RESEARCH ARTICLE

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

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ABSTRACT

The main purpose of this study is to look into how protective *Asparagus racemosus* leaves are *in-vitro*. Cancer is a major reason people die around the world. Many of the bad effects of the allopathic drugs that are now used to treat cancer have caused people to turn to plant medicine. Some of the solvents that were used in the sequential solvent extraction method were chloroform, ethanol and ethyl acetate. The chloroform extract had a higher percentage yield compared to the other extracts. Because it had anticancer activity *in-vitro*, the therapeutically active extract was picked to have anticancer activity in living things. The MTT test was used to see if the chloroform, ethyl acetate, and ethanol extracts could help fight cancer in HeLa cell types. The MTT assay showed that chloroform and ethyl acetate extracts had strong anticancer activity. These two extracts were chosen for study into how they work against cancer in living things.

Keywords: MTT assay, *Asparagus racemosus*, Anticancer activity, Cancer.

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INTRODUCTION

Folk and traditional medicines use plants and herbs that are now known to be one of the main sources of ideas for new chemoprevention drugs.[1] About 60% of the anticancer drugs we use today come from natural sources like plants, animals, and microbes.[2] Herbal medications are created using plant-based materials or preparations that contain raw or processed components from one or more plants that have therapeutic qualities. These systems include Ayurveda, Unani, and Siddha. They are taken as dietary supplements to treat or avoid common illnesses.[3,4] Throughout history, people have used goods made from plants for many reasons, including medicine. In a way, herbs can be thought of as biosynthetic chemical labs because they can make many different chemicals.[5,6] Herbal medicines can have anything from plant parts to separated, purified active ingredients. Leaves, roots, bark, seeds, and flowers could be some of their main food sources.[7] You can apply them to your skin, eat, drink, or breathe them in. As a way to fight off diseases and infections, larger plants make a number of secondary metabolites on their own.[8,9] The Ayurvedic medical system in India treats a variety of diseases, including cancer, primarily with plant-based medications or mixes. About 91% of the 877 new small molecule medicines that were released around the world between 1981 and 2002 got their start in nature.[10] Recent polls show that one-third of people regularly use medical natural goods, and it's possible that one-half of cancer patients do the same.[11] A quick look at Japanese plants that fight cancer shows that they contain phytochemicals like saponins, terpenoids, polyphenols, flavonoids, polysaccharides, alkaloids, and glycosides that have been shown to stop tumor growth.[12,13] A lot of research has been done in the last few years on how polyphenols, flavonoids, and triterpenes can help fight cancer.[14] In Indian traditional medicine, some plants are used to treat cancer, but most of these plants have not been studied in depth by scientists.[15] If a comprehensive ethnopharmacological investigation is conducted on one or more of the plants in the traditional system[16], it is certain that effective tumour drugs will be made. Because *Asparagus* is so popular, the current

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study's goal is to find out if *Asparagus racemosus* leaves can help fight cancer.[17]

The plant has been used in many traditional and ethnobotanical ways in many different countries.[18] Traditional medicine says that *A. racemosus* Willd. (Liliaceae) can help with many health problems, such as cancer, inflammation, epilepsy, night blindness, TB, leprosy, spasms, diarrhea, gonorrhea, piles, cough, diabetes, headaches, rheumatism, stomach problems, and more breastfeeding. The aerial parts can kill germs, fungi, cancer cells, and heart rhythm problems.[19] There is no scientific proof that the upper part can help treat cancer as it has been used in the past. So, the point of this study is to find out how the leaves of *A. racemosus* affect the body's ability to fight cancer.[20, 21]

MATERIALS AND METHODS

Collection of the Plant

A. racemosus leaves that were strong and new were brought back from India in September 2023. For anatomical studies, the leaves were quickly sealed with FAA, which stands for formalin, acetic acid, and ethyl alcohol.[22]

In-vitro Anticancer Activity

MTT assay

The MTT test, which is widely used in investigations on cell toxicity, is frequently employed and misinterpreted. A frequently employed method for assessing cellular metabolic activity is the MTT assay. Nevertheless, it is frequently implemented and understood inaccurately. The assay is commonly employed to assess the *in-vitro* cytotoxic effects of medicines on cell lines due to the correlation between total mitochondrial activity and the number of viable cells in most cell types.

Cell line and extracts used

We obtained the human cervical cancer cell line HeLa from the National Centre for Cell Science (NCCS) in Pune. The cells were cultured in Eagles minimum essential medium with 10% fetal bovine serum (FBS). At 37°C, the cells were kept in 95% air, 100% humidity, and 5% CO_2. Every week, maintenance cultures were changed, and the medium was changed twice a week.[23, 24]

Protocol for cell treatment

To make single-cell suspensions, trypsin-ethylene-diaminetetraacetic acid (EDTA) was used to hold the monolayer cells together. Then, a hemacytometer was used to count the living cells, and media containing 5% FBS was added until the final density reached 1×105 cells/mL. About 100 µL of the cell solution was put into 96-well plates so that there were 10,000 cells in each well. To aid in the cells' adhesion, the plates were subsequently placed in an incubator with the following settings: 37°C, 5% CO_2, 95% air, and 100% relative humidity. Over the course of 24 hours, the test samples were added to the cells in increasing amounts. First, they were mixed with dimethyl sulfoxide (DMSO). Then, a small amount of the

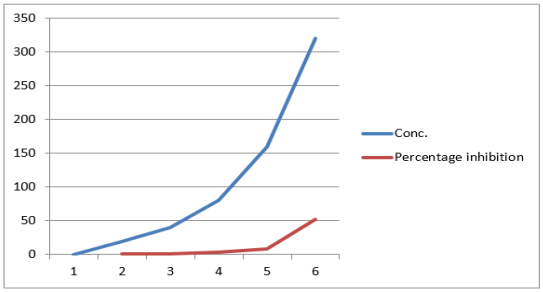

Figure 1: Chloroform extract of *A. racemosus* dose-response curve for HELA cell line using MTT test

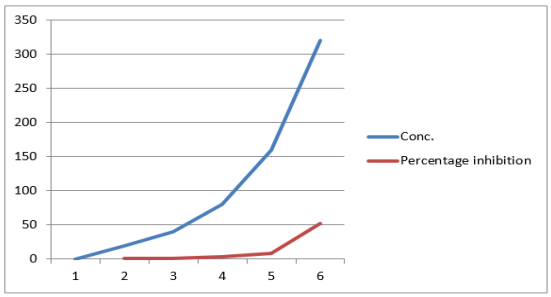

Figure 2: The MTT test was used to find the dose-response curve of an ethyl acetate extract of A. racemosus for HELA cells.

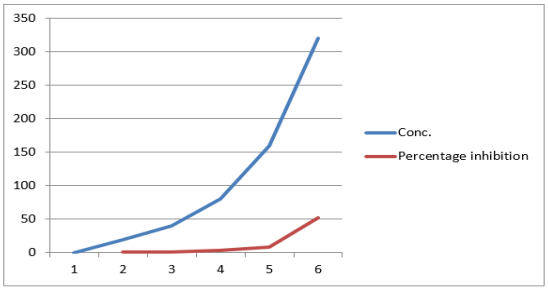

Figure 3: The MTT test was used to plot the dose-response curve of an ethanolic extract of *A. racemosus* for HELA cells.

sample solution was mixed with a serum-free medium until it was twice as strong as it was supposed to be for the final test. By doing four more series of dilutions, five sample amounts were found. The wells that were already holding 100 µL of medium were filled with 100 µL portions of each of these sample dilutions. This gave us the final sample amounts we needed. After the sample was added, the plates were kept at 37°C, 5% CO2, 95% air, and 100% relative humidity for another 48 hours. As a reference, three copies of the media without samples were kept for each concentration.[25-27]

Principle of MTT assay

The colorimetric assay relies on the tetrazolium ring's cleavage by succinate-dehydrogenase, an enzyme present in living cells' mitochondria. This leads to the development of an insoluble purple formazan product from 3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT), which is quantified using spectrophotometry. The number of viable cells and the amount of formazan produced are highly connected, and only living cells with functioning mitochondria can reduce the MTT.[28, 29]

Table 1: Chloroform extract of *A. racemosus* for MTT Assay

Extract of plant source	*Conc. (µg/mL)*	*Absor bance*	*Percentage inhibition*	*IC50 (µg/mL)*	R^2 *Value*
Asparagus racemosusus Chloroform exrtract	20	0.4152	3.1020	63.690	0.9989
	40	0.3762	13.123		
	80	0.1489	67.560		
	160	0.2401	95.635		
	320	0.0000	100.0		

Table 2: Ethyl acetate extract of *A. racemosus* for MTT Assay

Extract of plant source	*Conc. (µg/mL)*	*Absor bance*	*Percentage inhibition*	IC_{50} *(µg/mL)*	R^2 *Value*
A. racem osusus Ethyl acetate extract	20	0.4230	0.2500	88.963	0.9990
	40	0.4132	1.8230		
	80	0.3011	30.360		
	160	0.0230	96.352		
	320	0.0039	99.999		

Table 3: Ethanol extract of *A. racemosus* for MTT Assay

Extract of plant source	*Conc. (µg/mL)*	*Absor bance*	*Percentage inhibition*	IC_{50} *(µg/mL)*	R^2 *Value*
A. race mosus ethanol extract	20	0.4200	0.8123	236.8	0.9983
	40	0.4132	1.4521		
	80	0.4125	3.1250		
	160	0.3965	8.2500		
	320	0.2120	51.864		

Procedure

Following a 48-hour period, each well-received 15 µL of MTT (5 mg/mL) in phosphate-buffered saline (PBS). After that, the wells were kept at 37°C for an additional four hours. After turning off the MTT medium, the formazan crystals that had formed were broken up with 100 µL of DMSO. A 96-well plate counter was utilized to measure the absorbance at 570 nm. A nonlinear regression graph between the log concentration and the percentage of cell blockage was created using the GraphPad Prism software. This made determining the IC_{50} easier.[30-33]

RESULTS AND DISCUSSION

The following table shows how well the chloroform, ethyl acetate and ethanol extracts worked against cancer in the lab. Table 1 and Figure 1 comprise the chloroform extract of *A. racemosus* for MTT Assay.

Table 2 and Figure 2 represent the ethyl acetate extract of *A. racemosus* for MTT Assay**.**

Table 3 and Figure 3 represent the ethanol extract of *A. racemosus* for MTT Assay.

The anticancer properties of chloroform, ethyl acetate and ethanol extracts were evaluated in a laboratory setting using the MTT experiment. To determine the IC_{50} value, the extracts were tested to check if they killed HeLa cells at various concentrations.

Tables and figures are used to display the results. As the test chemical's concentration increased, so did the percentage of growth inhibition. On the HeLa cell line, the IC_{50} values for the extracts of chloroform, ethanol, and 88.963 and 236.8 µg/mL were determined, and the corresponding R2 values were 0.9989, 0.9990, and 0.9983. The ethanol extract was utilized for additional research on cancer in live things, but the chloroform and ethyl acetate extracts were far more successful in eliminating cancer cells in the lab.

CONCLUSION

Cancer is a major reason people die around the world. Many of the bad effects of the allopathic drugs that are now used to treat cancer have caused people to turn to plant medicine. Because of this, it was important to find plant sources of natural cancer drugs. *A. racemosus* Willd., was picked for this study because it has been used for a long time to treat cancer. A search of the books turned up almost nothing about the leaves of this plant. Because there isn't much information on leaves, the study was justified by the hope of discovering new phytochemical profiles and pharmacological activities. Because it had anticancer activity *in-vitro*, the therapeutically active extract was picked to have anticancer activity in living things. The MTT test was used to see if the chloroform, ethyl acetate, and ethanol extracts could help fight cancer in HeLa cell types. The MTT assay showed that chloroform and ethyl acetate extracts had strong anticancer activity. These two extracts were chosen for study into how they work against cancer in living things.

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