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RESEARCH ARTICLE

Antioxidant and Anticancer Activity of *Asparagus racemosus* on MCF-7 Cell line

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ABSTRACT

To investigate the *in vitro* antioxidant and anticancer activity of *Asparagus racemosus* rhizome on MCF-7 Cell line. Phytochemicals of methanol and aqueous extract of *Asparagus racemosus* were analysed by using standard methods. *In Vitro* antioxidant studies were carried out for the methanol extracts of the *Asparagus racemosus* using various free radical models such a DPPH, Reducing power assay and Hydrogen peroxide scavenging assay. *In vitro* cytotoxic assay such MTT assays were carried out in methanolic extract against EAC cell line. The results of the present study demonstrated that the qualitative phytochemical analysis of the methanol and aqueous extract of *Asparagus racemosus* showed the presence of alkaloids, tannin, flavonoids, saponin, glycosides, and phenolic compounds. The antioxidant activity of methanolic extract of rhizome *Asparagus racemosus* confirmed by free radical scavenging activity and reducing (FRAP) and it was found to be significant. The result revealed that the methanolic extract of *Asparagus racemosus* showed pronounced anticancer activity against MCF-7cell line. It revealed that the crude methanolic extract of *Asparagus racemosus* has anticell proliferative activity against MCF-7 cell lines. From our study it is concluded that, the phytochemicals present in the *Asparagus racemosus* possess antioxidant and anticancer activity.

KEYWORDS

Asparagus Racemosus, Phytochemicals, Antioxidant, Anticancer

INTRODUCTION

Cancer is one of the most dangerous and most painful of all chronic illnesses, and is also the second most common cause of death after heart disease. It is a disease characterized by the uncontrolled growth and spread of abnormal cells. Cancer can develop from most types of cells in different parts of the body, and each cancer has its own pattern of growth and spread. Some cancers remain in the body for years without showing any symptoms. Others can grow, invade and spread rapidly, and are fatal in a short period of time.

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Cancer is a leading cause of mortality, and it strikes more than one-third of the World's population and it's the cause of more than 20% of all deaths. Among the causes for cancer are tobacco, viral infection, chemicals, radiation, environmental factors, and dietary factors[1]. Breast cancer is the most commonly occurring cancer in women, comprising almost one third of all malignancies in females. It is second only to lung cancer as a cause of cancer mortality and it is the leading cause of death for women between the ages of 40 and 55[2]. The lifetime risk of a woman developing invasive breast cancer is 12.6 % 2 one out of 8 females in the United States will develop breast cancer at some point in her life[3].

The use of natural products with therapeutic properties is as ancient as human civilization and, for a long time, mineral, plant and animal products were the main sources of drugs.

In recent years, there has been growing interest in alternative therapies and the therapeutic use of natural products, especially those derived from plants. Virtually every indigenous culture in the world uses medicinal plants in some form or other for treatment of ailments. The actual knowledge of medicinal plants is possessed by a select group of practitioners, who determine the nature of the ailments and then prescribe remedies. In recent periods, traditional medicine has made a major come-back. It has been realized that a number of important modern pharmaceuticals have been derived from, or are plants used by indigenous people[4].

Antioxidant compounds in food play an important role as a health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease[5].

Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. There are a number of clinical studies suggesting that the antioxidants in fruits, vegetables, tea and red wine are the main factors for the observed efficacy of these foods in reducing the incidence of chronic diseases including heart disease and some cancers[6]. Keeping in view, the aim of the study is to investigate antioxidant and anticancer activity of *Asparagus racemosus* rhizome extract.

It is widely used by traditional practitioners as an analgesic, antidyspeptic, astringent and liver stimulant and is known to have wound healing property. Flowers are used in semen debility. It is also used in brain disorders[7] and scientifically this plant is validated for hepatoprotective[8], anti-hyperglycemic, antioxidant, anti-inflammatory and anticancer activity[7].

MATERIALS AND METHOD

Collection of Plant Sample

The fresh rhizome of *Asparagus racemosus* were collected from Thanjavur, Tamil Nadu, India. The plant was authenticated by the Department of herbal Government Arts College for Men, Kumbakonam. The rhizomes were shade dried for five days. The dried rhizome were powdered with electrical blender and stored in air tight container at room temperature for our study.

Methanol Extraction

100g of powdered dried rhizome was macerated with 70 % methanol (500 ml) with occasional stirring at 25 ± 2°C for 3 days. The extract was then filtered using a Buchner funnel and a sterilized cotton filter. The solvent was completely removed by rotary evaporator and methanolic extract of rhizome was obtained.

Aqueous Extraction

100 g of dried plant material was macerated with 500 ml distilled water with occasional shaking at room temperature for 2 days. As the preservative a small amount of sodium azide added. The extract was then filtered using a sterilized cotton filter. The distilled water is completely removed by using rotary evaporator.

Qualitative Analysis of Phytochemicals

Phytochemical analysis was carried out qualitatively to identify the presence of various secondary metabolites[9].

In Vitro Antioxidant Activity

Antioxidant activity measured by using DPPH radical scavenging assay method[10], Reducing Power assay[11], Hydrogen peroxide radical scavenging activity[12]. Tests were carried out in triplicate for 3–5 separate experiments. The amount of extract needed to inhibit free radicals concentration by 50%, IC_{50}, was graphically estimated using a nonlinear regression algorithm.

In Vitro Cytotoxicity Assay[13]

Methodology

The human breast cancer cell line (MCF 7) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum

Essential Medium (EMEM) containing 10% fetal bovine serum (FBS). All cells were maintained at 37^0 C, 5% CO_2, 95% air and 100% relative humidity.

Cell Treatment Procedure

The monolayer cells were detached with trypsin-ethylene diamine tetra acetic acid (EDTA) to make single cell suspensions and viable cells were counted by tryphan blue exclusion assay using a hemocytometer. The cell suspension was diluted with medium containing 5% FBS to give final density of $1x10^5$ cells/ml. one hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37^0C, 5% CO_2, 95% air and 100% relative humidity. After 24 hrs the cells were treated with serial concentrations of the test samples. They were initially dispersed in neat dimethylsulfoxide (DMSO) and diluted to twice the desired final maximum test concentration with serum free medium. Additional four, 2 fold serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100 µl of these different sample dilutions were added to the appropriate wells already containing 100 µl of medium, resulted the required final sample concentrations. Following drug addition the plates were incubated for an additional 48 hrs at 37^0 C, 5% CO_2, 95% air and 100% relative humidity. The medium containing without samples were served as control and triplicate was maintained for all concentrations.

MTT Assay

3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyl tetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, crhizome the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells.

After 48hrs of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37^0C for 4hrs. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader.

The % cell inhibition was determined using the following formula.

$$\% \text{ of Cell inhibition} = \frac{100 - \text{Abs (sample)}}{\text{Abs (sample)}} \times 100$$

Nonlinear regression graph was plotted between % Cell inhibition and Log concentration and IC50 was determined using GraphPad Prism software.

RESULTS AND DISCUSSION

Table 1 indicates that the qualitative analysis shows the presence of alkaloids, tannin, flavonoids, saponin, glycosides, and phenolic compounds in methanol and aqueous extract of *Asparagus racemosus*. The various phytochemicals present in *Asparagus racemosus* which are naturally occurring biochemical in plants that give plants their colour, flavor, smell and texture. They may help to prevent diseases like cancer and heart diseases besides their role to inhibit the microorganisms causing many diseases in human beings. Some of the secondary metabolites act as scavengers of free radicals and reduce the damage due to oxidants by neutralizing the free radicals[14].

Table 1: Phytochemical compositions of methanol and aqueous extract of *Asparagus racemosus*

S.No	Name of the phytochemicals	Methanol extract	Aqueous extract
1.	Alkaloids	+	+
2.	Flavanoids	+	+
3.	Tannins	+	+
4.	Glycosides	+	+
5.	Phenols	+	+
6.	Steroids	+	+

7.	Terpenoids	+	+
8.	Carbohydrate	+	+
9.	Protein	+	+
10.	Phlobatannins	+	+
11.	Anthroquinone	-	-

(+) Present (-) Absent

Table 2 and Figure 1 indicates the percent inhibition of DPPH radicals by *S.racemosus* extract. The percentage of DPPH radical scavenging activity of *S.racemosus* rhizome extract found to be increased in dose dependent manner. Free radicals and other reactive oxygen species (ROS) are formed constantly in human body during normal metabolic processes. They help to destroy micro-organisms and fight against infections. However accumulation of ROS is toxic. Increased ROS results in oxidative stress, which may lead to extensive cellular damage through covalent binding and lipid peroxidation[15].

The ability of the plant extract in combating the free radicals mainly due to the secondary metabolites of *S.racemosus.* Reactive oxygen species (ROS) are the intermediate products resulting from univalent reduction of molecular oxygen[16]. And these differ significantly in their interactions and can cause extensive cellular damage such as nucleic acid strand scission, modification of polypeptides and lipid peroxidation. Many of these free radicals have been also implicated in the pathology of various human diseases.

Table 2: Antioxidant activity of methanolic extract of *Asparagus racemosus* by DPPH method

S.No	Concentration (µg/ml)	% of scavenging activity	
		Standard	Plant Extract
1.	200	2.0 ± 0.14	13.33 ± 0.93
2.	400	18.0 ± 1.26	17.80 ± 1.24
3.	600	40.0 ± 2.8	22.22 ± 1.55
4.	800	60.0 ± 4.2	31.11 ± 2.17
5.	1000	92.0 ± 6.44	35.57 ± 2.48

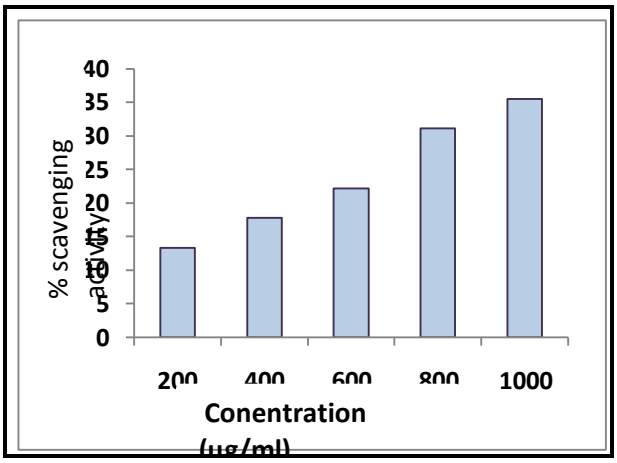

Figure 1: Free radical scavenging of methanolic extract of *Asparagus racemosus* by DPPH method

Table 3 and Figure 2 shows the reducing power capabilities of the plant extract. The antioxidant property of *S.racemosus* extract was determined through FRAP using ascorbic acid as standard. The percentage of free radical scavenging activity of the fractions was found to be 86.68% and 83.33%. The extracts displayed good reducing power which was found to rise with increasing concentration of the extracts. A higher absorbance indicates greater reducing power ability[17]. Several reports have conclusively shown close relationship between total phenolic content and antioxidative activity of the fruits and vegetables. Phenolic compounds, as natural antioxidants exhibit therapeutic potential in multiple diseases including cardiovascular disease, aging and cancer[18].

Table 3: Antioxidant activity of methanolic extract of *Asparagus racemosus* by FRAP method

S.No	Conc^{n} (µg/ml)	% of scavenging activity	
		Standard	Plant extract
1.	200	25.0 ± 1.75	33.37 ± 2.33
2.	400	40.0 ± 2.8	66.68 ± 4.66
3.	600	50.0 ± 3.5	77.79 ± 5.44
4.	800	57.1 ± 3.99	83.33 ± 5.83
5.	1000	66.6 ± 4.66	86.68 ± 6.06

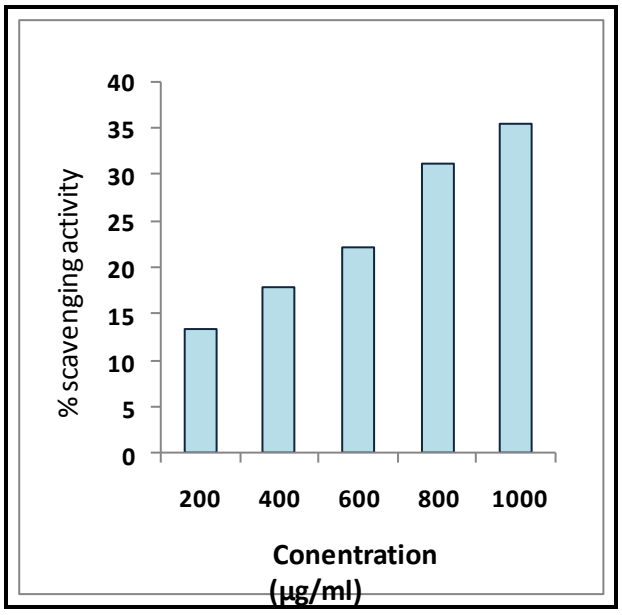

Figure 2: Free radical scavenging of methanolic extract of *Asparagus racemosus* by FRAP method

Table 4 and Figure 3 shows the scavenging activity of *S.racemosus* rhizome extract. Antioxidant activity of the total scavenging of hydrogen peroxide was found to be 89.28% and 78.57%. The absorbance value was noted for a concentration of the standard[19]. The reducing power of the plant extract may be due to the secondary metabolites present in the rhizome. Flavanoids and other phenolic compounds have been reported as scavengers of free radicals and reduce the damage due to oxidants by neutralizing the free radicals before they can attack the cells and prevent damage to lipids, enzymes, carbohydrate and DNA[20].

Table 5 exhibited the anticancer activity of methanol extract of *S.racemosus* by MTT assay. The cytotoxicity of total methanol rhizome extract of *Asparagus racemosus* was examined on malignant cell lines MCF- 7 in a different concentration was assessed by MTT assay. The IC50 value of the *S.racemosus* was found to be 300ug/ml. The inhibitory effect of *S.racemosus* was less than that of the standard drug taxol. Similar observation made by previous researchers shown that the administration of methanol extract *S. racemosus* of rhizome as well as the standard drug exhibited ability to reduce the tumour cells[13].

Table 4: Antioxidant activity of methanolic extract of *Asparagus racemosus* by H2O2 method

S.No	Conc^{n} (µg/ml)	% of scavenging activity	
		Standard	Plant extract
1.	200	22.2 ± 1.55	46.43 ± 3.25
2.	400	33.0 ± 2.31	57.14 ± 3.99
3.	600	50.0 ± 3.5	67.86 ± 4.75
4.	800	66.6 ± 4.66	78.57 ± 5.49
5.	1000	77.7 ± 5.43	89.28 ± 6.24

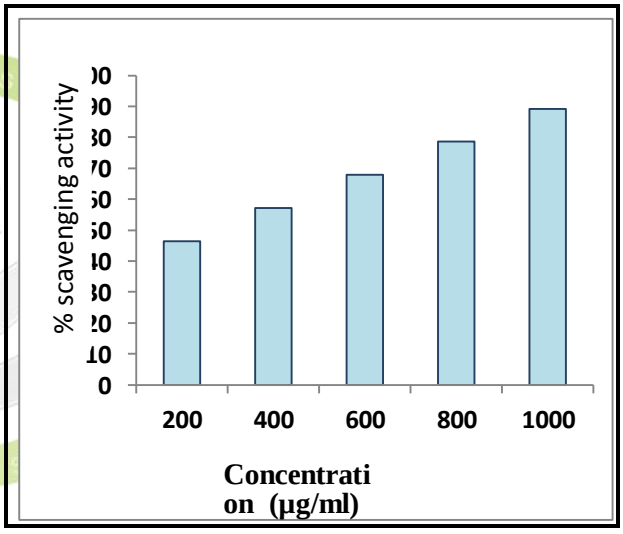

Figure 3: Free radical scavenging of methanolic extract of *Asparagus racemosus* by H2O2 method

Kim *et al*.,[21] reported that, MTT assay has been most widely used in different cancers, and is sensitive, accurate, and efficient in the *in vitro* evaluation of anticancer or immunological agents prior to the preclinical and clinical testing. Some research groups have used MTT assay to guide individual adjuvant chemotherapy for gastric cancer, showing that the therapy based on the chemosensitivity testing can improve the clinical outcomes of cancer patients. Several studies are in tune with our findings where extracts are altered the viability of the cells.

Table 5: Effect of methanolic extract of *Asparagus racemosus* on MCF-7 cell line.

S.No	Concentration (µg)	Standard	Concentration (µg)	Plant extract
		% inhibition		% inhibition
1.	0.001	14.02 ± 0.002	18.75	2.18 ±0.002
2.	0.01	35.24 ±0.003	37.5	10.65 ±0.005
3.	0.1	51.27 ±0.010	75	11.02 ±0.012
4.	1	75.59 ±0.007	150	14.11 ±0.005
5.	10	92.53 ±0.010	300	13.47 ±0.004

The results of the phytochemical analysis for the extracts of *Asparagus racemosus* from the two different extracts namely methanol and aqueous showed that carbohydrates, phenol and flaonoids are strongly present in the extracts. Carbohydrate, protein, alkaloids, glycoside, Terpenoids, tannin, phenol and flavonoid content are found high in aqueous extract. The antioxidant activity of *Asparagus racemosus* was determined by the DPPH (2,2Diphenyl picryl hydrazyl) assay, FRAP (ferric reducing antioxidant power), and H_2O_2 radical scavenging activity. From the above results it is observed that the methanolic extract has significant antioxidant activity. Different concentration of the plant extracts was tested for its cytotoxic effect on MCF-7 by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay using taxol as standard (IC_{50} 300 µg/ml). The result revealed that the methanolic extract of *Asparagus racemosus* showed pronounced anticancer activity against MCF-7cell line. This study revealed that the antioxidant and anticancer properties of *Asparagus racemosus* might be due to the phytochemicals present in it.

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