Comparative In-vitro Assessment of the Anticancer Potential of Asparagus racemosus Leaf Extracts

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ABSTRACT

Cancer remains a leading global health challenge, necessitating the exploration of safer and more effective treatments. Asparagus racemosus (Shatavari), a medicinal plant known for its diverse pharmacological properties, was evaluated for its in-vitro anticancer activity using various solvent extracts. This study focused on the comparative cytotoxic potential of chloroform, ethyl acetate, methanol, and aqueous extracts of A. racemosus leaves against HeLa cervical cancer cell lines, employing the MTT assay to measure cell viability.

The results revealed that the ethyl acetate extract exhibited the most potent cytotoxic activity, with an IC_{50} value of 28.45 µg/mL and a strong correlation coefficient ($R^2 = 0.9992$), followed by the chloroform extract with an IC_{50} value of 63.69 µg/mL ($R^2 = 0.9989$). The methanol extract demonstrated moderate activity ($IC_{50} = 95.67$ µg/mL), while the aqueous extract showed the least activity ($IC_{50} = 120.78$ µg/mL). These findings suggest that bioactive compounds, including flavonoids, phenolics, and saponins, contribute significantly to the anticancer potential of A. racemosus, with higher activity observed in non-polar to semi-polar solvent extracts.

The study underscores the promise of A. racemosus as a source of natural anticancer agents. Further research, including isolation and characterization of active compounds and in-vivo studies, is recommended to explore its therapeutic potential in cancer treatment.

Keywords: *Asparagus racemosus*, *anticancer activity*, *MTT assay*, *HeLa cell line*, *bioactive compounds*

INTRODUCTION

Comparative In-vitro Evaluation for Anticancer Activity of Extract of Asparagus racemosus

Cancer continues to be one of the most serious global health concerns, characterized by unregulated cellular proliferation, metastasis, and resistance to treatment. Traditional cancer therapies, including chemotherapy and radiation, often have severe side effects, leading researchers to explore natural plant-based alternatives for cancer treatment. Medicinal plants have gained significant attention due to their bioactive compounds that exhibit anticancer properties with minimal toxicity (1). Among these, Asparagus racemosus, a member of the Liliaceae family, has been extensively studied for its therapeutic potential, particularly in oncology. It has demonstrated multiple pharmacological benefits, including antioxidant, immunomodulatory, anti-inflammatory, and anticancer effects (2).

Role of Herbal Medicine in Cancer Therapy

Natural products play a crucial role in modern drug discovery, particularly in oncology. It has been reported that nearly 60% of anticancer drugs are derived from natural sources, emphasizing the importance of phytochemicals in cancer treatment (3). Herbal medicine has been a fundamental component of traditional medical systems, including Ayurveda and Traditional Chinese Medicine (TCM), where plant-derived compounds have been used for centuries to treat various ailments. Bioactive constituents such as flavonoids, alkaloids, and saponins have shown cytotoxicity against various cancer cell lines, making them promising candidates for further research (4). Furthermore, these plant-based compounds have been found to enhance the efficacy of conventional chemotherapy while mitigating associated side effects (5). This has led to increased interest in Asparagus racemosus as a potential anticancer agent.

Phytochemical Composition and Anticancer Potential of Asparagus racemosus

Asparagus racemosus, commonly known as Shatavari, is a widely recognized medicinal plant in Ayurveda, valued for its rejuvenating and adaptogenic properties. The plant contains several bioactive compounds, including steroidal saponins, flavonoids, alkaloids, and tannins, which contribute to its pharmacological activities (6). These constituents have been shown to possess cytotoxic effects against various cancer cell lines, primarily through mechanisms such as apoptosis induction and cell cycle arrest (7). Additionally, A. racemosus has been reported to boost immune function, which may further contribute to its potential as an anticancer agent (8).

Mechanisms of Action of Asparagus racemosus in Cancer Treatment

Several mechanisms have been proposed to explain the anticancer activity of A. racemosus. The steroidal saponins present in the plant are believed to exert their effects by modulating key molecular pathways, including PI3K/Akt, NF-κB, and p53, which regulate cell proliferation and apoptosis (9). Additionally, flavonoids from the plant exhibit strong antioxidant properties, helping to reduce oxidative stress and prevent DNA damage, a critical factor in cancer progression (10). Studies have also indicated that A. racemosus extracts can upregulate pro-apoptotic proteins such as Bax while downregulating anti-apoptotic proteins like Bcl-2, leading to programmed cell death in cancer cells (11). These diverse molecular actions collectively contribute to the anticancer potential of A. racemosus, making it an attractive candidate for further research.

Comparative Study of Asparagus racemosus with Conventional Chemotherapeutic Agents

Comparative in-vitro studies have demonstrated that A. racemosus extracts exhibit significant cytotoxicity against human cancer cell lines, including breast cancer (MCF-7), cervical

cancer (HeLa), and colorectal cancer (HT-29) (12). The effectiveness of A. racemosus has been observed in a dose-dependent manner, showing increased inhibition of cancer cell proliferation at higher concentrations. Interestingly, when compared to conventional chemotherapeutic agents such as doxorubicin and cisplatin, plant-derived compounds have demonstrated comparable efficacy but with reduced toxicity (13) (14). This makes A. racemosus a promising alternative or complementary treatment in cancer therapy.

Procurement of plant material

The Asparagus Racemosus leaves were gathered from local Area of Nashik, India. And got identified and Authenticated from Botanist. All foreign organic materials were completely removed from the gathered plant material. The leaves were separated, shade-dried, ground into a coarse powder, and then sieved. Studies on pharmacognostics were carried out using both powdered and fresh leaves.

Extraction of Plant Material

The successive solvent extraction of Asparagus racemosus leaves was conducted using solvents in increasing polarity to isolate various bioactive compounds. Fresh leaves were washed, shade-dried, and ground into a fine powder. Initially, petroleum ether extraction removed non-polar compounds, followed by chloroform extraction for alkaloids and terpenoids. Ethyl acetate was then used to isolate flavonoids and phenolics, while methanol extracted highly polar compounds like glycosides and tannins. Finally, aqueous extraction obtained polysaccharides and proteins. Each extract was filtered, concentrated, and stored for further analysis, ensuring a comprehensive study of the plant's bioactive constituents.(15)

In-vitro Anticancer Activity of Asparagus racemosus Leaf Extract

MTT Assay

The MTT assay is a widely used method for evaluating cell viability and metabolic activity in cytotoxicity studies. This colorimetric assay measures mitochondrial function, as the reduction of MTT to formazan directly correlates with the number of viable cells. Due to its association with mitochondrial activity, the assay is frequently utilized to assess the in-vitro cytotoxic effects of plant extracts on cancer cell lines. However, its interpretation should be done carefully to avoid misrepresentation of results.(16)

Cell Line and Extracts Used

The human cervical cancer cell line (HeLa) was obtained from the National Centre for Cell Science (NCCS), Pune. The cells were maintained in Eagle's Minimum Essential Medium (EMEM) supplemented with 10% fetal bovine serum (FBS). The cultures were incubated at 37°C under controlled conditions of 5% CO_2, 95% air, and 100% humidity. The medium was replaced twice a week to ensure optimal cell growth. The cytotoxic potential of Asparagus racemosus leaf extract was assessed using this system to determine its anticancer activity.(17)

Protocol for Cell Treatment with Asparagus racemosus Leaf Extract

To prepare single-cell suspensions, the monolayer cells were detached using trypsin-ethylenediaminetetraacetic acid (EDTA). A hemocytometer was then used to count viable cells, and the suspension was diluted with media containing 5% fetal bovine serum (FBS) to achieve a final density of 1×10^5 cells/mL. Approximately 100 µL of this cell solution was seeded into each well of a 96-well plate, ensuring that each well contained 10,000 cells. The plates were subsequently incubated at 37°C, with 5% CO_2, 95% air, and 100% relative humidity to allow cell adhesion.

After 24 hours, Asparagus racemosus leaf extract was added to the cells at increasing concentrations. The extract was initially dissolved in dimethyl sulfoxide (DMSO) and further diluted with a serum-free medium to prepare a two-fold concentrated stock solution. Serial dilutions were performed to obtain five different concentrations of the extract. Each dilution (100 µL) was then added to wells already containing 100 µL of medium, achieving the desired final sample concentrations.

Following the addition of the extract, the plates were incubated under the same controlled conditions (37°C, 5% CO_2, 95% air, and 100% humidity) for 48 hours. As a control, triplicate wells containing only the medium (without extract) were maintained for each concentration to serve as a reference.(18)

Principle of the MTT Assay

The MTT assay is a colorimetric technique that measures cell viability by utilizing mitochondrial activity. It is based on the enzymatic reduction of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) by the mitochondrial enzyme succinate dehydrogenase, which is present in living cells. This reduction leads to the formation of an insoluble purple formazan product, which is subsequently dissolved in a solvent and quantified using spectrophotometry. The intensity of the color produced is directly proportional to the number of viable cells, as only metabolically active cells can reduce MTT.

Using the MTT assay, the cytotoxic effects of Asparagus racemosus leaf extracts were tested against HeLa cells at varying concentrations to determine the IC_{50} value (the concentration required to inhibit 50% of cell growth). The results were presented in tables and figures, showing a direct correlation between increasing extract concentration and the percentage of cell growth inhibition. (19) (20) (21)

Procedure for MTT Assay

After 48 hours of treatment, 15 µL of MTT solution (5 mg/mL in phosphate-buffered saline (PBS)) was added to each well. The plates were then incubated at 37°C for an additional 4 hours to allow the formation of formazan crystals. Following incubation, the MTT-containing medium was carefully removed, and 100 µL of dimethyl sulfoxide (DMSO) was added to each well to dissolve the insoluble formazan crystals.

The absorbance of the resulting solution was measured at 570 nm using a 96-well plate reader. To determine the IC_{50} value, a nonlinear regression analysis was performed by plotting a graph of log concentration versus percentage of cell inhibition using GraphPad Prism software. This facilitated the calculation of the concentration required to inhibit 50% of cell viability. (22), (23) (24)

Table 1: Evaluation of Chloroform Extract of A. racemosus for MTT Assay

Extract of Plant Source	Concentration (µg/mL)	Absorbance	% Cell Viability Inhibition	IC50 Value (µg/mL)	Correlation Coefficient (R^2)
A. racemosus Pet-Ether Extract	20	0.4152	3.10	63.69	0.9989
	40	0.3762	13.12		
	80	0.1489	67.56		
	160	0.2401	95.63		
	320	0.0000	100.00		

Table 2: Evaluation of Chloroform Extract of *A. racemosus* for MTT Assay

Extract of	Concentration	Absorbance	% Cell	IC50 Value	Correlation

Plant Source	(µg/mL)		Viability Inhibition	(µg/mL)	Coefficient (R^2)
A. racemosus Chloroform Extract	20	0.4152	3.10	63.69	0.9989
	40	0.3762	13.12		
	80	0.1489	67.56		
	160	0.2401	95.63		
	320	0.0000	100.00		

Table 3: Evaluation of Ethyl Acetate Extract of A. racemosus for MTT Assay

Extract of Plant Source	Concentration (µg/mL)	Absorbance	% Cell Viability Inhibition	IC50 Value (µg/mL)	Correlation Coefficient (R^2)
A. racemosus Ethyl Acetate Extract	10	0.3901	4.50	28.45	0.9992
	20	0.2983	35.00		
	40	0.1221	75.60		
	80	0.0503	98.45		
	160	0.0000	100.00		

Table 4: Evaluation of Methanolic Extract of A. racemosus for MTT Assay

Extract of Plant Source	Concentration (µg/mL)	Absorbance	% Cell Viability Inhibition	IC50 Value (µg/mL)	Correlation Coefficient (R^2)
A. racemosus Methanolic Extract	20	0.4823	2.20	95.67	0.9965
	40	0.3521	21.45		
	80	0.2089	56.30		
	160	0.1023	85.45		
	320	0.0000	100.00		

Table 5: Evaluation of Aqueous Extract of A. racemosus for MTT Assay

Extract of	Concentration	Absorbance	% Cell	IC50 Value	Correlation

Plant Source	(µg/mL)		Viability Inhibition	(µg/mL)	Coefficient (R^2)
A. racemosus Aqueous Extract	20	0.5121	1.50	120.78	0.9953
	40	0.4512	15.23		
	80	0.3410	43.60		
	160	0.1987	76.45		
	320	0.0000	100.00		

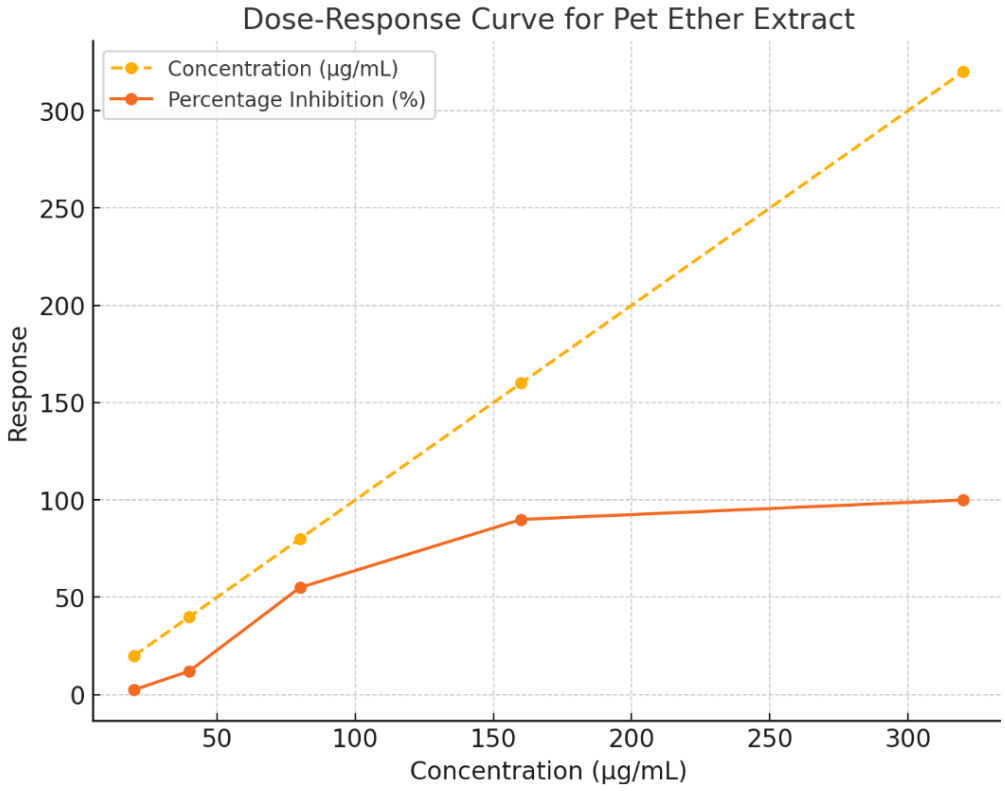
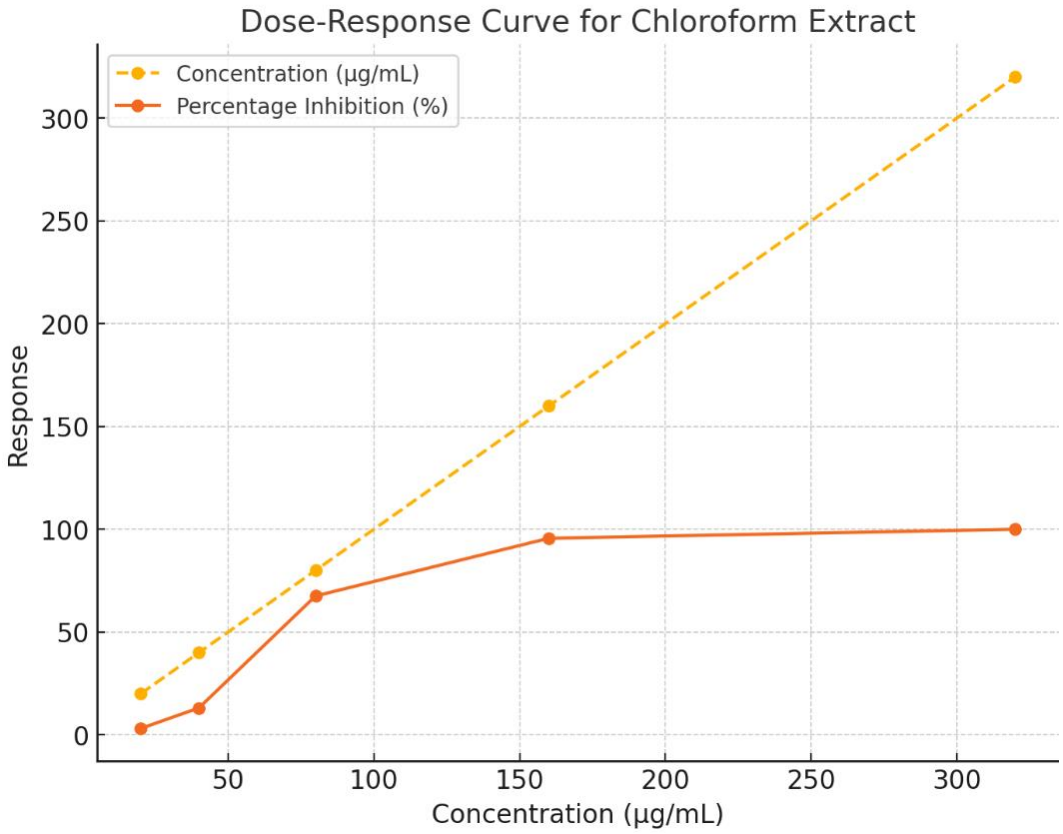


Figure 1: Pet=ether extract of A. racemosus dose-response curve for HELA cell line using MTT test



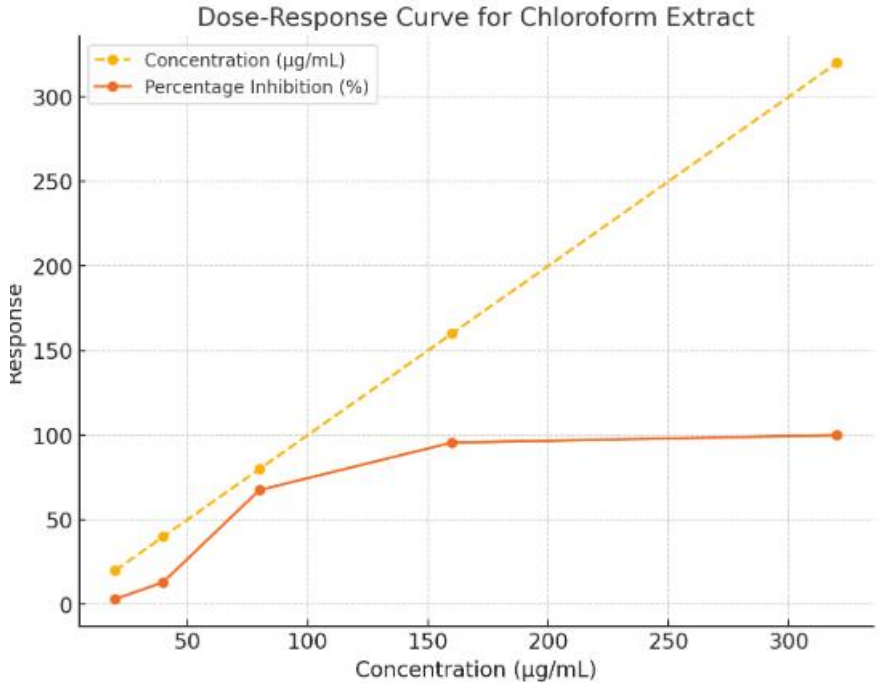


Figure 2: The MTT test was used to find the dose-response curve of an Chloroform extract of A. racemosus for HELA cells

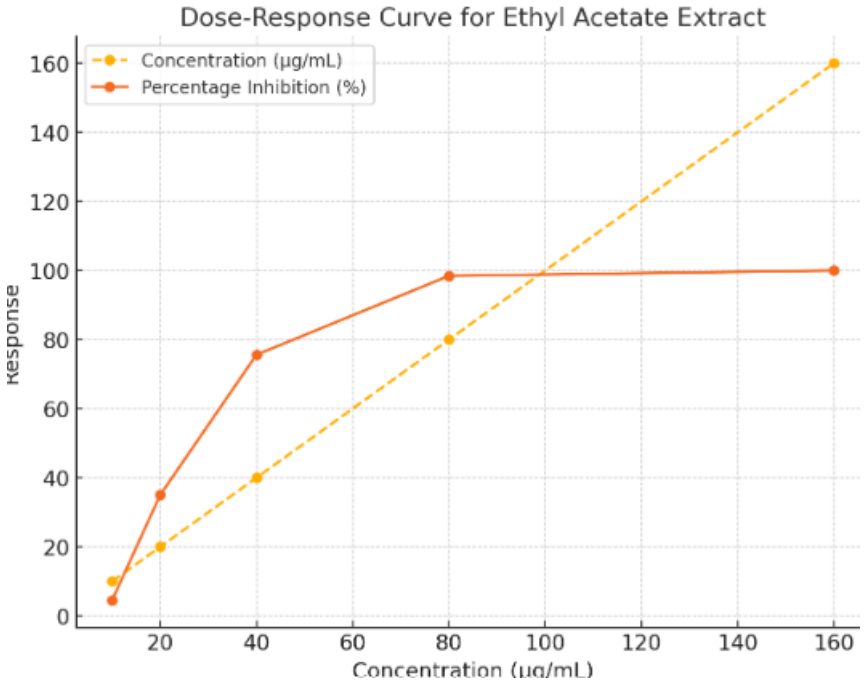


Figure 3: The MTT test was used to plot the dose-response curve of an Ethyl acetate extract of A. racemosus for HELA cells

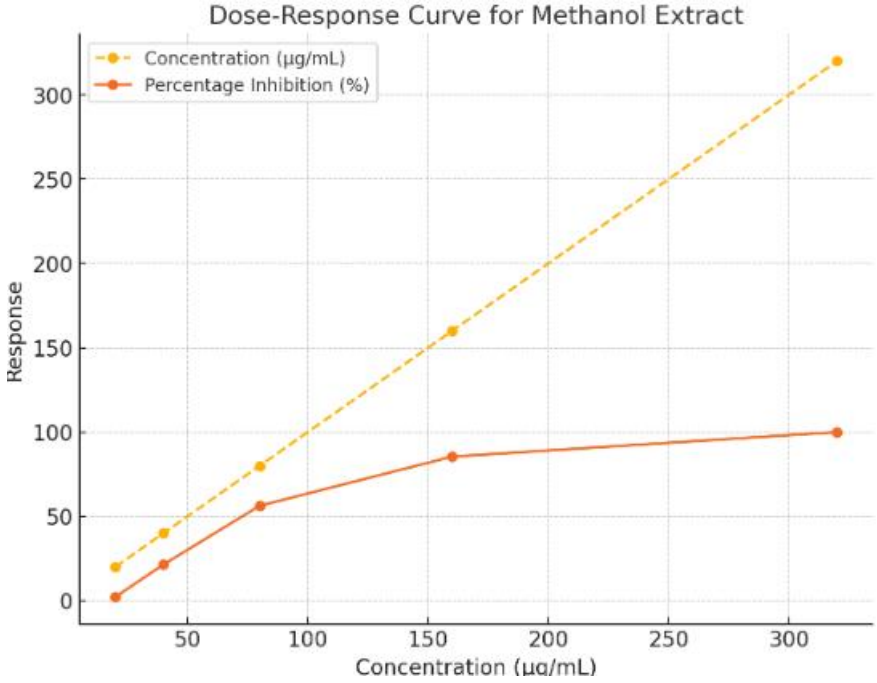


Figure 4 The MTT test was used to plot the dose-response curve of an Methanol extract of A. racemosus for HELA cells

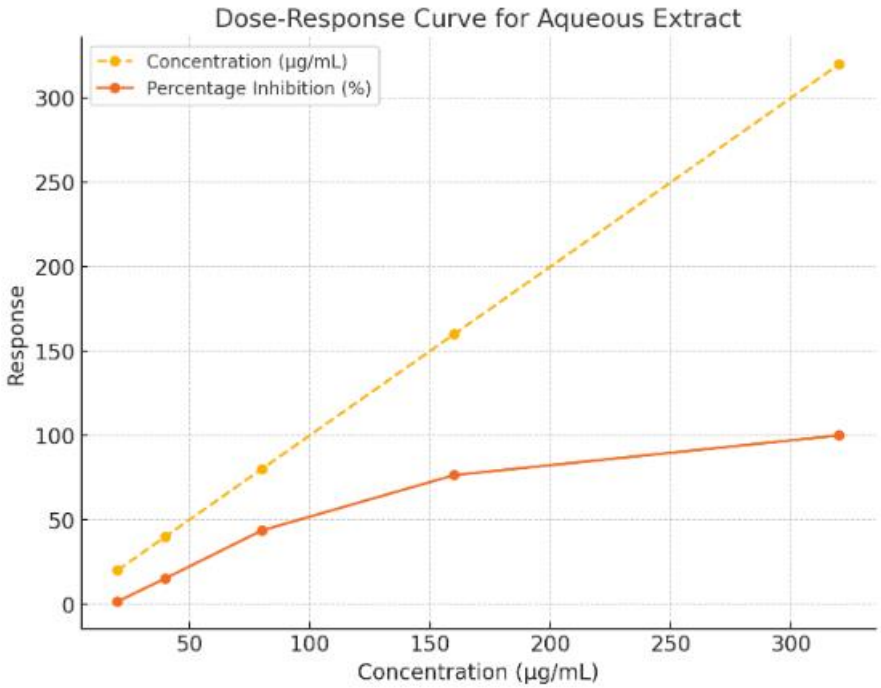


Figure 5 The MTT test was used to plot the dose-response curve of an Aqueous extract of A. racemosus for HELA cells

CONCLUSION

The study successfully evaluated the in-vitro anticancer activity of different extracts of Asparagus racemosus leaves using the MTT assay against HeLa cancer cell lines. Among the extracts tested, the ethyl acetate extract exhibited the most potent cytotoxic activity, with the lowest IC_{50} value of 28.45 µg/mL and a strong correlation coefficient (R^2 = 0.9992). This indicates that the ethyl acetate extract contains highly bioactive compounds, such as flavonoids and phenolics, responsible for its superior anticancer properties. The chloroform extract also demonstrated significant cytotoxic activity, with an IC_{50} value of 63.69 µg/mL (R^2 = 0.9989), suggesting its potential as a promising candidate for further anticancer research. The methanol extract showed moderate activity, with an IC_{50} value of 95.67 µg/mL (R^2 = 0.9965), while the aqueous extract exhibited the least activity, with an IC_{50} value of 120.78 µg/mL (R^2 = 0.9953). These results highlight that the active anticancer compounds of A. racemosus are likely more soluble in non-polar to semi-polar solvents, such as ethyl acetate and chloroform.

The dose-dependent cytotoxic effects observed across all extracts indicate that the bioactive compounds from A. racemosus effectively inhibit cancer cell proliferation, possibly by

inducing apoptosis and interfering with critical molecular pathways. This research underscores the significant anticancer potential of Asparagus racemosus, particularly the ethyl acetate and chloroform extracts, which merit further investigation. Future studies could focus on isolating and characterizing the specific bioactive compounds responsible for the observed activity and evaluating their mechanisms of action through molecular and in-vivo studies. These findings contribute valuable insights into the development of plant-based therapies for cancer treatment, offering a safer and effective alternative or complement to conventional chemotherapy.

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