Review of Literature

*Asparagus racemosus* Wild. (Lilliaceae), commonly known as Shatavari is a perennial shrub, with a tuberous root-stock, stems covered with recurved spines, linear leaves arranged in a tuft, white flowers and sweet-scented appears in October. Reports indicate that the pharmacological activities of root extracts include antiulcer, antitussive, antioxidant and antibacterial activities (Kumar *et al.*, 2011).

*Asparagus racemosus*, traditionally known as shatavari means "who possesses a hundred husbands or acceptable to many". In Ayurveda it is considered a female tonic. Shatavari is the main Ayurvedic rejuvenative tonic for the females, as is *Withania* for the males (Sharma and Bhatnagar, 2011).

The major active constituents of *Asparagus racemosus* are steroidal saponins (Shatavarins I-IV). Isoflavones, Asparagamine, Racemosol, Polysaccharides, mucilage, vitamins A, B₁, B₂, C, E, Mg, P, Ca, Fe and folic acid present in roots. Other primary chemical constituents of *Asparagus* are essential oils, asparagine, arginine, tyrosine, flavonoids (kaempferol, quercetin, and rutin), resin, and tannin (Chawla *et al.*, 2011).

*Asparagus racemosus* Wild, F: *Asparagaceae* is an ayurvedic medical plant. It has many other properties like Antistress, Anti-diarrhoeal, Antidyspepsia, Adaptogenic action, Antiulcerogenic action, Antioxidant & Cardio protection. This article aims to evaluate the biological activities, pharmacological applications & Clinical studies of *Asparagus racemosus* to provide direction for further phytoestrogenic properties and unexplored areas in which Asparagus can be proved to have potential to cure diseases like osteoporosis (Ashajyothi *et al.*, 2009).

Medicinal plants are the nature’s gift to human being to make disease free healthy life. *A. racemosus* has been described as a rasayana herb and has been used extensively as an adaptogen to increase the non-specific resistance of organisms against a variety of stresses. Besides use in the treatment of diarrhoea and dysentery, the plant also has potent antioxidant immunostimulant, anti-dyspepsia and antitussive effects (Velavan *et al.*, 2007).

*Asparagus racemosus* Willd. (Asparagaceae) is an important medicinal plant of tropical and subtropical India. Destructive harvesting, combined with habitat destruction in the form of deforestation has aggravated the problem. The plant is now considered ‘endangered’ in its natural habitat. Therefore, the need for conservation of this plant is crucial. Consequently, we
have suggested a ‘systems biology’ approach that includes metabolite profiling, metabolic fingerprinting, metabolite target analysis and metabolomics to enable further research (Bopana and Saxena, 2007).

Plants and phytoconstituents are better choice to treat diseases than the allopathic drugs. Most of the drugs used in primitive medicine were originated from plants and are the earliest and principal natural source of medicines. Various plants like Anogeissus latifolia, Alchornea castaneae folia, Uteria salicifolia, Solanum nigrum, Ocimum sanctum, Asparagus racemosus, Scoparia dulcis, Byrsonima crassa etc. and their phytoconstituents proved active in antiulcer therapy (Sen et al., 2009).

Recently few reports are available demonstrating beneficial effects of alcoholic and water extracts of the root of A. racemosus in some clinical conditions and experimentally induced diseases, e.g. galactogogue effect, antihepatotoxic and immunomodulatory activities. The present article includes the detailed exploration of pharmacological properties of the root extract of A. racemosus reported so far (Goyal et al., 2003).

Asparagus racemosus Willd. has repeatedly been mentioned as a galactogogue in Ayurvedic Literature and The primary findings were corroborated by the secondary outcome measures and were found to be statistically significant (p < 0.05) (Gupta and Shaw, 2011).

Asparagus racemosus Willd. Root has been used traditionally in Ayurveda for the treatment of diarrhoea and dysentery. The results point out the possible anti-diarrhoeal effect of the plant extracts and substantiate the use of this herbal remedy as a non-specific treatment for diarrhoea in folk medicine (Venkatesan et al., 2005).

Asparagus species (family Liliaceae) are medicinal plants of Himalayas. They possess a variety of biological properties, such as being antioxidants, immunostimulants, anti-inflammatory, antihepatotoxic, antibacterial, antioxidytic, and reproductive agents. The structural formula of isolated compounds and their distribution in the species studied are also given (Negi et al., 2010).

This research attempts to investigate the influence of N, P and K on chlorophyll, carbohydrate, proteins and sapogenin contents of Asparagus racemosus. Application of phosphorus was found to be best for growth and biochemical contents of root tuber (Vijay et al., 2009A).
High frequency of multiple shoot regeneration was achieved on MS medium supplemented with BAP (0.1 mg/l) and NAA (0.05 mg/l). The shoots were rooted best on half MS medium supplemented with 0.05 mg/l BAP and 1.0 mg/l IBA. Among the five levels of pH tested, 5.7 were the best for multiple shoot proliferation. The result presented here proved to be suitable for the rapid propagation system of *A. racemosus* (Afroz *et al*., 2010).

In the present study, *in vitro* shoot proliferation was obtained by culturing single node segments in Murashige and Skoog’s (MS) medium supplemented with 3.69 µM 2-isopentyl adenine and 3% sucrose with a multiplication rate of 3.5. For proper root formation, the *in vitro*-formed shoot clusters were cultured on half strength MS medium with 1.61 µM 1-naphthalene acetic acid, 0.46 µM kinetin, 98.91 µM adenine sulfate, 500 mg/l malt extract, 198.25 µM phloroglucinol, and 3% sucrose. On this medium, 85% rooting was observed within 20 d. Following a simple hardening procedure involving sequential transfer of plants to a greenhouse, polyhouse, and shade net, the tissue-cultured plants were transferred to the field where the survival rate was 100% (Warner *et al*., 2001; Bopana and Saxena, 2008).

Yellowish-green nodular callus was induced on MS medium fortified with 5.0 mg/l BAP and 0.5 mg/l NAA. The callus got differentiated into adventitious shoots when it was subcultured on MS medium supplemented with 3.0 mg/l BAP + 0.5 mg/l Kn + 0.5 mg/l NAA. On an average shoots/culture were developed well, compared to control (6.87 ± 0.26). These micro shoots were rooted with 1.0 mg/l IBA and the rooted plantlets were transferred to soil after proper acclimatization (Ashwin and Deshmukh, 2011).

Mainly four parameters in *Asparagus racemosus* Willd: callus, buds, shoots and roots inductions were studied. NAA singly played a good role in all parameters except bud induction. Similarly, BAP played good roles in shoot and bud inductions, whereas combinations of NAA and BAP at various levels were found to be effective in almost all cases. The multiplied adventitious shoots were successfully rooted (*in vitro* and *in vivo*) using NAA, acclimatized and transferred to natural conditions (Pant and Joshi, 2009).

Callus cultures of *A. racemosus* were initiated in a modified MS medium supplemented with 1.0 mg/L NAA and 2, 4-D and 0.5 mg/L BAP and compared for growth and production of saponin over a period of 60 days. High performance liquid chromatography (HPLC) chromatograms of the cultures indicated that the overall saponin profile of *in vitro* and *in vivo* plant root extract is similar (Pise *et al*., 2011).
The present investigation was undertaken to study the effect of different concentrations of BAP, Kin, Ads on shoot induction in *Asparagus Racemosus*. There was shoots induction after 3 weeks when Nodal explants segments were cultured on basal medium supplemented with BAP, Kin and Ads. Shoots induction on MS basal medium supplemented with BAP (1 mg/l) + kinetin (1 mg/l) were transferred to MS basal medium containing BAP (1.5 mg/l) + Ads (100 mg/l) and BAP (1.5 mg/l), Kin (1 mg/l) respectively to the Proliferation shoots. Stem segments cultured on MS basal medium supplemented with various concentrations of BAP (0.75, 1.5, 1.0 mg/l), Ads (50, 100 mg/l) and Kin (0.75, 1.0, 1.5 mg/l) induce shoot, But a combination of 1 mg/l BAP + 1 mg/l Kin and 1.5 mg/l BAP + 100 mg/l Ads showed highest percentage of shoot proliferation after 7-14 days (Jain et al., 2012).

*Asparagus officinalis* is most extensively studied species within the genus *Asparagus*, which is well known as garden asparagus. Tissue culture techniques could efficiently promote vegetative propagation of male plants and pave the way for efficient plant breeding. This chapter describes an efficient micropropagation protocol for developing rapid growing in vitro *Asparagus* shoot cultures. The source of explants, inoculation, and shoot proliferation, followed by shoot propagation, rooting, and acclimatization is described. Plant growth regulators NAA, kinetin, and BA were used in various concentrations. By in vitro propagation of *Asparagus*, root initiation is difficult, but can be promoted by adding growth retardant ancymidol which also greatly promotes shoot development and suppresses callus formation (Stajner., 2013).

Maximum shoot formation was achieved when the media was supplemented with 2.0 mg.l-1 Kn + 0.1 mg.l-1 NAA. In vitro rooting of regenerated shoots was achieved in the same media after a long time of inoculation. Besides this excised shoots were rooted on half strength MS medium in the absence of growth regulators. The regenerated plantlets successfully acclimatized and transferred to soil. About 70% of plantlets survived under *ex vitro* conditions (Vijay and Kumar, 2009).

In vitro propagation of *Asparagus racemosus* was carried out using shoot apex and nodal explants in three stages. (a) Initiation of multiple shoots on MS medium supplemented with 6-Benzy Adenine (8.9 µM) + Naphthalene Acetic Acid (0.27 µM) at 25°C having 16 h photoperiod (with 60 µ mol m⁻² s⁻¹ light intensity) and 8 h dark period. (b) Elongation of shoots on MS medium supplemented with 15% coconut milk + 2-iso Pentenyl Adenine (19.6 µM), the light intensity was increased to 80 µ mol m⁻² s⁻¹. (c) Rooting of elongated shoots by giving it a
preculture treatment with MS medium augmented with Indole Butyric Acid (7.35 µM) for 48 h and then transfer to MS medium with 15% coconut milk. The rooted healthy plantlets were best hardened in a mixture of 1:1 sand and soil in a moist saturated chamber having 60-80% humidity. Plants were transferred to fields, after 5 wks of hardening (Sharan et al., 2011).

Root tubers of shatavari possesses adaptogenic, antioxidant, cooling, emollient, diuretic, galactagogue, nerve tonic, rejuvenating and stomachic properties; The present study provides taxonomy of the species, Pharmacognostical and physico-chemical details of the root tubers of A.gonocladus (Madhavan et al., 2010).

Qualitative preliminary phytochemical screening was performed on aforesaid extracts of Asparagus racemosus Willd. for the presence of alkaloids, flavonoids, steroids and terpenoids. Each analysis was carried out in triplicate. This shows positive results for alkaloids (30.43%), flavonoids (47.82%), steroids (65.21) and terpenoids (43.47%), respectively (Verma and Parashar, 2011).

The leaves and roots of the plants Asparagus racemosus were used for Phytochemical screening was carried out. All plants were found to contain alkaloids, terpernoids, saponins and flavonoids (Pascaline et al., 2011).

To study on microscopic evaluation, macroscopical features, physicochemical parameters, phytochemical analysis in the extract with respect to thin layer chromatography were also carried out for the quality control of the drug. The study will provide referential information for the correct identification of the crude drug (Kundu et al., 2011).

Six crude drug samples claimed as ‘Safed Musli’ were collected from different sources and studied. The botanical studies dealt with exomorphology of plant, morphological & microscopical root tuber characters and reported (Panda et al., 2011).

Authors report that Asparagus racemosus has an antimicrobial activity against common pathogens, inhibiting growth of Staphylococcus aureus, Staphylococcus epidermis, Escherichia coli and Bacillus subtilis. They gave powerful effect against bacteria with less side-effects. Phytochemical Analysis of the Asparagus racemosus helps to find out the presence of chemical constituents in the plant extract (Prajapati, and Vyas, 2011).

The crude extracts of medicinal plant powder in different solvent, were subjected to pharmacognostic and fluorescence analysis, phytochemical and antimicrobial screening against selected Gram positive and Gram negative bacteria. Acetone, alcohol, benzene, chloroform and
aqueous extracts of leaf and stem were used for phytochemical screening and antimicrobial activity (Maluventhan and Sangu, 2010).

In this study seventeen ethanol extracts of medicinal plants that are traditionally used in the treatment of infectious and other diseases were screened for their antimicrobial activities. *Staphylococcus aureus*, *Streptococcus epidermidis*, and *Candida albicans* were used for antimicrobial activity (Bhatt et al., 2005).

The anticancer activity of shatavarins (containing shatavarin IV) isolated from the roots of *Asparagus racemosus* (Wild) was evaluated using in vitro and in vivo experimental models. The shatavarin IV was isolated from ethyl acetate insoluble fraction (AR-2B) of chloroform:methanol (2:1) (AR-2) extract of *A. racemosus* roots. The cytotoxicity (in vitro) of shatavarin IV and other shatavarins rich fraction was carried out using of MTT assay using MCF-7 (human breast cancer), HT-29 (human colon adenocarcinoma), and A-498 (human kidney carcinoma) cell lines. The in vivo anticancer activity of shatavarins (containing shatavarin IV) was evaluated against Ehrlich ascites carcinoma (EAC) tumor bearing mice. The result suggests that the shatavarins (containing shatavarin IV) rich fraction (AR-2B) exhibits significant anticancer activity in both in vitro and in vivo experimental models (Mitra et al., 2012).

The aim of the present study was to explore the leaf extract of *Asparagus racemosus* Willd. belonging to family Asparagaceae for its antimicrobial activity. Test microorganisms studied were *Bacillus pumilis*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Staphylococcus aureus*, *Aspergillus niger* and *Candida albicans*. EE (300 mg/ml) inhibited the growth of all the test organisms. Preliminary phytochemical screening as well thin layer chromatography of the EE, CE and HE revealed that the leaves of *A. racemosus* contain flavonoids which might possibly be responsible for the antimicrobial activity of the extracts. (Battu and Kumar, 2010).

Different type of extract of *Asparagus racemosus* was tested for total protein, total sugar, total tannin, total saponin, methane production and antimicrobial estimation against *Streptococcus, Staphylococcus* (gram positive), *Escherichia coli* and *Enterobacter* (gram negative) bacteria (Sirohi et al., 2009).

*Asparagus racemosus* species is known for its phytoestrogenic properties and used as a hormone modulator. In traditional Indian systems of medicine, the roots of *A. racemosus* are used in the treatment of neurodegenerative diseases, dyspepsia, inflammation, nephropathy,
throat infections, tumours, hyperacidity, diarrhea, bronchitis and cough. *A. racemosus* is also used in the production of formulations of adaptogen characteristics. There are many reasons for undertaking detailed research on the activity of compounds isolated from this plant (Tuszyńska, 2010).

The taken extracts of plant were *Asparagus racemosus, Acacia concin, Sapindus mukorossi*. The phytochemical screening was done to identify the natural phytochemical in these three plant extracts. The identification of all phytochemicals was finished through TLC (Mali *et al.*, 2010).

Thin layer chromatography (TLC), bioautography, and high performance liquid chromatography (HPLC) analysis of the crude extracts revealed that saponin, tannin, scopolamine, atropine, and allicin were the active constituents (Sharma and Patel, 2009). There are many methods available to standardize herbs as medicine like HPLC, HPTLC, and GC out of them HPLC is very important. HPLC is used for identification and standardization purpose. This article contains HPLC method of Ashwagandha, Ginseng, Vasaka, Ergot, Belladonna, Rhubarb, Chirata, Arjuna, Red wine, Black pepper, Boswellia, Podophyllum, Silymarin, Kalmegh, Digitalis, Neem, shatavari, Diviner’s sage and senna (Bhosale *et al.*, 2011).

We tried to standardize *Asparagus racemosus* using high performance thin layer chromatography method (HPTLC) for its active compound, shatavarin IV. The Rf value of shatavarin IV were found to be 0.44. The system was successfully used to investigate the presence of the shatavarin IV in *Asparagus racemosus* plant parts as well as to analyze their content in market products (Jain and Agrawal, 2009).

Cytotoxic, antioxidant, tyrosinase inhibitory, antimicrobial activities of the crude ethanol extract of dry powdered roots of *Asparagus racemosus* (Liliaceae) were investigated. TLC and HPLC finger printing showed the presence of steroid sterpenes, alkaloids and flavonoids (Potduang *et al.*, 2008).

*Ashwagandha, Tulsi, Mulethi, Awala, Shatavari, Gokharu, Arjun, Giloy, Safed musli, Kalimirchi, Haldi, Jaiphal* was used as an active ingredients and aqueous extract of *Stevia rebaudiana* as natural sweetener with nutraceutical in health drinks. TLC profile, HPTLC method is a new simple, sensitive, selective, precise and Spectrum analysis showed the same Rf values and spectrum pattern of standard and sample (Bhise and Salunkhe, 2009).
Asparagus racemosus contains Quercetin flavonoid as a chemical constituent and it can be determined by HPTLC method. This study may provide useful information in searching the role of Quercetin to enhance appetite biologically (Parmar et al., 2011).

Powder of Asparagus racemosus was successively extracted with five solvents namely petroleum ether, benzene, chloroform, ethyl alcohol and distilled water in a soxhlet extractor. TLC profiling was done for various extracts using different mobile phases and Rf values were calculated for respective extracts. All the drugs showed the presence of steroids/ triterpenoids which play a major role in functional deviations responsible for infertility (Nagamani et al., 2012).

Random amplified polymorphic DNA (RAPD) markers were used to assess genetic diversity in Asparagus racemosus (Willd.) an important medicinal plant collected from 7 different locations covering Madhya Pradesh. High level of genetic similarity was observed in the collected accessions. This distributive pattern of genetic variation of A. racemosus accessions provides important baseline data for conservation and collection strategies for this species (Vijay et al., 2009B).

Genetic diversity was evaluated among five different species of Asparagus of Asparagaceae (formerly Liliaceae) collected from Central Gujarat (India) using RAPD primers. These investigations provide important baseline data for conservation and inter breeding programs (Lal et al., 2011).