Isolation, structure elucidation and biotransformation studies on secondary metabolites from *Asparagus racemosus*

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Abstract - *Asparagus racemosus* is important medicinal plant of tropical and subtropical India, is a potent phytoestrogen, which is used extensively in the treatment of menopausal symptoms, diarrhea, dyspepsia, and neurodegenerative disorders. The multiple uses of this species have increased its commercial demand resulting in over-exploitation. Consequently, the plant is recognized as being "critically endangered" in its natural habitat. Development of an efficient Isolation, structure elucidation and biotransformation studies protocol will not only play a significant role in meeting the requirement of planting material for commercial cultivation, but also in aiding the conservation process.
Keywords- *Asparagus racemosus,* phytochemical, HPLC, UV, Chemical analysis

Asparagus racemosus

Shatavari, *Asparagus racemosus*, is a climbing plant which grows in low jungles areas throughout India. This sweet and bitter herb is particularly balancing to Pitta Dosha. In India, Shatavari is considered the women's equivalent to Ashwagandha. The name translates to "she who possesses 100 husbands", referring to the herbs rejuvanitive effect upon the female reproductive organs. In Australia the herb is more often used to treat gastrointestinal disorders and as an external wash for wounds. The healing qualities of Shatavari are useful to a wide array of ailments. It is well known for it effects on the female reproductive system. It is also effective in a number of other systems of the body and is therefore of use to both men and women. Shatavari is perhaps best known as a female rejuvanitive. It is useful for infertility, decreased libido, threatened miscarriage, menopause, leucorrhea and has the ability to balance pH in the cervical area. Dry membranes, such as those on the vaginal wall, are also brought into balance through the herbs demulcent action. Men may benefit from the herb as well in the treatment of impotence and general sexual debility. In addition to it's applications for reproductive organs, Shatavari is also quite effective for stomach ulcers, hyperacidity and diarrhea. Dry and irritated membranes in the upper respiratory tract are soothed by this herb making it useful in cases of bronchitis and chronic fevers. It is believed to bring into balance all of the body's fluids. The most important herb in Ayurvedic medicine for women; used internally for infertility, loss of libido, threatened miscarriage, menopausal problems. It both nourishes and cleanses the blood and the female reproductive organs. It is a good food for menopause or for those who have had hysterectomies, as it supplies many female hormones. It nourishes the ovum and increases fertility. This herb is known to increase Sattva, or positivity and healing power. It also enhances the feelings of spiritual love, and increases Ojas. The male reproductive system will also benefit from Shatavari. It may be used in cases of sexual debility, impotence, spermatorrhea, and inflammation of sexual organs and useful for hyperacidity, stomach ulcers, dysentery, and bronchial infections [1].

Definition of the problem

Indigenous rural communities in the tropics manage parasitic diseases, like malaria and leishmaniasis, using herbal drugs. The efficacy, dosage, safety and active principles of most of the herbal preparations are not known. The investigations demonstrated the efficacy and safety of some extracts of *Asparagus racemosus* plants that are used by rural indigenous communities for the treatment of parasitic diseases [2]. The results of the biochemical determinations also show that pretreatment on rats with the aqueous extract of *Asparagus racemosus* leads to the amelioration of oxidative stress and hepatotoxicity brought about by treatment with diethylnitrosamine. These results prove that the aqueous extract of the roots of *Asparagus racemosus* has the potential to act as an effective formulation to prevent hepatocarcinogenesis induced by treatment with diethylnitrosamine [3]. Five steroidal saponins, shatavarins VI-X, together with five known saponins, shatavarin I (or asparoside B), shatavarin IV (or asparinin B), shatavarin V, immunoside and schidigerasaponin D5 (or asparanin A), are the chemical constituent available roots of *Asparagus racemosus* [4]. Three more steroidal saponins, racemosides A (1), B (2) and C (3), are seen in the fruits of *Asparagus racemosus* [5,6].

Importance of the proposed project in the context of current status

The protective effects of *Asparagus racemosus* against myelosuppression [8]. The powdered dried root of *Asparagus racemosus* (Shatavari) is used in Ayurveda for dyspepsia (amlapitta or acid regurgitation) and to increase milk secretion in a lactating woman. A mixture 'Lactare', containing *Asparagus racemosus* as its major component, has been reported to cause significant rise in scrum prolactin levels [9]. The alcoholic extract of *Asparagus racemosus* has been shown to increase the

prolactin levels in female rats [10, 11]. However, no scientific proof justifying aforementioned uses of root extract of *A. racemosus* is available so far. 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 [12].

Technological utility and Socio- economic relevance

Asparagus racemosus (Shatavari) is recommended in Ayurvedic texts for prevention and treatment of gastric ulcers, dyspepsia and as a galactogogue. *A. racemosus* has also been used successfully by some Ayurvedic practitioners for nervous disorders, inflammation, liver diseases and certain infectious diseases. This project work includes the detailed exploration of pharmacological properties of the root extract of *A. racemosus* reported so far. Development of an efficient Isolation, structure elucidation and biotransformation studies protocol will not only play a significant role in meeting the requirement of planting material for commercial cultivation, but also in aiding the conservation process.

Methodology for semantic approach

Plant material

The roots of *Asparagus racemosus* wild are collect for the biochemical analysis. The plant is identified by routine pharmacognostical studies including organoleptic tests, and macroscopic and microscopic observations. The voucher specimen (NV-168) has been retain in laboratory for future reference. The collected roots were air-dried and pulverise using mechanical grinder.

Preparation and phytochemical study of extracts

The roots are coarsely powder and subjected to successive solvent extraction with 95% ethanol and water. A semi-solid extract is obtained after complete elimination of solvent under reduced pressure. The extracts will store in desiccators and used for further experiment after suspending in aqueous Tween 80 solution (0.5%). The chemical constituents of the extracts will identify by qualitative chemical tests and further confirmed by thin layer chromatography study for the presence of alkaloids, sterols and/or terpenes and flavonoids.

HPLC

High-performance liquid chromatography (or High pressure liquid chromatography, HPLC) is a form of column chromatography used frequently in biochemistry and analytical chemistry to separate, identify, and quantify compounds.

UV

Ultraviolet-visible spectroscopy or ultraviolet-visible spectrophotometry (UV-Vis or UV/Vis) involves the spectroscopy of photons in the UV-visible region. The absorption in the visible ranges directly affects the color of the chemicals involved. In this region of the electromagnetic spectrum, molecules undergo electronic transitions. This technique is complementary to fluorescence spectroscopy, in that fluorescence deals with transitions from the excited state to the ground state, while absorption measures transitions from the ground state to the excited state. UV/Vis spectroscopy is use in the quantitative determination of solutions of transition metal ions and highly conjugated organic compounds.

Animals

Inbreed Albino Wistar rats of either sex weighing between 200 and 260 g are use. They are housed in polyacrylic cages and fed with standard rodent pellet diet and given water ad libutum. The animals are housed under standard laboratory environmental condition for an acclimatization period of 14 days prior to perform the experiments.

Castor oil induced diarrhoea

Rats are divide into eight groups (n = 6) and, fasted for 18 h and water is provide ad libitum. The ethanol and aqueous extracts of *Asparagus racemosus* (150, 200 and 250 mg/kg, p.o.) are administered orally to the first six groups of rats. The frequency of defecation is noted in transparent plastic dishes placed beneath the individual rat cages upto 4 h (13).

Statistical analysis

The data analyse statistically using one-way analysis of variance followed by Dunnett's `t' test. The data are expressed as mean ± s.e.m. P-values less than 0.05 imply significance.

Chemical analysis

The results of the preliminary phytochemical screening of ethanol and aqueous extracts of *A. racemosus* root. The antidiarrhoeal activity of flavonoids has been ascribed to their ability to inhibit intestinal motility and hydro-electrolytic secretion, which are known to be altered in this intestinal condition. In vitro and in vivo experiments have shown that flavonoids are able to inhibit the intestinal secretary response, induced by prostaglandins E2. In addition, flavonoids present antioxidant properties, which are presumed to be responsible for the inhibitory effects exerted upon several enzymes including those involved in the arachidonic acid metabolism. As a consequence, it is possible to suggest that the antisecretary and antioxidant properties of flavonoid could contribute to the observed anti-diarrhoeal effect.

Molecular modeling and structure analysis

Molecular modeling is the science (or art) of representing molecular structures numerically and simulating their behavior with the equations of quantum and classical physics. Molecular modeling is a collective term that refers to theoretical methods and computational techniques to model or mimic the behaviour of molecules from A. racemosus. The techniques are used in the fields of computational chemistry, computational biology and materials science for studying molecular systems ranging from small chemical systems to biological molecules and material assemblies obtained from A. racemosus. The simplest calculations for *A. racemosus* secondary metabolites can be performed by hand, but inevitably computers are required to perform molecular modelling of any reasonably sized system. The common feature of molecular modelling techniques is the atomistic level description of the molecular systems; the lowest level of information is individual atoms (or a small group of atoms). Quantum chemistry also known as electronic structure calculations, where electrons are consider explicitly for secondary metabolites and benefit of molecular modelling is that it reduces the complexity of the system, allowing many more particles (atoms) to be considered during simulations.

Future scope and objectives of research

1. Isolation of secondary metabolites from *Asparagus racemosus*
2. Structure elucidation of secondary metabolites from *Asparagus racemosus*
3. Biotransformation studies of secondary metabolites
4. Molecular modeling and structural analysis of secondary metabolites

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