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SHORT COMMUNICATION

Asparagus racemosus (Shatavari) targeting estrogen receptor α: - An *in-vitro* and *in-silico* mechanistic study

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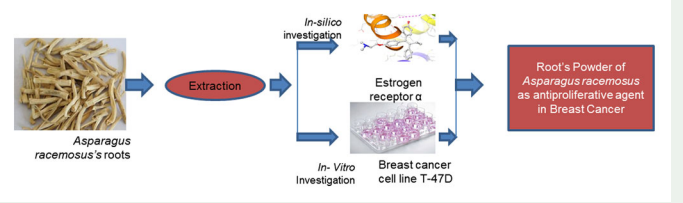
ABSTRACT

Breast cancer is a disease where cells in the tissue of the breast, grow and divide without normal control. Breast cancer is second major cause for death in world wide. Importance of natural product increase due to adverse effect of existing synthetic drugs. *Asparagus racemosus* comprises phytoestrogens which can be used for the treatment of breast cancer. In the current study, *In vitro* antiproliferative activity of the extracts of *A. racemosus* is performed in T47D cancer cell lines. Outcomes of the result indicated that aqueous methanol and methanol extract showed excellent antiproliferative activity as compared to bazedoxifene (standard), ethyl acetate and petroleum ether extract. *In silico* study of reported phytochemical constituents of *A. racemosus* was performed for understand the molecular mechanism and prospect pharmacophore development. Furthermore, compound **26** (rutin) which has been earlier reported and isolated from alcoholic extract exhibited the remarkable binding profile with estrogen receptor α. For that reason, our study proposed that *A. racemosus* could be used as a new source for the treatment of breast cancer.



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1. Introduction

8.2 million People die from cancer worldwide every year and new cancer cases are expected to rise 70% within the next two decade (Siegel et al. 2016). Breast cancer (BC) is a second leading cause of death after cardiovascular diseases. Breast cancer is categorized by the uncontrolled growth of irregular cells in the milk generating glands

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of the breast or in passages (Ducts) that deliver milk to the nipples (Beckmann et al. 1997). The major types of BC's are ductal carcinoma in situ, invasive ductal BC, invasive lobular BC and inflammatory BC (Weigelt and Reis-Filho 2009). Estrogen receptor (ER) is a group of protein (a 12 helix protein) present inside the cells of the female reproductive tissues or located in nucleus of cells. These receptors are activated by hormone estrogen (17β-estradiol) (Heldring et al. 2007). There are mainly two types of ER's which play an important role in BC i.e. ERα and ERβ. Overexpression of ERα causes the proliferation of cells in breast tissue (Duffy 2006). Therefore, targeting ERα is an effective approach for decreasing the proliferation of cells in breast tissue. Numerous drugs are used for treatment of BC such as selective estrogen receptor modulator (tamoxifen, raloxifene, Toremifene and Bazedoxifene), selective estrogen receptor down regulator (Fulvestrant), chemotherapy and targeted therapy for BC like Pertuzumab, Tratuzumab and Lapatinib have their own side effects (Shapiro and Recht 2001). Bazedoxifene is used as a standard drug for *in vitro* and *in silico* studies (Komm et al. 2012). It has been testified that more than 1000 species of plants are used for treatment of BC. *Asparagus racemosus* (Shatavari) is one of the important medicinal herbs that contain estrogen like chemical constituents called phytoestrogens (Ashajyothi et al. 2009; Singh and Geetanjali 2016). Phytoestrogens have structure resemblance to endogenous estradiol having less activity. Previous study indicated that n-butanol, methanol, ethyl acetate and water extracts had significant antiproliferative activity on different cell lines (Četojević-Simin et al. 2010). Another study was carried out to evaluate the anticancer activity of major shatavarins (Shatavarin IV) from methanolic extract of *A. racemosus* roots. *In vitro* cytotoxicity study using MCF7, HT29, and A498 cell lines showed potent activity with methanolic extract of *A. racemosus* roots (5.05% Shatavarin IV) (Mitra et al. 2012).

Earlier study demonstrated that root extract of this plant obtained by extraction with chloroform and methanol (1:1) led to significant decrease in the incidence of cancer (Bopana and Saxena 2007). Based on these consideration, the current study is aim to study the antiproliferative activity of *A. racemosus* (Shatavari) on T47D cancer cell lines and to analyse the mechanism of action behind the antiproliferative activity.

2. Results and discussion

2.1. In- vitro *antiproliferative activity*

Four different extracts of *A. racemosus* comprising petroleum ether (APE), ethyl acetate (AEA), methanol (AME) and methanol water (AMW) have been considered for inhibition of proliferation of cells. The antiproliferative activity was evaluated by using MTT assay by using T47 D breast cancer cell line (Figure S1). It was found that most polar AMW extract showed good activity with IC_{50} 8.89 μg/ml followed by AME with IC_{50} 27.79 μg/ml (Table S1). In another study, cytotoxicity of total methanol extract of *Asparagus racemosus* was observed to be 300 ug/ml on malignant MCF- 7 cell lines (Le Son and Anh 2013). It was evident from our study that *Asparagus racemosus* have potent antiproliferative activity. The AMW extract which has shown impressive activity is known to be source of Rutin, quercetin, Kaempferol, genistein and Daidzein (Simin et al. 2013). To recognize the mechanism behind the activity of extracts was performed.

2.2. Molecular simulation study

The mechanism behind the antiproliferative activity of phytochemical constituents of *Asparagus racemosus* is determined by help of Maestro software. The target selected for the study was ERα. Total 30 molecules were selected for molecular docking study, Most of molecules were phytoestrogens. Determine the validation of docking stimulation protocol, the co-crystallized ligand present in structure of estrogen receptor was redocked. The root mean square deviation (RMSD) found after superposition of the docked position of the ligand with crystal structure was 0.11 A°. (Figure S2).

Phytoestrogens present in *Asparagus racemosus* that were described in literature to act as antiproliferative agent by regulate the ER were used to conduct the docking protocol. All constituents of *Asparagus racemosus* have better binding affinity in the range (−13.7 to −2.95 Kcal/mol) as compared to bazedoxifene (−7.331 Kcal/mol) which was used as reference standard in *In vitro* antiproliferative activity in case of ERα. Compound 26 (Rutin) shown best docking score (−13.7 Kcal/mol) as compared to standard drug bazedoxifene (−7.331 Kcal/mol) with ERα (Table S2). So phytoestrogens capable of forming broad hydrogen bonding were exhibiting excellent affinity as compared compound which have less polar group. Binding pattern of rutin with ERα indicate that hydrogen bonding contributes (−5.66 Kcal/mol) of the total binding energy with receptor site. The crucial amino acid contributing in the interaction were ASP_{351}, GLU_{351}, GLY_{420} and APG_{394} for ERα. Additional binding energy predictable by lipophilic and electrostatic interaction are shown in Figure S3. Standard bazedoxifene was docked with rutin at receptor site and can been observed that rutin occupied the similar binding position, where bazedoxifene binds in case of ERα. The binding cavity of ERα is lined by polar group amino acids which are responsible for hydrogen bonding interaction (Figure S4).

ADME study was performed by using Quikprop software of maestro. ADME study suggests that most of phytoestrogens shown moderate to low oral absorption. Rutin shown 80% oral absorption. Rutin and other compounds have been reported to be present in high concentration and have been isolated from alcoholic extract of *Asparagus racemosus* (Chua 2013). Dock score, hydrogen binding, lipophilicity and electrostatic energy for all phytochemical constituents have been shown in Table S2. Structures of phytochemicals are shown in Figure S5.

3. Experimental

All experimental procedures are described in the supplementary material section.

4. Conclusion

From *In-vitro* investigation, we have concluded that AMW extract of *Asparagus racemosus* showing excellent antiproliferative activity as compare to bazedoxifene (Standard). These extracts can serve as a potent source of compounds which have a better therapeutic agent for targeting ERα. From the *In silico* investigation, we have observed that compound 26 (Rutin) showing better binding affinity & docking score as compare to

bazedoxifene. Therefore, our study has provided comprehensive understanding at the molecular level of *Asparagus racemosus* extracts potential as an antiproliferative agent.

Disclosure statement

No potential conflict of interest was reported by the authors.

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