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## Modulation of steroid hormone synthesis by alcoholic extract of *Asparagus racemosus* on MCF-7 cells

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### ABSTRACT

The objective of the study was to elucidate the effect of alcoholic extract of *A. racemosus* on oestrogen and progesterone synthesis from MCF-7 cells. The root tubers of *A. racemosus* was collected, dried, extracted in soxhlet, solvent evaporated and dried and was used for study. Qualitative phytochemical analysis of the extract was done. The IC<sub>50</sub> was determined by doing MTT assay in MCF-7 cell lines. MCF-7 cells cultured in RPMI-1640 were exposed to IC<sub>50</sub>, half and double the doses of IC<sub>50</sub> for 96 hours and the media collected every 48 hours to determine the concentration of oestrogen and progesterone using ELISA. The qualitative phytochemical analysis of the extract revealed the presence of steroids, alkaloids, diterpenes, triterpenes, tannins, glycosides and saponins. The IC<sub>50</sub> was found to be 267 µg/mL. The extract of *A. racemosus* caused an increase in the concentration of oestrogen and progesterone secreted by MCF-7 cells in a dose and time dependent fashion. There was an increase in the secretion of progesterone in a dose dependent fashion compared to untreated cells, whereas the secretion decreased at 96 hours compared to 48 hours. From the study, it could be concluded that alcoholic extract of *A. racemosus* caused a positive modulation of steroid hormone synthesis.

**Keywords:** *Asparagus racemosus*, MCF-7, Oestrogen, Progesterone.

### INTRODUCTION

The oestrogens and progestogens are the major reproductive hormones in females, that act through the nuclear receptors and bring about ligand induced transcription of key genes associated with reproduction. The role of oestrogen in growth and differentiation of various bodily targets especially those associated with reproduction, maintenance of bone mass, cardiovascular protection and brain integrity has been clearly understood [1]. The role of these female reproductive steroid hormones in the pathogenesis of different cancers has been elucidated long back. Moreover, these hormones and their modulators are used as therapeutic agents in these conditions.

Research on plants that can modulate the synthesis or action of these hormones in various target tissues is gaining significance. Various phytochemicals like coumestrol, genistein, daidzein, resveratrol [2] naringenin [3], have been shown to have oestrogenic or antioestrogenic properties depending up on their concentrations or site of action. Bioflavonoids are a part of the diet and many of them are characterised as phytoestrogens as they bind and activate oestrogen receptors [4]. Hence the effect of such flavonoids can be regarded as selective modulation of oestrogen receptors.

*Asparagus racemosus* belongs to family Asparagaceae; Liliaceae and is commonly as called Satavari, among the 22 species of *Asparagus* recorded in India; *Asparagus racemosus* is the most commonly used one in traditional medicine [5]. The roots of *A. racemosus* were used in diabetes [6, 7] nervous disorders, dyspepsia [8], diarrhoea, dysentery [9], tumors [10], inflammations, hyperdipsia, neuropathy, urolithiasis [11] hepatic tumors [12] hepatotoxicity [13] cough, bronchitis [14], hyperacidity, gastric ulcers [15] and also as a galactagogue [16]. The present study aimed to investigate the effect of alcoholic extract of root tubers of *Asparagus racemosus* on the synthesis of oestrogen and progesterone.

### MATERIALS AND METHODS

#### Plant Extraction

The tubers of *A. racemosus* were collected locally, from Mannuthy and was dried in shade until they were dry. The tubers were coarsely powdered using an electric pulveriser and the powder obtained was extracted using a Soxhlet extraction apparatus with methanol. The methanol extract was then concentrated using a rotary vacuum evaporator under reduced pressure and temperature (40°C). The

yield of the extract was calculated and kept under refrigeration in an airtight container after complete evaporation of the solvent until further use.

### Phytochemical analysis

The qualitative phytochemical analysis was performed [17].

### Assessment of effect of extracts on viability of MCF-7 cells and Calculation of IC<sub>50</sub>.

The MTT assay was done using methanolic extracts of tubers of *A. racemosus* in MCF-7 cells as per [18]. The T25 flask with MCF-7 cells on attaining 70-80 per cent confluency was trypsinized and seeded in a 96 well plate and exposed to 1280, 640, 320, 160, 80, 40, 20 and 10 µg/mL concentrations of the extract. After 24 hours of incubation with the extract, the media were carefully pipetted out and ten microliters of MTT (5 mg/mL prepared in DPBS) was added to all wells including blanks and covered with aluminium foil and incubated at 37°C for 4 hours, in CO<sub>2</sub> incubator. After incubation, the media containing MTT was removed. Added 200 µL of DMSO to all the wells to dissolve to formazan crystals formed. The plates were gently agitated on orbital shaker for 10 minutes. The absorbance was measured using microplate reader (Varioskan Flash, Thermofischer Scientific, Finland) at a wavelength of 570 nm. Per cent cell viability was found out using the formulae and IC<sub>50</sub> was calculated using online software My curvefit.com.

### Culture of cells for steroid analysis

Adherent human breast adenocarcinoma cell line, MCF-7 received as a gift from Amala Cancer Research Centre, Thrissur was used for *in vitro* hormone assays. Cells were adapted to grow in Rosewells Park Memorial Institute (RPMI-1640) media supplemented with 10 per cent foetal bovine serum and 1 per cent gentamicin (50 mg/mL). The cells were maintained in a humidified incubator at 37°C with five per cent carbon dioxide (CO<sub>2</sub>). Charcoal stripped FBS was used for studies involving modulation of oestrogenic activity. Cell lines were sub cultured by enzymatic digestion with one per cent trypsin/1mM EDTA solution when they reached approximately 70 to 80 per cent confluency and these trypsinized cells were used for the studies. The cells at a concentration of 1x10<sup>5</sup> cells/mL of media was plated into six well plates and incubated at 37°C for 24 hours. Once the cells reached confluency, they were treated with the extracts of the plant. The collected media was stored at -80°C and used for the estimation of Progesterone and Oestrogen.

### Assay for hormones

The MCF-7 cells were exposed to extracts of *A. racemosus* in the concentrations 534, 267 and 133.5 µg/mL (2 times IC<sub>50</sub>, IC<sub>50</sub> and half dose of IC<sub>50</sub>) for 96 hours. The culture media were collected every 48 and 96 hours and replaced with fresh media containing the extract. The assay was done in triplicates.

The total progesterone level in the cell culture media was estimated using Progesterone ELISA kit provided by Abnova Coorporation, USA as per manufactures protocol. The absorbance was measured using microplate reader (Varioskan Flash, Thermofischer Scientific, Finland) at a wavelength of 450 nm. The mean absorbance values were calculated.

The total oestrogen level in the cell culture media was estimated using Enzyme-Immunoassay kit provided by Omega diagnostics as per manufactures protocol. The absorbance was measured using microplate reader (Varioskan Flash, Thermofischer Scientific, Finland) at a wavelength of 450 nm. The mean absorbance values of standard and samples were calculated.

The standard curve was plotted for both hormones using the mean absorbance of each standard on Y-axis and the concentration on X-axis. Online curve fitting software AAT Bioquest was used for plotting the 4 Parameter logistic Curve for ELISA and the regression equation was derived.

### Statistical Analysis

The results were analysed using repeated measures ANOVA using SPSS V 24 and post hoc analysis was done by Latin Square Design. Data on cell viability was analysed using student 't' test.

## RESULTS

### Phytochemical Analysis

The qualitative phytochemical analysis revealed the presence of steroids, alkaloids, diterpenes, triterpenes, tannins, glycosides and saponins

### Assessment of effect of extracts on viability of MCF-7 cells and Calculation of IC<sub>50</sub>

The per cent inhibition of cell proliferation as evaluated by MTT assay 24 hours post treatment with methanolic extract of *A. racemosus* (MAR) in MCF-7 cell line is presented in table 1. Maximum inhibition was shown when cells were exposed to 160 µg/mL of MAR with values of 47.23±3.83 per cent whereas the inhibition of the cells exposed to 5 µg/mL was 14.11±4.63 per cent. The viability of cells at 10, 20, 40 and 80 µg/mL were 26.29±2.66, 24.91±3.45, 22.85±4.08 and 39.91±4.71 respectively.

The IC<sub>50</sub> of *A. racemosus* was calculated by using the per cent cell inhibition obtained from MTT Assay. A curve was plotted using the values in AAT Bioquest and the graph obtained is represented in the table1. The IC<sub>50</sub> value was found to be 267 µg/mL (Figure 1).

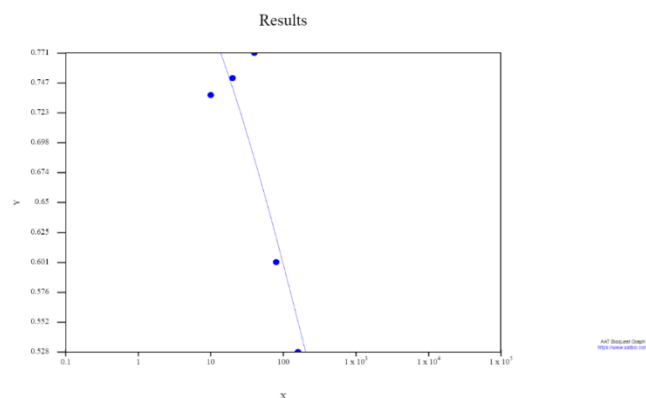
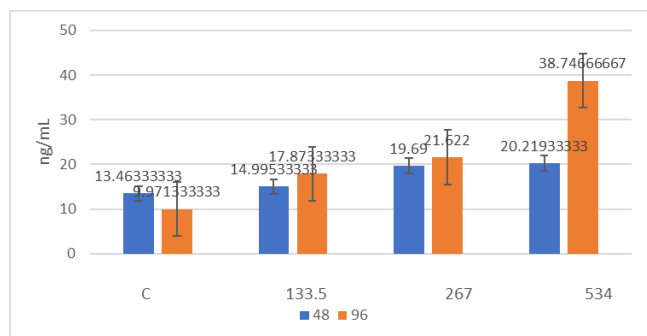


Figure 1: IC<sub>50</sub> of *A. racemosus*

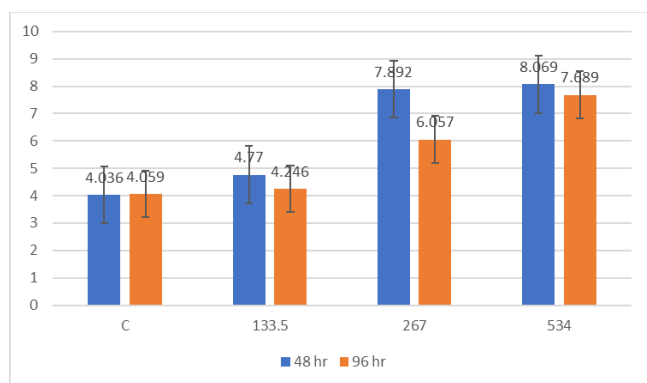
### Effect on methanolic extract of *A. racemosus* on Oestrogen concentration

The maximum oestrogen concentration after 48 hours of treatment was observed when MCF-7 cells were treated with MAR at the dose of 534 and 267 µg/mL which was 20.21±0.63 and 19.69±0.26 ng/mL respectively (Figure 2). At the dose of 133.5 µg/mL the oestrogen concentration was 14.99±0.15 ng/mL. There was an increase in the oestrogen concentration when cells were exposed to MAR at both time intervals and maximum concentration was at 96 hours. At 96 hours, the oestrogen concentration were 38.74±0.55, 21.62±0.40 and 17.87±0.24 ng/mL at the doses of 534, 267 and 133.5 µg/mL respectively.



**Figure 2:** The effect of methanolic extract of *A. racemosus* on oestrogen secretion by MCF-7 cells

There was a dose dependent increase in the concentration of progesterone secreted from the MCF-7 cells that were treated with 267 and 534 µg/mL of the methanolic extract of *A. racemosus*. The increase was relevant during the first 48 hours of treatment as compared to the total 96 hours of treatment (Figure 3). After 96 hours of treatment, there was a decrease in the concentration of progesterone, at each dose, as compared to the concentrations at 48 hours, but there was an increased secretion compared to the untreated cells.



**Figure 3:** The effect of methanolic extract of *A. racemosus* on progesterone secretion by MCF-7 cells

### DISCUSSION

Oestrogens and progestins are the endogenous hormones concerned with numerous physiological functions in the body including developmental effects, maintenance of fertility, pregnancy, lactation and metabolism of minerals, carbohydrates, protein and lipids. The concentrations of oestrogen and progesterone are important in maintaining the fertility status of an individual. The clinical uses of these hormones and their antagonists or selective receptor modulators

differ in different clinical situations, which find their clinical efficacy even in cancers. Both the hormones arise from the same precursor, cholesterol, whose transport from cytoplasm to mitochondria is the rate limiting step in the synthesis. Cholesterol is converted to pregnenolone, then progesterone which gets converted to testosterone and then to oestrogen, which is mediated by the enzyme aromatase.

These hormones acts through nuclear receptors, which act as ligand activated transcription factors, which regulate the activity of target genes. Oestrogen act via other mechanism also like the G-protein coupled receptor, GPR 30 which is thought to mediate the rapid effects of oestrogen. There are many reports on the association of GPR 30 and many of the oestrogen dependent cancers.

In the present study, the methanolic extract of tubers of *A. racemosus* caused a dose and time dependent increase in the secretion of oestrogen from MCF-7 cells whereas the secretion of progesterone was enhanced only in the higher doses. There was also a time dependent decrease in the secretion of progesterone, which indicated that the effect on estrogen secretion was more pronounced as compared to progesterone. The effect in the case of progesterone was significant, as the secretion from control cells remained unchanged even after 96 hours of culture. The decrease in the secretion of progesterone was evident in the IC<sub>50</sub> dose compared to all other treatments whereas there was a significant increase in the secretion of oestrogen at the higher dose. These results indicated that the extract of *A. racemosus* modulated the activity of both oestrogen and progesterone.

The phytochemical analysis of the extract showed presence of steroids, saponins and glycosides, the activity of which might have contributed to the effect on the steroidogenesis. There are various reports on the activity of bioflavonoids and carboxylic acids acting as weak progestins or antiprogestins [19]. It was reported that certain phytoestrogens like biochanin A and genistein significantly increased the synthesis of progesterone at lower concentrations whereas the synthesis was inhibited at higher concentrations [20], which was seen in the present study also. The decrease in the synthesis of progesterone at higher concentrations over time can be due to the enhanced metabolism of the hormone or enhanced synthesis of oestrogen. It has already been proven that phytochemicals like flavones and isoflavones especially apigenin, chrysin, biochanin A are oestrogenic and cause cell proliferation in *invitro* systems [21].

There are various reports on the biphasic response of phytochemicals on oestrogen secretion. Genistein was shown to have oestrogenic properties at concentrations below 1µM, but antagonist activity is seen at concentrations above 10 µM [22]. The biphasic effect on daidzein on cultured cells were thought to be associated with influence on cell cycle regulatory protein [23]. The phytoestrogens can be used as alterantives to oestrogen replacement therapy as they show oestrogenic effects. Many of the biological effects of oestrogen like regulation of cell growth, migration, apoptosis and regulation of cardiac and vascular hypertrophy in response to ischemia are mediated through GPER [24]. Recently, it was elucidated that genistein is an agonist of both GPER and ERα and can induce arterial vasodilation in humans, pigs and rats [25].

In conclusion, the potential oestrogenic and progestogenic activity of *Asparagus racemosus* was elucidated in the human breast cancer cell lines. The alcoholic extract of *A. racemosus* caused a dose and time dependent increase in the secretion of oestrogen where as a dose dependent increase in progesterone synthesis was noticed which

decreased over time. Since the oestrogenic and progestogenic activities, are beneficial not only on reproductive aspects, but also on the other tissues bearing oestrogen receptors, identification of the potent molecule is warranted.

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### Conflict of Interest

None of the authors have any potential conflict of interest associated with this research.

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None declared.

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