

Review

Approaches for Conservation of an Ethnomedicinal Plant: *Asparagus racemosus* Willd.

Shubha Thakur, Kishan Lal Tiwari and Shailesh Kumar Jadhav

School of Studies in Biotechnology, Pt. Ravishankar Shukla University, Raipur (C.G.), India

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Corresponding Author:

Shailesh Kumar Jadhav

School of Studies in

Biotechnology, Pt. Ravishankar

Shukla University Raipur

(C.G.), India

Email: jadhav9862@gmail.com

Abstract: India is one of the megadiversity regions over the globe, gifted with the immense forest resources having the richness of the medicinal plants. The increasing demand of herbal medicines has led to destructive harvesting practices hampering the existence of natural population. *Asparagus racemosus*, a female-friendly herb, is one such asset to Indian flora having numerous pharmacological applications. Steroidal saponins are the major constituents which impart the therapeutic properties to the plant. The pace with which the plant is being over-exploited is alarming. Biotechnological approaches comprising *in vitro* tools, molecular profiling along with chemoprofiling can help to conserve the species and also to provide well authenticated elite plant material for cultivation. The reviews published earlier mainly emphasized on the pharmacological attributes of the plant and less concern is addressed to reveal the need of conservation strategies. This review is compiled by emphasizing on all the aspects of the plant viz. its phytoconstituents, pharmacological importance, *in vitro* conservation and molecular characterization studies done till date which could help light up the direction for further research.

Keywords: *Asparagus racemosus*, Shatavarin, Conservation, *In vitro*

Introduction

Medicinal plants have been providing a tremendous source of natural medicines since time immemorial. With the advances in science and technology this task was taken over by allopathic treatments. But since recent decades, the trend is reverting and the trade and demands of plant based medicines is increasing with a great pace. The most acceptable part of which is their safety and usually not attributing any side effects. Even in Western countries about 40% of pharmaceuticals are directly or indirectly obtained from natural resources (Rout *et al.*, 2000). Major part of this demand is met by the wild populations threatening the survival of many species. One such species belonging to genus *Asparagus* is *Asparagus racemosus* which might struggle for its survival in near future if proper concern for its conservation is not provided.

The Genus-*Asparagus*

The genus *Asparagus* is a member of Asparagaceae family (APG III, 2009), a large genus containing approximately 200 species distributed throughout the

world (Dahlgren *et al.*, 1985). It consists of herbs, shrubs and vines that are known all over the world for its medicinal importance apart from being consumed as food. It is characterized as herbaceous perennials, tender wood shrubs and presence of photosynthetic stems (cladodes) (Obermeyer, 1983). The genus is largely divided into three subgenera namely *Asparagus*, *Protasparagus* and *Myrisphyllum* (Clifford and Conran, 1987). *Asparagus racemosus* is one of the important medicinal plant in Ayurvedic system of medicine belonging to the genus *Asparagus*.

Asparagus racemosus Willd.

Taxonomy

Kingdom: Plantae

Clade: Angiosperms

Clade: Monocots

Order: Asparagales

Family: Asparagaceae

Subfamily: Asparagoideae

Genus: *Asparagus*

Species: *A. racemosus*

Vernacular Names

Asparagus racemosus is commonly called Satavari, Satawar or Satmuli in Hindi; Satavari in Sanskrit; Shatamuli in Bengali; Shatavari or Shatmuli in Marathi; Satawari in Gujarati; Toala-gaddalu or Pilli-gaddalu in Telegu; Shimaishadavari or Inli-chedi in Tamil; Chatavali in Malayalam; Majjigegadde or Aheruballi in Kannada; Kairuwa in Kumaon; Narbodh or Satmooli in Madhya Pradesh; and Norkanto or Satawar in Rajasthan (Anonymous, 1987).

General Properties

A. racemosus is an important rasayana herb of Indian system of medicine. The plant is also known as Shatavari and is a part of most of the Ayurvedic rasayana preparation including Chyawanprash, an outstanding adaptogenic preparation (Ali, 1998; Bopana and Saxena, 2007; Gautam *et al.*, 2009). Shatavari is known for its phytoestrogenic properties and is extensively used in combating menopausal symptoms and increasing lactation (Sabnis *et al.*, 1968; Mitra *et al.*, 1999).

In Sanskrit, Shatavari means "she who possesses a hundred husbands", implying its ability to help support fertility and vitality. Its principle constituents include saponins, alkaloids, proteins and tannins. The plant contains triterpene saponins called Shatavarin I-X, which support the body's own natural production of estrogen.

Morphology and Habitat

A. racemosus is an important species found in tropical and subtropical regions in India.

Stem and Leaves

A. racemosus is a thorny, climbing under shrub with woody stems. Stems are delicate and brittle. The leaves of the plant are reduced to scales and spine called cladodes.

Roots

Roots are tuberous, finger-like and clustered ranging from 30 cm to 1 m in length, smooth and tapering at both ends.

Flower and Fruit

The inflorescence has tiny white flowers, in small spikes. Fruits are globular, or vaguely three lobbed, pulpy berries, purplish black when ripe, seeds with hard and brittle testa.

Phytoconstituents

The major bioactive compound of *A. racemosus* is steroidal saponins. However entire plant contains saponins known as Shatavarin (I-X) but roots are the richest source. Shatavarins are produced only after flowering which takes three years and mature plants can then be harvested (Rao, 1952; NMPB, 2002). Shatavarin IV is a glycoside of sarsasapogenin having two molecules of rhamnose and one molecule of glucose (Fig 1). Important bioactive constituents of *A. racemosus* have been summarized in Table 1.

Kumeta *et al.* (2012) strongly suggest that *A. racemosus* does not contain asparagine A and previous reports of isolation of this compound was likely to be caused by misidentification of *Stemona* plants as *A. racemosus*. Justification of this fact yet needs further research.

Ethnobotany and Traditional Uses

India has a diverse heritage of cultural traditions. A major part of which is pursued by the ethnic and tribal people residing in various parts of the country. Medicinal plants are a part of their livelihood and tribals contribute maximum to conserve the knowledge of using these plants in a numerous disorders. Many reports have cited the use of *Asparagus racemosus* by various tribals all over the country and also in different countries (Kala, 2009; Singh *et al.*, 2012).

Table 1. Important bioactive constituents present in *Asparagus racemosus*

Compound	Belongs to	Present in	References
Shatavarin I-IV	Steroidal saponins	Roots, leaves	Ravikumar <i>et al.</i> , 1987
Shatavarin V		Roots	Hayes <i>et al.</i> , 2006
Shatavarin VI-X		Roots	Hayes <i>et al.</i> , 2008
Racemofuran (α,α -diphenyl- β -picrylhydrazyl)	Furan	Roots	Wiboonpun <i>et al.</i> , 2004
Racemosol	Dihydrophenanthrene derivative	Roots	Sekine <i>et al.</i> , 1997
Asparagine	Polycyclic alkaloid	Roots	Sekine <i>et al.</i> , 1994
Racemoside A, B, C	Steroidal saponin	Fruits	Mandal <i>et al.</i> , 2006
8-methoxy-5,6,4'-trihydroxyisoflavone 7- <i>o</i> - β -D-glucopyranoside	Isoflavone	Roots	Saxena and Chourasia, 2001
Quercitin, rutin, hyperoside	Flavonoids	Flower and fruits	Sharma, 1981
Sitosterol, 4,6-dihydroxy-2-O (-2 hydroxy isobutyl) benzaldehyde	Sterols	Roots	Singh and Tiwari, 1991
Kaepfrol and Sarsasapogenin	Saponin	Roots	Ahmad and Jain, 1991

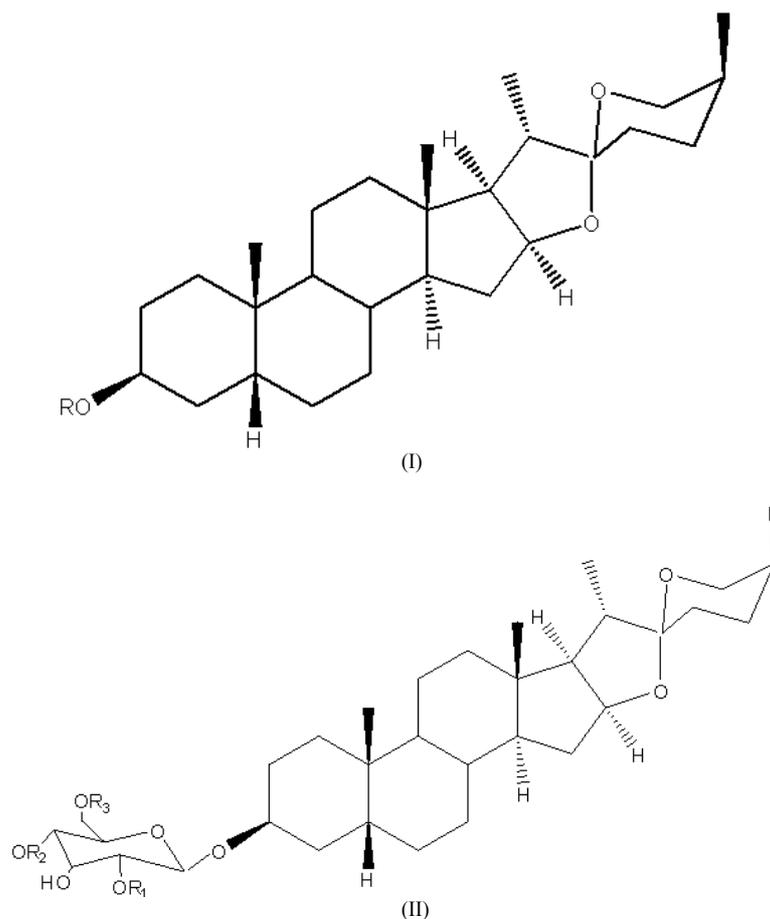


Fig. 1. (I) Chemical structure of sarsasapogenin and shatavarin IV (I) Sarsasapogenin (II) Shatavarin IV with R1- β -D-Glc, R2- α -L-Rham, R3-H

The plant has been mainly used for increasing milk secretion in lactating women for which root decoction or dried root powder is given to the patients. The plant is further used with *Azardichta indica* by the tribal communities for controlling blood sugar level (Swarnkar and Katewa, 2008). In eastern part of India the plant has attained religious virtue and the people believe that the plant will protect them from bad spirits (Sharma and Pegu, 2011).

Pharmacological Applications

The roots of *A. racemosus* are found to be containing steroidal saponins exhibiting variety of properties and thus find numerous pharmacological applications.

Phytoestrogens

Phytoestrogens are the naturally occurring compound that mimics the structure and function of estrogens. They are defined as a plant substance that are structurally and functionally similar to 17 β -estradiol or that produce estrogenic effects (Knight and Eden, 1995). Phytoestrogens

consists of a number of classes, including lignans, isoflavones, coumestans, resorcylic acid lactones, etc. *A. racemosus* is known for its phytoestrogenic properties. The herb is an asset for female reproductive system and is used in combating many female related problems like dysfunctional uterine bleeding, dysmenorrhea, menopausal symptoms, gonorrhoea (Thomson, 2002). It is also beneficial in female infertility as it enhances folliculogenesis and ovulation, prepares the womb for conception and prevents miscarriages.

Ayurveda has called Shatavari the Queen of herbs and is the foremost herb recommended to promote maternal health. The plant is used to increase milk secretion during lactation and thus used as galactagogue. Joglekar *et al.* (1967) examined the effect of *Asparagus racemosus* in the form of Ricalex® tablets (Aphali Pharmaceuticals; 40 mg concentrated root extract per tablet) to women suffering from deficient milk secretion. Sabnis *et al.* (1968) reported a significant increase in the milk yield on administration of alcoholic extract of *A. racemosus* to rats.

Immunomodulant

Rao (1952) reported *A. racemosus* as an immunomodulant. Sharma *et al.* (2011) made a similar observation and found a new saponin molecule in roots of *A. racemosus* functioning as immunostimulator. Thakur *et al.* (2011) worked to screen the activity of polysaccharide fraction of *A. racemosus* as immunomodulant. Phytochemical evaluation confirmed the presence of 26.7% fructo-oligosaccharide (FOS). Natural Killer (NK) cell activity was evaluated as NK cell activity is considered to be important attribute of the immune system. The results indicate that FOS from *A. racemosus* potentiates the NK cell activity and this could be an important mechanism underpinning the Rasayana properties of this plant.

Antidepressant

Singh *et al.* (2009) evaluated antidepressant activity of *A. racemosus* in rodent models. They made use of methanolic extract of roots of plant. The results show that methanolic extract of *A. racemosus* decreases immobility in Forced Swim Test (FST) and increases avoidance response in Learned Helplessness test (LH) indicating antidepressant activity.

Adaptogen

Adaptogens are the natural substances that are known to enhance the immunity and physical well being of a person. *A. racemosus* extract has been found to have excellent adaptogenic properties. Rege *et al.* (1999) orally administered aqueous, standardized extract of *A. racemosus* to experimental animals and then exposed them to various biological, physical and chemical stimuli that cause stress. Using a model of cisplatin they induced alterations in gastrointestinal motility. They also tested the ability of *A. racemosus* extract to normalize them, irrespective of the direction of pathological change. The results of this experiment found that the extract reversed some of side effects of cisplatin such as gastric emptying and intestinal hypermotility.

Anticarcinogen

Anticancer drugs isolated from plants have made a remarkable success in treatment of cancer. More and more plants are being screened for anticarcinogenic activity. Many reports support the presence of compounds in *A. racemosus* which can be helpful in treatment of cancer. Bhutani *et al.* (2010) conducted a study on steroidal saponins of *A. racemosus* for apoptosis inducing study. They investigated the anti-proliferative activity of steroidal constituents isolated from *A. racemosus* on human colon carcinoma cells. They concluded that the steroidal saponin have the potential of being used for the development of potential cancer therapeutics.

In Neural Disorders

Reactive Oxygen Species (ROS) are among the major risk factors that initiate and promote neurodegeneration. Parihar and Hemnani (2004) investigated the potential of extract of *A. racemosus* against Kainic Acid (KA) - induced hippocampal and striatal neuronal damage. They concluded that *A. racemosus* extract could eventually result in protective effect on the KA induced excitotoxicity.

Anti-HIV

Sabde *et al.* (2011) has evaluated *A. racemosus* for anti-HIV activity based on the use of plant as potent immunomodulator. Ninety-two extracts were prepared from 23 different plants. In a human CD4⁺ T-cell line, anti-HIV activity was measured. The ethanol, butanol and aqueous extract showed promising anti-HIV potential with butanol extract showing 87.2% inhibition of HIV-1 at noncytotoxic concentration.

Conservational Needs

Asparagus racemosus due to its numerous applications is an important constituent of many ayurvedic formulations. The demand of the plant is on significant rise and thus the annual growth rate is 15.1% (NMPB, 2002). It is being overharvested for its roots containing its core properties and is thus regarded as vulnerable in its natural habitat (Warrier *et al.*, 2001). The growing demand of plant has caused a serious reduction in native populations.

The extensive use of *A. racemosus* has brought it to the list of 32 prioritized medicinal plants for conservation and development by National Medicinal Plant Board (NMPB, 2002). Conservation of this species thus assumes paramount urgency.

Biotechnological Interventions for Conservation

In vitro Approaches

Asparagus racemosus can be propagated both by seeds and vegetative propagation. The germination percentage of seeds is low and vegetative propagation is a slow process and needs extensive labour. In addition to this, the metabolite content varies as many factors are responsible for the quantity of active principles in the plant. Thus, the need of uniform and elite plant material is foremost. *In vitro* tools along with the chemoprofiling of plant through HPLC can be beneficial. Many reports have been published standardizing the micropropagation protocol in *A. racemosus*.

Kar and Sen (1985) made the first attempt to cultivate *A. racemosus in vitro*. Shoot proliferation was induced from callus culture of *A. racemosus* with 2-4 D and kinetin using stem disc as explants.

Table 2. *In vitro* regeneration of *Asparagus racemosus*

Mode of propagation	Explant used	Reference
Indirect organogenesis via callus culture	Stem disc	Kar and Sen, 1985
Axillary branching	Nodal portion	Bopana and Saxena, 2008
Adventitious shoot bud regeneration and through callus culture	Nodal portion	Kumar and Vijay, 2008
Indirect organogenesis via callus culture	Shoot explants (node, internode, shoot tips), cladodes, roots callus	Pant and Joshi, 2009
Adventitious shoot bud regeneration	Shoot apex and nodal portion	Sharan <i>et al.</i> , 2011

Another more efficient protocol was given for axillary branching with nodal explants (Bopana and Saxena 2008). Normally, *in vitro* rooting in *A. racemosus* is a tedious and generalized protocol and hormones are not enough to induce roots. But this report has been successful in root induction by the use of phloroglucinol. They used MS medium supplemented with 3.69 μ M 2-isopentyl adenine and 3% sucrose with a multiplication rate of 3.5. Root formation was induced with various combinations of hormone but most optimum was found to be half strength MS medium with 1.61 μ M naphthalene acetic acid, 0.46 μ M kinetin, 98.91 μ M adenine sulphate, 500 mg/L malt extract, 198.25 μ M phloroglucinol and 3% sucrose.

The *in vitro* propagation reported in *Asparagus racemosus* have been summarized in Table 2.

Molecular Characterization

For efficient conservation and management, the genetic composition of species in different geographic locations needs to be assessed (Vijay *et al.*, 2009). For detecting genetic polymorphism in several plant populations, fingerprinting methods based on RAPD, ISSR markers have been increasingly utilized. The accessions collected from Madhya Pradesh as well as Himachal Pradesh and Tamil Nadu (Ginwal *et al.*, 2009) showed high genetic similarity. The results of their RAPD analysis concluded that it is needed to take individuals from more different populations so as to preserve their diversity. Moreover, species specific RAPD markers were identified (Singh *et al.*, 2013) which could probably used for genotype identification of this species from its adulterants.

Scaling Up Strategies of Important Metabolite

Steroidal saponins are the major phytoconstituents in *A. racemosus* which impart major properties to it as immunomodulant, galactogouge, adaptogen, antitussive, anticarcinogenic, antioxidant and antidiarrheal (Pise *et al.*, 2011a). Extraction of saponin by *in vitro* tools can indirectly help in reducing the overexploitation of the plant. Also the scaling up of the saponin content in the *in vitro* cultures with the use of

elicitors could be beneficial in quality enhancement of the drugs. This can be done by cell suspension cultures, adventitious root cultures and through development of hairy root culture system. One promising attempt has been made (Pise *et al.*, 2011a) to obtain Shatavarin *in vitro* by optimizing the conditions for production of shatavarins in cell culture of *A. racemosus* in MS medium. Maximum levels of saponin and biomass accumulation was recorded on day 25 of culture cycle at pH 3.4 and 5.6.

Another report by (Pise *et al.*, 2011b) suggested that root calli produces more saponin compared to nodal calli and maximum accumulation was found to be 10.38 \pm 0.14 mg/ g of callus after 60 days of inoculation. Commercial utilization of these protocols and *in vitro* extraction of saponin can better serve the purpose leading to less harvest of the plant from wild populations.

Conclusion

Asparagus racemosus is an important medicinal plant of Indian flora. The pace with which this plant is being exploited is of great concern. The plant is known primarily to promote female health apart of being having tremendous applications as adaptogen, antioxidant, antidiarrheal, immunomodulant, antitussive. Many links are also available suggesting the plant having anticancerous properties. Numerous applications of this plant in various formulations have raised the demand of the plant leading to its overexploitation. Due to this, alternative strategies for propagation and conservation are urgently required to prevent the species being threatened. Moreover less number of putative sequences availability has made it more difficult to get an insight into the metabolic pathways of formation of active compounds responsible for its properties. Systems biology and functional genomics approach could be beneficial in this regard which could directly or indirectly helpful in quality and quantity enhancement of the metabolites of the plant. *In vitro* conservation measures like short term, medium term and long term conservation methods needs to be adopted along with the sustainable use of the existing population.

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Author's Contributions

Shubha Thakur: Manuscript preparation.

Kishan Lal Tiwari: Formatting of manuscript.

Shailesh Kumar Jadhav: Guidance for preparation and publication of manuscript.

Ethics

The authors declare that there is no conflict of interest regarding publication of this paper.

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