Pharmacognostical Studies for *In vivo* and *In vitro* Production of *Asparagus racemosus* Willd.

**Introduction**

*Asparagus racemosus* Willd. (family-Asparagaceae) also known by the name “Shatavari” means "who possesses a hundred husbands or acceptable to many" has been used as a medicine (Prajapati, and Vyas, 2011). The genus *Asparagus* includes about 300 species around the world in eastern Asia including India, sri Lanka, Indonesia and Southern part of China (Gomase and Sherkhane, 2010), Korea and Japan (Kim et al., 2005). Shatavari is the main Ayurvedic rejuvenative tonic for the female, as is *Withania* for the male (Sharma and Bhatnagar, 2011). It grows wild in forests and is planted in gardens. Some of the European species to be mentioned are *A. officinalis*, *A. sprengeri* and *A. acutifolius*. *A. officinalis* is reported to be popular vegetable consumed in many parts of the world (Afroz et al., 2010; Goyal et al., 2003).

It is a woody climber which grows to a height of 1-2 m. The leaves are of pine needle shape, small, soft spines but uniform. In July, it produces minute, tiny white flowers arranged in the form of small spikes, fragrant and profuse in simple or branched racemes, in September it produce fruits blackish-purple, globular berries. The roots are tuberous, bearing numerous fusiform, Succulent tuberous roots are 30-100 cm long & 1-2 cm thick, finger shaped & clustered and the stem is woody pale grey or brown in colour and armed with strong spines (Mandal et al., 1996; Sharma et al., 2000; Prajapati and Vyas, 2011; Sharma and Bhatnagar, 2011).

Previously was reported that *A. racemosus* contains Steroidal saponins (Hayes et al., 2006), known as shatavarsins I-IV that are present in the roots. Shatvarin I is the major glycoside with 3-glucose and rhamnose moieties attached to sarsapogenin, polycyclic alkaloids (Negi, et al., 2010), proteins, starch, tannin, isoflavones polysaccharides (Kamat et al., 2000), sapogenins (Ch et al., 2008; Oketch Rabah, 1998), Polyphenols, polyacids and vitamin-C (Visavadiya and Narasimhacharya, 2005). Recently, Shatavarin V, Asparinins, Asparosides, Curillins, Curillosides have also been reported (Sharma and Bhatnagar, 2011; Patricia et al., 2006). Other active compounds such as quercetin, rutin and hyperoside are found in the flowers and fruits; while diosgenin and quercetin-3 glucuronide are present in the leaves (Bopana and Saxena, 2007). This plant also contains vitamins A, B$_1$, B$_2$, C, E, Mg, P, Ca, Fe, and folic acid. Other primary chemical constituents of *Asparagus* are essential oils, asparagine, arginine, tyrosine and flavonoids (Oketch-Rabah, 1998; Shao et al., 1997; Chawla et al., 2011; Negi, et al., 2010).
The positive effects of the essential nutrients are requiring on the good crop yield and yield improvement has been reported (Carasky and Iwuafor, 1999). N, P and K are among the common major nutrients, which are essential for the growth and development of all the plant species. There are various ways for improving yield and quality of Asparagus but the best way to improve yield and quality is to apply appropriate amount of fertilizers and to select high yielding varieties. The present investigation was undertaken with nitrogen, phosphorus to investigate their effects on growth and biochemical contents of root tubers of Asparagus racemosus willd. (Vijay et al., 2009A).

Plant tissue culture techniques have become a powerful tool for studying and solving basic and applied problems in plant biotechnology. The Asparagus plant is regarded as an easy to propagate plant, but its propagation through the in vitro micropropagation technology is advantageous that may provide methods for both large-scale propagation, improvement of plants in a comparatively short time compared to in vivo (Chand et al., 1997), disease free, true-to-type plants independent of seasonal and other environmental conditions in a comparatively smaller space. The growing demand for the plant has caused a serious reduction in native populations due to overharvest and deforestation. Therefore, this is one of those several medicinal plants for which sustainable conservation methods are required on a priority basis. So it is recognized as ‘vulnerable’ (Warner et al., 2001; Pise et al., 2011).

Pharmacognostic analysis of Asparagys racemosus including the macroscopic and microscopic study of plant. It is commonly performed by examining cells and tissues by sectioning and staining, followed by examination under microscope. Histology is an essential tool of biology and medicine (Kokate et al., 2005).

Phytochemicals are non-nutritive plant chemicals that contain protective and disease preventing compounds. More than 900 different phytochemicals have been identified as components of food, and many more phytochemicals continue to be discovered today. Many primary metabolites and Secondary metabolites like carbohydrate, protein, alkaloids, terpenoids, glycosides, Phenols are essential for to cure different diseases in animal bodies. Considering all these facts, present investigation is designed to find out Phytochemical analysis of Asparagus racemosus a plant which evokes various therapeutic effects.

Approach of over work involved is to explore the Physico-chemical analysis (proximate analysis) of medicinal plants and which gives valuable information and help to access the quality
of the sample. It provides information on total ash, extractive value, moisture content, foreign organic matter, etc (Shah and Seth, 2010; WHO, 1998).

Many medicinal plant powder show the fluorescence when the sample is exposed to ultraviolet radiation. Evaluation of crude drugs based on fluorescence in daylight is not much used as it is usually unreliable due to the weakness of the fluorescence effects. Fluorescence lamps are fitted with suitable filters, which eliminate visible radiation from the lamp and transmit ultraviolet radiation of definite wavelength. Several crude drugs show characteristic fluorescence useful for their evaluation (Mukherjee, 2002).

The bacterial organisms including Gram positive and Gram negative like different species of *Bacillus, Staphylococcus, Salmonella* and *Pseudomonas* etc. are the main source to cause severe infections in humans. Traditional healers claim that their medicine is cheaper and more effective than modern medicine so in developing countries, low-income people such as farmers, people of small isolate villages and native communities use folk medicine for the treatment of common infections (Rojas et al., 2006). In addition only a limited number of *in vivo* and *in vitro* studies on antimicrobial properties of herbal products have been published (Tambekar et al., 2007; Cupp, 1999).

Thin layer chromatography (TLC) is a chromatography technique used to separate mixtures of compound in different parts of medicinal plant like root, stem, and leaf and identification of all phytochemicals. High performance thin layer chromatography (HPTLC) is an enhanced form of thin layer chromatography (TLC). HPTLC is now-a-days applied to obtain “Finger-print” patterns of herbal formulations, quantification of active ingredients and also detection of adulteration. HPTLC is a powerful analytical technique due to its merits of reliability, simplicity, reproducibility and speed.

Genetic variation is essential for long term survival of species and it is a critical feature in conservation. The objective of the present study is to assess genetic diversity of *A. racemosus* to provide genetic data and a theoretical basis for protection of the species. Therefore, RAPD polymorphism is the reflection of variation of the whole genomic DNA and would be a better parameter to measure the pattern of genetic diversity of the rare and endangered plants (Vijay et al., 2009B; Lal et al., 2011).

This study will be helpful in micro propagation, conservation of the endangered medicinal plant as well and use of secondary metabolites in pharmaceutical interest.