribose, glycerin, octanoic acid, hexyl ester were present in small amounts (Table 3).

## Antioxidant assays

Table 4a explains the dose-dependent ferric reducing antioxidant potential of *A. racemosus*. In acetone extract, it was ranging from 0.407 in 100 µg/mL to 2.950 in 1600 µg/mL, while in methanolic extract it ranged from 0.363 in 100 µg/mL to 1.867 in 1600 µg/mL. The antioxidant activity was relatively more effective in acetone solvent compared to methanolic solvent.

In the present study, the ABTS scavenging activity of *A. racemosus* extract (Table 4a) in acetone ranged from 14.30% at 100 µg/mL to 87.9% at 6400 µg/mL concentration. Methanol extract showed a minimum of 5.52% scavenging activity at 100 µg/mL while it was 87.40% at 6400 µg/mL. Although the methanol extract was slow in scavenging ABTS initially, it stabilized and was almost on par with acetone extract from 800 µg/mL concentration. Table

**Table 3: GC-MS profile showing the phytochemicals of *A. racemosus* in methanol.**

| Sl. No. | Compound | Retention Time | Area % | Pharmacological activity |
|---|---|---|---|---|
| 1 | 5 hydroxy methyl furfural | 10.944 | 12.83 | Cytotoxic and antioxidant property.[26] |
| 2 | 3-Deoxy-d-mannoic lactone | 16.643 | 6.69 | No anticancer activity. |
| 3 | Heptyl caprylate | 27.794 | 6.36 | Cytotoxic compound.[27] |
| 4 | Dihydroxyacetone | 5.639 | 6.25 | Cytotoxic and antioxidant.[28] |
| 5 | 6,6-Dideutero-Nonen-1-Ol-3 | 14.888 | 6.08 | Cytotoxic and antioxidant property.[29] |
| 6 | Xanthosine | 14.523 | 5.96 | Anti-tumor activities.[30] |

**Table 4a: Antioxidant scavenging potential of the acetone and methanol extracts of *A. racemosus*.**

| Conc of extract µg/mL | FRAP value* | | % of DPPH scavenged* | | % of ABTS scavenged* | |
|---|---|---|---|---|---|---|
| | Acetone | Methanol | Acetone | Methanol | Acetone | Methanol |
| 100 | 0.407±0.21 | 0.363±0.19 | - | - | 14.30±0.12 | 5.52±0.21 |
| 200 | 0.506±0.80 | 0.465±0.45 | - | - | 17.70±0.22 | 7.68±0.33 |
| 400 | 0.676±0.20 | 0.531±0.72 | 4.46±0.21 | 1.02±0.12 | 24.30±0.43 | 18.32±0.75 |
| 800 | 1.455±0.14 | 0.855±0.31 | 10.50±0.54 | 2.15±0.32 | 38.10±0.14 | 22.31±0.82 |
| 1600 | 2.950±0.69 | 1.867±0.87 | 17.50±0.85 | 4.86±0.18 | 48.08±0.36 | 37.60±0.23 |
| 3200 | - | - | 29.13±0.46 | 11.04±0. 23 | 67.97±0.22 | 61.01±0.56 |
| 6400 | - | - | 59.00±0.59 | 22.70±0.34 | 87.40±0.12 | 87.80±0.16 |
| Average | 1.198±0.95 | 0.816±0.55 | | | | |

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

**Table 4b: 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)).

5 illustrates that the $IC_{50}$ value of *A. racemosus* at which 50% of DPPH scavenged was 5416±0.87 compared to 378.32±0.23 µg/mL in L-ascorbic acid which was used as standard reference. No DPPH activity was observed in methanol extract. The $IC_{50}$ value of *A. racemosus* in the removal of ABTS was 2149 µg/mL in acetone and 2880 µg/mL in methanol, respectively, compared to L-ascorbic acid (215 µg/mL).

### Anticancer activity

*A. racemosus* extracts in both acetone and methanol demonstrated potential anticancer activity and efficiently reduced cell viability in all the tested concentrations. The cell viability in acetone extract was maximum 100 µg/mL (87.1%) and a marked decrease (18.9%) was observed at 1600 µg/mL. Similar trend was also observed in the methanol extract which exhibited 89.3% cell viability at 100 µg/mL which decreased to 15.4% at 1600 µg/mL (Figure 1a and b).

As the concentration of the extracts increased from 100 to 1600 µg/mL, there was a noticeable reduction in cell viability of HeLa cells, reflecting cytotoxic effect of *A. racemosus* extracts. The $IC_{50}$ values for the acetone and methanol extracts were 771.44 µg/mL and 768.38 µg/mL, respectively (Table 6). 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.

## DISCUSSION

Phenolic compounds potentially regulate signaling pathways related to cancer and moderate key signaling pathways involved in cancer, including the PI3K/Akt and MAPK pathways which are crucial for cell survival and proliferation. They induce apoptosis by upregulating pro-apoptotic proteins like Bax and downregulating anti-apoptotic proteins such as Bcl-2.[21] Phenolic compounds inhibit cancer proliferation; induce cancer cell death, by disrupting the tumor vasculature.[31] Tannins exhibit antioxidant activity predominantly by chelating metal ions and prevent the formation of reactive oxygen species. Their ability to bind and precipitate proteins also contributes to their anticancer effects by inhibiting enzymes involved in cancer progression.[21] Terpenoids effectively scavenge free radicals and exert anti-cancer effects

**Table 5: $IC_{50}$ values of acetone and methanol extracts of *A. racemosus* compared to L-ascorbic acid (Control) at which 50% of DPPH and ABT radicals are removed.**

| DPPH* | | | ABTS* | | |
|---|---|---|---|---|---|
| L-Ascorbic acid | Acetone | Methanol | L-Ascorbic acid | Acetone | Methanol |
| 378.32±0.23 | 5416±0.87 | - | 215±0.32 | 2149±0.39 | 2880±0.57 |

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

**Table 6: Cell viability at different concentrations of *A. racemosus* in acetone and methanol extract compared to standard anticancer reference drug Cisplatin.**

| Sl. No. | *A. racemosus* extract | Concentration (µg/mL) | % Cell viability | $IC_{50}$ (µg/mL) |
|---|---|---|---|---|
| 1 | Acetone | 100 | 87.10±0.32 | 771.44±1.26 |
| | | 200 | 75.09±0.19 | |
| | | 400 | 62.03±0.63 | |
| | | 800 | 40.07±0.54 | |
| | | 1600 | 18.80±0.38 | |
| 2 | Methanol | 100 | 89.32±0.51 | 768.38±1.54 |
| | | 200 | 73.68±0.87 | |
| | | 400 | 62.18±0.56 | |
| | | 800 | 43.30±0.34 | |
| | | 1600 | 15.39±0.26 | |
| 3 | Cisplatin | 5 | 70.08±0.35 | 23.98±0.21 |
| | | 10 | 61.45±0.42 | |
| | | 20 | 50.87±0.78 | |
| | | 40 | 25.37±0.39 | |
| | | 80 | 18.13±0.43 | |

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

by modulating various signaling pathways in carcinogenesis.[32] Other phytochemicals like flavonoids, glycosides and alkaloids observed in the present study, were also reported to exert similar mechanisms cited above in cancer therapy.

From Table 1, A. *racemosus* has important phytochemicals that have potential therapeutic effects in cancer treatment. Therefore, we evaluated their antioxidant and anti-cancer activity using He La cells, in pursuit of alternative treatment strategies for cervical cancer. The phytochemical profile of the acetone and methanolic extracts of *A. racemosus* was presented in Table 2. In acetone extract, compounds like diacetone alcohol (21.93%), oleic acid (10.19%), (2S*, Benzyloxy) methyl) propyl)-2,3-dihydro-4H-pyran-4-one (pyran derivative) (7.34%), palmitic acid (6.40%), n-Octadecane (5.44%), Di-n-decylsulfone (5.12%) and smilagenin were present which are reported to possess antioxidant and anticancer properties. For instance, high amounts of diacetone alcohol (>80%) was reported[33] in the extracts from three cyanobacterial species (*Anabaena oryzae, Oscillatoria* sp. and *Stigonema ocellatum*). These extracts showed high toxicity 49.1, 56.7 and 59 µg/L against MCF-7 cell line, respectively, establishing the cytotoxic effect of diacetone alcohol against human breast adenocarcinoma cells. Probably, the presence of high concentration of diacetone alcohol observed in the present study (21.93%) and $IC_{50}$ value of 771.44 µg/mL suggests that this compound could have induced cytotoxicity against HeLa cells.

Recent reports suggest that oleic acid-rich oils play a critical role as a hydrophobic substrate in the biological production of sophorolipids, a class of glycolipid bio surfactants with anticancer activity.[19] Previous research also highlighted the anticancer activity of these compounds against a variety of human

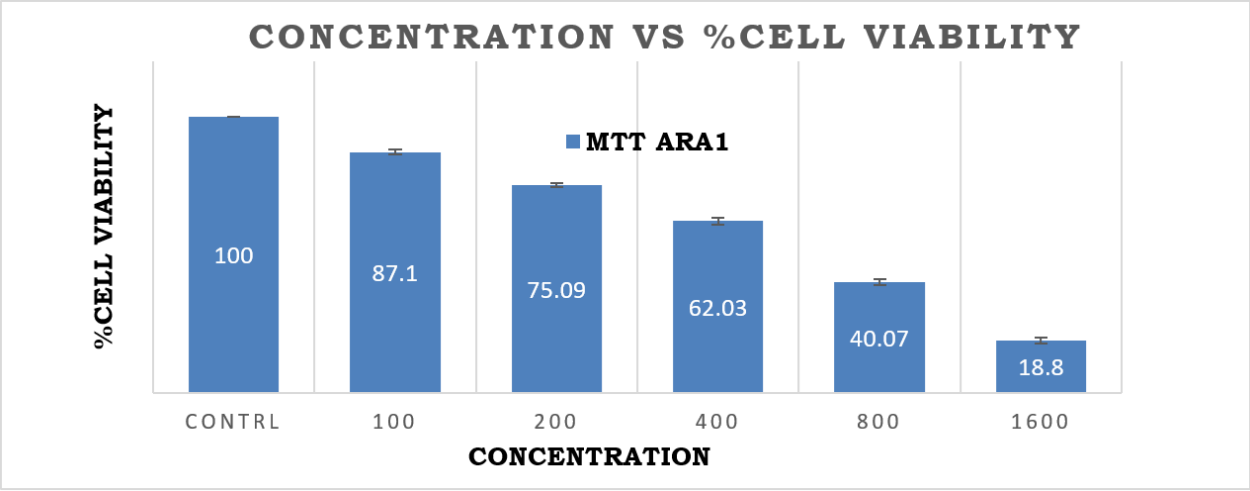


**Figure 1a:** MTT assay results in different concentrations (µg/mL) of acetone extract.

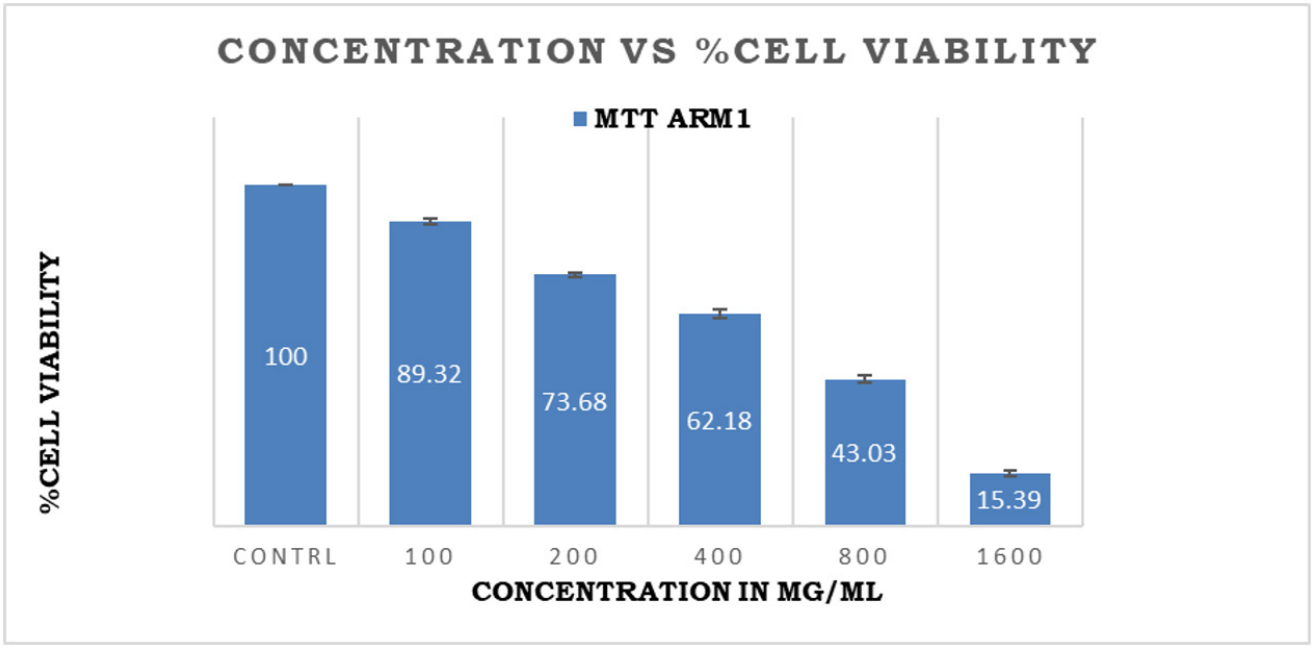


**Figure 1b:** MTT assay results in different concentrations of methanol extract.

cancer cell lines such as acute monocytic leukemia, THP-1, cervical carcinoma HeLa, colon carcinoma HCT 116, lung adenocarcinoma A549, breast adenocarcinoma MCF-7, pancreas PANC-1, and squamous carcinoma A431.[34] In our study, presence of oleic acid (10.19%) was observed next to diacetone alcohol. We hypothesize that oleic acid could play a role in the synthesis of sophorolipids that have anticancer activity. This observation was in accordance with an earlier study[35] who investigated the *in vitro* and *in vivo* anticancer activity of sophorolipids to human cervical cancer cells.

In our study, GC-MS profile shows the presence of (2S*,1'S*)-2-(1'-((Benzyloxy)methyl) propyl)-2,3-dihydro-4H-pyran-4-one (7.34%). Whilst research on its specific anticancer activity may be limited, anticancer potential of similar compounds in pyranone family suggests its possible role in combating cancer. Presence of palmitic acid (6.40%) could also play a key role in cytotoxic effect against HeLa cells. The anticancer activity of palmitic acid was highlighted in a latest study[36] which reported that palmitic acid inhibits endometrial cancer cell proliferation and invasion, by increasing the formation of cellular lipid droplets, which in turn reduces tumor growth. Palmitic acid has been shown to induce cell cycle arrest and promote apoptosis in human neuroblastoma and breast cancer cells.[21] In our study, the acetone extract contained n-octadecane (5.44%) which could contribute to the anticancer activity. Previous research also demonstrated the antioxidant properties and anticancer activity of octadecanoic acid.[23] Similarly, octadecenoic acid (n-Octadecane) and its methyl esters prevented tumor growth in HeLa cells.[24] Antimicrobial and anticancer activity of Di-n-decylsulfone was also reported.[37] From Table 2, the acetone extract of *A. racemosus* has phytochemical constituents with established anticancer and antioxidant activities. The cytotoxicity against HeLa cells in the present study could be attributed to the presence of these compounds.

On the other hand, GC profile of the methanolic extract of *A. racemosus* showed the presence of 5 hydroxy methyl furfural (12.83%) followed by 3-Deoxy-d-mannoic lactone (6.69%), heptyl caprylate (6.36%), dihydroxy acetone (6.25%), 6,6-Dideutero-Nonen-1-Ol-3 (6.08%) xanthosine (5.96%), and many other compounds in small amounts (Table 2). The cytotoxic and antioxidant property of the above compounds was established against different types of cancers.[26,27,30] In our study, the antioxidant and cytotoxic activity of the methanolic extract of *A. racemosus* could be due to presence of the above compounds.

In the present study, FRAP activity was relatively more effective in acetone solvent compared to methanolic solvent (Table 4a). *A. racemosus* consistently demonstrated a dose-dependent relationship between the concentration of plant extracts and their ferric reducing power, establishing its antioxidant capacity. The antioxidant capacity could be due to the plant's rich phenolic content. It is well known that phenolic compounds effectively scavenge free radicals and reduce metal ions. These findings support the medicinal use of *A. racemosus* in dealing oxidative stress-related conditions, including cancer.

The DPPH scavenging activity of *A. racemosus* in acetone range from 4.46% at 400 µg/mL to 59% at 6400 µg/mL concentration. On the other hand, in methanol solvent, the plant extract could only scavenge 22.7% even at 6400 µg/mL concentration (Table 4a). These results when compared to ascorbic acid (Table 4b) indicate that methanolic extract could not scavenge DPPH radical effectively. This suggests that acetone is more efficient solvent for extracting antioxidant compounds from *A. racemosus* compared to methanol, which could be attributed to acetone's ability to dissolve a wider range of polar and non-polar compounds, including various antioxidants.

From Table 5, the acetone extract of *A. racemosus* showed an $IC_{50}$ value of 5416 µg/mL for DPPH radical scavenging, which is notably higher compared to L-ascorbic acid (378.32 µg/mL), a well-known antioxidant. On the other hand, methanolic extract could only scavenge up to 23% even at 6400 µg/mL. The high $IC_{50}$ value of the acetone extract specifies that a higher concentration of this extract is required to achieve 50% DPPH scavenging activity compared to L-ascorbic acid. Although *A. racemosus* exhibited significant antioxidant activity, it is less potent compared to L-ascorbic acid. These results are in alignment with earlier research[38] which supports the concept that acetone extracts are more potent than methanol extracts.

In ABTS assay, the initial scavenging activity of the methanol extract was low which indicates that it takes more time to extract the antioxidant compounds compared to acetone. However, both solvents demonstrated a dose-dependent upsurge in scavenging activity at high concentrations. This suggests that both extracts contain equal amounts of active antioxidants, and the methanol extract eventually matches the acetone extract's performance at higher concentrations (Table 5). This underscores that maximum scavenging potential can be attained with sufficient concentration of phytochemicals in the plant.

The dose-dependent antioxidant activity suggests that higher concentrations of the acetone extract showed significant antioxidant effects, which could be due to presence of potential phytochemicals observed in this investigation (Tables 1, 4a and 4b). In our study, the $IC_{50}$ value of *A. racemosus* is 2149 µg/mL in acetone and 2880 µg/mL in methanol compared to L-ascorbic acid (215 µg/mL). Overall, the higher $IC_{50}$ values for the acetone and methanol extracts of *Asparagus racemosus* illustrate that higher concentrations of extracts are required to achieve 50% ABTS scavenging activity compared to L-ascorbic acid.

In the present study, the anticancer activity of *A. racemosus* in both acetone and methanol solvents was evaluated by MTT assay using HeLa cells. The plant extracts in both solvents demonstrated significant dose-dependent cytotoxic effects on HeLa cell lines, validating their potential as anticancer agents. The cell viability

in acetone extract was maximum 100 µg/mL (87.1%) and a significant decrease (18.9%) was observed at 1600 µg/mL. Similar trend was also observed in methanol which exhibited 89.3% cell viability at 100 µg/mL which decreased to 15.4% at 1600 µg/mL (Figure 1a and b). The $IC_{50}$ values for the acetone and methanol extracts were 771.44 µg/mL and 768.38 µg/mL, respectively (Table 6), underlining the comparable cytotoxic efficiency of the two solvent extracts against HeLa cells. These findings advocate that both extracts of *A. racemosus* comprise phytochemicals with good anti-proliferative effects on HeLa cells. Previous studies have emphasized the potential of flavonoids, terpenoids, and other phenolic compounds in *A. racemosus* to its anticancer activity.[39] The findings of our study supported by earlier reports accentuates the therapeutic potential of *A. racemosus* in cervical cancer treatment.

Although the results of our present study are encouraging, more research is required to isolate and identify the specific compounds responsible for the observed cytotoxicity which can be used as adjuvants in cancer therapy. Moreover, studies on the mechanistic aspects could provide deeper understanding into how these extracts induce cell death in cervical cancer cells. The potential therapeutic application of *A. racemosus* extracts in cervical cancer treatment requires further investigation.

## CONCLUSION

This study convincingly demonstrated the antioxidant and anticancer properties of *A. racemosus*. The Ferric Reducing Antioxidant Power (FRAP) assay demonstrated that acetone extracts of *A. racemosus* exhibited superior ferric reducing potential compared to methanol. Similar trend was observed in DPPH scavenging activity, wherein acetone extract showed a significant increase compared to methanolic extract. The ABTS assay results demonstrated a maximum scavenging activity of up to 87.9% in both the extracts.

In terms of anticancer activity, *A. racemosus* exhibited significant cytotoxic effects against HeLa cells, with dose-dependent increase in cell mortality. The observed cytotoxic effects are putative due to induction of apoptosis and cell cycle arrest. These findings suggest that *A. racemosus* holds substantial promise as a therapeutic agent and could potentially augment the cancer treatment strategies. Further research is necessary to fully elucidate the mechanisms of action and optimize its application in cancer therapy.

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## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethics approval was not sought as the study is conducted *in vitro* using commercially procured cell lines. Consent to participate is not applicable since study does not involve any animal/human participation.

## CONFLICT OF INTEREST

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

## ABBREVIATIONS

**GC-MS:** Gas Chromatography- Mass Spectroscopy; **MTT assay:** 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide assay.

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