

## *In vitro* cytotoxic activity of *Asparagus racemosus* on ovarian carcinoma cell lines (SKOV-3) by 3-(4, 5dimethylthiazol2-yl)-2, 5-diphenyl tetrazolium bromide assay

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### ABSTRACT

**Introduction:** Natural products have been used since ancient times for the treatment of many diseases. Biodiversity and traditional medical knowledge had provided useful lead compounds for cancer chemotherapy. In the Indian medicine system, *Asparagus racemosus*, commonly called Shatavari has been used as a nutritive tonic, general female reproductive tonic, fertility tonic, treatment for sexual debility, and as an aphrodisiac. **Aim:** The aim of the present study is to study the *in vitro* cytotoxic effect of *A. racemosus* extracts on SKOV3 cells line (HTB77) using 3-(4, 5dimethylthiazol2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay. **Materials and Methods:** SKOV3 cells line (HTB77) commercially purchased from the American Type Culture Collection were cultured in McCoy's 5A modified medium supplemented with fetal bovine serum (heat inactivated, 10%) and penicillin-streptomycin (1%) at 37°C in 5% CO<sub>2</sub>. MTT colorimetric assay that measures the reduction of yellow MTT by mitochondrial succinate dehydrogenase is used to check the viability of the cells. **Result:** It was observed that the extract showed promising anticancer activity toward the selected cancer cell line on dose-dependent manner. **Conclusion:** *A. racemosus* contains a rich source of steroidal saponins which are important secondary metabolites with cytotoxic activity. Furthermore, research should be directed toward the isolation of bioactive compounds from these plants.

**KEY WORDS:** 3-(4, 5dimethylthiazol2-yl)-2, 5-diphenyl tetrazolium bromide assay, *Asparagus racemosus*, Cytotoxic, SKOV3 cells line

### INTRODUCTION

Cancer is a major burden on the health-care system worldwide not only in the developed countries but also in the developing countries. Anticancer, or antineoplastic, drugs are used to treat malignancies or cancerous growths. Drug therapy may be used alone or in combination with other treatments such as surgery or radiation therapy. Anticancer drugs are used to control the growth of cancerous cells.<sup>[1]</sup> Around the world, tremendous research is being taking place for anticancer therapy. Cytotoxic agents are recognized to good anticancer agents in anticancer therapy and certain drugs are being developed. Natural products

have been used since ancient times for the treatment of many diseases. Before the 20<sup>th</sup> century, the majority of medicines used to treat human and animal illness were obtained from the leaves, barks, and roots of medicinal plants. Biodiversity and traditional medical knowledge had provided useful lead compounds for cancer chemotherapy. The recognition of natural products as prominent mediators of the critical pathways involved in the development and progression of cancer has renewed interest in their potential as chemopreventive and chemotherapeutic anticancer agents.<sup>[2]</sup>

*Asparagus racemosus*, commonly called Satavar, Shatavari, or Shatamull, is native to the Himalayas in India. It is successfully used in Ayurveda to cure many diseases. Its habitat is common at low altitudes in the shade and tropical climates throughout Asia, Australia, and Africa. Out of several species of *Asparagus* grown

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Website: [jprsolutions.info](http://jprsolutions.info)

ISSN: 0975-7619

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Received on: 08-01-2018; Revised on: 12-02-2019; Accepted on: 16-03-2019

in India, *A. racemosus* is most commonly used in indigenous medicine. The root is used medicinally. In the Indian medicine system, Shatavari is said to “give her capacity to have a hundred husbands.” In traditional Ayurvedic Gynecological prescribing, this herb has been used as a nutritive tonic, general female reproductive tonic, fertility tonic, treatment for sexual debility, and as an aphrodisiac.<sup>[3,4]</sup> It has also been used traditionally as a tonic for lactating women to improve the quality and quantity of breast milk. People use *A. racemosus* for dyspepsia, constipation, stomach spasms, and stomach ulcers. It is also used for fluid retention, pain, anxiety, cancer, diarrhea, bronchitis, tuberculosis, dementia, and diabetes. Some people use it to ease alcohol withdrawal.<sup>[5,6]</sup>

Pharmacologic research has found that the key constituents of Shatavari are steroidal saponins, including Shatavarin-I, alkaloids, and mucilage. The presences of the steroidal saponins suggest its activity as an estrogen modulator and a menstrual cycle regulator.<sup>[7]</sup> This plant also contains Vitamins A, B<sub>1</sub>, B<sub>2</sub>, C, E, Mg, P, Ca, Fe, and folic acid. Other primary chemical constituents of *Asparagus* are essential oils, asparagine, arginine, tyrosine, flavonoids (kaempferol, quercetin, and rutin), resin, and tannin. Shatavari has adaptogenic and immunomodulating properties and can be used as a very useful tonic herb for women with stress-induced and immune-mediated infertility.<sup>[8]</sup> It has antibacterial action; hence, it should be considered a general reproductive tonic for any woman who has a history of genitourinary infections.<sup>[9]</sup> In the present study, the *in vitro* cytotoxic effect of *A. racemosus* extracts (ARE) on SKOV3 cells line (HTB77) was studied using 3-(4, 5dimethylthiazol2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay.

## MATERIALS AND METHODS

### Chemicals and Reagents

McCoy's 5A medium, heat-inactivated fetal bovine serum (FBS), and penicillin-streptomycin were purchased from Thermo Fisher Scientific (USA). MTT and dimethyl sulfoxide (DMSO) were purchased from SD Fine Chemicals.

### Culture Medium

SKOV3 cells line (HTB77) commercially purchased from the American type culture collection were cultured in McCoy's 5A modified medium supplemented with FBS (heat inactivated, 10%) and penicillin-streptomycin (1%) at 37°C in 5% CO<sub>2</sub>.

### Cell Viability Assay

MTT Assay is a colorimetric assay that measures the reduction of yellow MTT by mitochondrial succinate dehydrogenase. Cancer cells were seeded at the density of  $2 \times 10^5$  cells/well was plated on

into 6 well plates and treated with the ARE for 24 h. The cells were permitted to adhere for 24 h, and the growth medium (MEM) removed using micropipette and the monolayer of cells washed twice with MEM without FBS to remove dead cells and excess FBS. 1 ml of medium (without FBS) containing different dilution of drugs/standards were added in respective wells; 200 µl of MTT (5 mg/ml in PBS) were added to each well, and the cells incubated further for 6–7 h in 5% CO<sub>2</sub> incubator. After removal of the medium, 1 ml of DMSO was added to each well. The effect of extracts on cell growth inhibition was assessed as percent cell viability, where vehicle-treated cells were taken as 100% viable. The cells were then exposed to with the medium alone (as negative control) and cisplatin (as positive control). The final dried extract was dissolved in 10% of DMSO, stored at 4°C, and subjected to MTT assay. Concentrations of the ARE ranging 50, 100, 250, 500, and 1000 µg/ml were used. The supernatant was removed and 50 µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, colored (dark purple) formazan product. The plates were placed on a shaker for 15 min and the absorbance was read on an enzyme-linked immunosorbent assay reader at 570 nm.<sup>[10,11]</sup> The cell mortality was checked for 24–48 h. Each experiment was carried out in triplicate and the half maximal inhibitory concentration (IC<sub>50</sub>) of ARE as the percentage survival of the cells was calculated according to the formula provided below: Percentage of viable cell concentration was calculated thus:

$$\text{Viability (\%)} = (\text{Mean test OD/control OD}) \times 100$$

## RESULTS AND DISCUSSION

In this study, the cytotoxic activity of ARE was determined using MTT assay in SKOV3 cells line (HTB77) exposed to 50–1000 µg/ml of extracts and the results are given in Table 1 and Figure 1. It was observed that the extract showed promising anticancer activity toward the selected cancer cell line on dose-dependent manner.

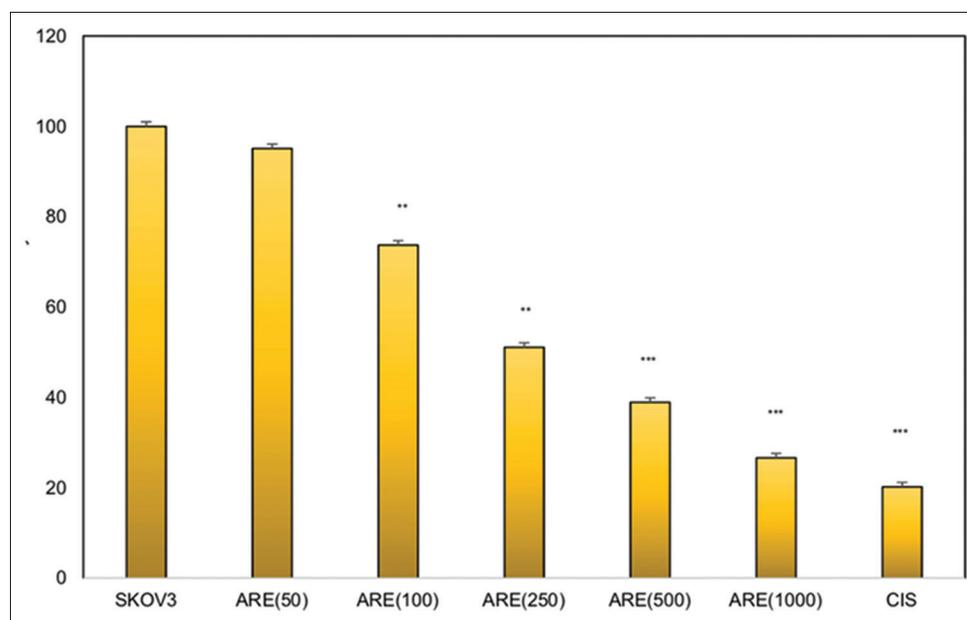
Herbal medicines have played an important role in treating different diseases since ancient times and they have made an impact on both global health and international trade. Medicinal plants play an important role in the health-care system. The herbal products have been classified under “dietary supplements” and are included with vitamins, minerals, amino acids, and other products intended to supplement the diet. In fact, there are several medicinal plants all over the world, including India, which are being used traditionally for the prevention and treatment of cancer.<sup>[12]</sup> However, only a few medicinal plants have attracted the interest

**Table 1: Cytotoxic effect of different concentration of ARE on SKOV3 cells**

| S. No. | Treatment        | Concentration of the extract (µg/ml) | Absorbance 570 nm |
|--------|------------------|--------------------------------------|-------------------|
| 1      | Negative control | Negative control                     | 0.518±0.10        |
| 2      | ARE              | 50                                   | 0.493±0.34        |
| 3      |                  | 100                                  | 0.382±0.21*       |
| 4      |                  | 250                                  | 0.265±0.22**      |
| 5      |                  | 500                                  | 0.202±0.21***     |
| 6      |                  | 1000                                 | 0.138±0.12***     |
| 7      | Cisplatin        | 10                                   | 10 0.105±0.04***  |

Values are expressed as Mean±SEM (n=3), \*P<0.05; \*\*P<0.01; \*\*\*P<0.001 statistically significant as compared with negative control.

ARE: *Asparagus racemosus* extract



**Figure 1:** Percentage of cell viability changes. Results were expressed as Mean± SEM (n = 3) \*p < 0.001 statistically significant as compared with SKOV3 cells untreated

of scientists to investigate the remedy for neoplasm (tumor or cancer). The plants consist of various phytochemicals, which block various hormonal actions and metabolic pathways that are associated with the development of cancer. A whole variety of phenolic compounds; in addition to, flavonoids are widely distributed in grains, fruits, vegetables, and herbs.<sup>[13]</sup> Phenolic compounds such as caffeic and ferulic acids, sesamol, and vanillin have been reported to exhibit antioxidant and anti-carcinogenic activities and inhibit atherosclerosis. *A. racemosus* is widely used in Ayurveda for its medicinal properties and its anticancerous property has been implicated in the regulation of cell proliferation and apoptotic gene products. Asparagus root is a rich source of steroidal saponins which are important secondary metabolites with cytotoxic activity.<sup>[14]</sup>

## CONCLUSION

Herbal components having anticancerous and immunomodulatory activity are required to be identified for cancer treatment. Many Ayurvedic formulations are recognized for their ability as

anticancer, anti-inflammatory, immunomodulatory, and many other therapeutic uses. Furthermore, research should be directed toward the isolation of bioactive compounds from these plants and to identify how these natural extracts could be used as an alternative therapy to currently used chemotherapies for different types of carcinomas.

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Source of support: Nil; Conflict of interest: None Declared