



PHYTOCHEMICAL ANALYSIS AND ANTI-BACTERIAL ASSESSMENT OF ROOT EXTRACT OF ASPARAGUS RACEMOSUS (SHATAVARI) FOUND IN AMBIKAPUR, SURGUJA (CHHATTISGARH)

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ABSTRACT *Asparagus racemosus* (Shatavari) are recommended in Ayurvedic texts for prevention and treatment of gastric ulcers, dyspepsia and as a galactagogue. *A. racemosus* has also been used successfully by some practitioners of Ayurvedic for nervous disorders, inflammation, liver diseases and certain infectious and various kinds of diseases. However, no scientific proof justifying aforementioned uses of root extract of *A. racemosus* (Shatavari) is available so far. Recently few reports are available demonstrating beneficial effects of alcoholic and water extracts of the root of *A. racemosus* (Shatavari) in some clinical conditions and experimentally induced diseases, e.g. galactagogue effect, antihepatotoxic and immunomodulatory activities. The present study includes the detailed phytochemical analysis of the root extract of *Asparagus racemosus* reported so far.

KEYWORDS : Medicinal plant, Screening, root extract ,Phytochemical,

Introduction-

Phytochemicals (Greek word phyto, meaning plant) are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than, those attributed to macronutrients and micronutrients.⁽¹⁾ Medicinal plants are a rich source of bioactive phytochemicals or bionutrients. They protect plants from disease and damage and contribute to the plant's color, aroma and flavour. In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals.^(2,3) The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents.⁽⁴⁾ Phytochemicals have two categories i.e., primary and secondary constituents. Primary constituents have chlorophyll, proteins sugar and amino acids. Secondary constituents contain flavonoids, terpenoids, , phenols and alkaloids... etc.⁽⁵⁾

The Medicinal plants have antifungal, antibacterial and anti-inflammation activities. Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have different mechanism and protect from various diseases.⁽⁶⁾ The purpose of the present review is to provide an overview of the extremely diverse phytochemicals presents in medicinal plants. After centuries of empirical use of herbal preparation, the first isolation of active principles alkaloids such as morphine, strychnine, quinine etc. in the early 19th century marked a new era in the use of medicinal plants and the beginning of modern medicinal plants research. Emphasis shifted away from plant derived drugs with the tremendous development of synthetic pharmaceutical chemistry and microbial fermentation after 1945. Plant metabolites were mainly investigated from a phytochemical and chemotaxonomic viewpoint during this period. Numerous research groups have also reported such studies whole world.⁽⁷⁻¹¹⁾ Thus this study deals with the analysis based on phytochemical test of *A. racemosus* (Shatavari) medicinal plant occurring in northern part of newly Chhattisgarh state in Surguja region. The medicinal plant *A. racemosus* (Shatavari) for identifying their phytochemical constituents. This plant different bioactivities which were later correlated with the presence of some specific phytoconstituents

A. racemosus is an important plant in traditional medicine in tropical and subtropical India. Shatavari or Shatamull is the common name of the *A. racemosus* and belong to the family *Asparagaceae*. It has much medicinal value and used as medicines for centuries. *A. racemosus* (Shatavari) are the mostly used for the treatment of gastric ulcers and dyspepsia, and uterine tonic as a galactagogue *A. racemosus* (Shatavari) has also been used by some traditional Indian practitioners for nervous disorders and as a best general health tonic.

The *Asparagus* genus is considered to be of medicinal importance

because of the presence of steroidal saponins and sapogenins in various parts of the plant. *A. racemosus* (Shatavari) is commonly mentioned as a rasayana in the Ayurveda. Rasayanas are those plant drugs which promote general well being of an individual by increasing cellular vitality or resistance. It is a woody climber which grows to a height of 1-2 m. The leaves are of pine needle shape, small but uniform and have tiny white flowers arranged in the form of small spikes. The roots are tuberous, succulent, finger shaped and clustered.

Materials and Methods-

STEPS INVOLVED IN PLANT COLLECTION

- (i) Collection of plant material- Fresh root of *A. racemosus* (family Asparagaceae) was collected locally from Ambikapur, Surguja (C.G.) India and got identified by Prof. Sabita Roy, Head, Department of Botany and Sr. Anita Minj, Asstt. Prof. Department of Home Science H.C. Women's College, Ambikapur, C. G., India.
- (ii) **Cleaning of Plants** -After plants collection they have to be cleaned properly, washed in distilled water. Cleaning has to be done by hands in order to get better results.
- (iii) **Drying** -The main purpose of drying is to remove the water content from plants so that the plants can be stored, then root were shadow dried individually for 15 days at room temperature.
- (iv) **Powdering**-After complete drying of plants they have to be powdered well for further analysis. The powdered samples are stored in a container for extraction.
- (v) **Plant-extract preparation-**
 - (a) **Water extract** - To prepare root extract 10grams of prepared powder from selected plant is individually placed in 250 ml conical flask by adding 100 ml of distilled water to it such that plant powder soaked in it and shaken well. The solution then filtered with the help of filter paper and filtered centrifuge at 20 minute. Then the solutions were labeled Extract of the selected plant samples were taken and used for further phytochemical analysis. The water extract was kept in refrigerator when not in use.
 - (b) **Alcoholic extract**- To prepare root extract 10grams of prepared powder from selected plant is individually placed in 250 ml conical flask by adding 100 ml of 95 percent alcohol to it such that plant powder soaked in it and shaken well. The solution then filtered with the help of filter paper and filtered centrifuge at 20 minute. Then the solutions were labeled Extract of the selected plant samples were taken and used for further phytochemical analysis. The alcoholic extract was kept in refrigerator when not in use.

Preliminary Phytochemical Analysis Preliminary phytochemical screening : The qualitative analysis of water and alcoholic extracts for the presence of various phytochemical was carried out using the standard procedure with little modification.

1- Test for proteins

(a)Millon's test- Crude extract when mixed with 2 ml of Millon's reagent, white precipitate appeared which turned red upon gentle heating that confirmed the presence of protein.

(b)Ninhydrin test- Crude extract when boiled with 2ml of 0.2% solution of Ninhydrin, violet colour appeared suggesting the presence of amino acids and proteins.

2-Test for saponins- Foam test Crude extract was mixed with 5ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.⁽¹²⁾

3-Test for carbohydrates

(a)Fehling's test - Equal volume of Fehling A and Fehling B reagents were mixed together and 2ml of it was added to crude extract and gently boiled. A brick red precipitate appeared at the bottom of the test tube indicated the presence of reducing sugars.

(b)Molisch's test- Crude extract was mixed with 2ml of Molisch's reagent and the mixture was shaken properly. After that, 2ml of concentrated H₂SO₄ was poured carefully along the side of the test tube. Appearance of a violet ring at the interphase indicated the presence of carbohydrate.

4- Test for Terpenoids- 5 ml of each extract was added to 2ml of chloroform and 3ml of con.H₂SO₄ to form a monolayer of reddish brown coloration of the interface was showed to form positive result for the terpenoids.⁽¹³⁾

5- Test for Flavonoids- Add a few drops of concentrated HCL and Mg turning to 1 ml of ethanol extract. Appearance of pink or magenta-red color indicates the presence of flavonoids.⁽¹⁴⁾

6- Test for Alkaloids-To the extract added 1% HCL and 6 drops of Mayer's reagent and Dragendorff reagent. Any organic precipitate indicated the presence of alkaloids in the sample.

7- Test for steroids-2 ml of acetic anhydride was added to 0.5 g of ethanolic extract of each sample with 2 ml of H₂SO₄. The colour change from violet to blue or green indicated the presence of steroids.

8- Test for cardiac glycoside-

(a) 5ml of each extract was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1ml of Conc. H₂SO₄. A brown ring of the interface indicated a deoxysugar characteristic of cardenolides. A violet ring might appear below the brown ring whereas acid layer, a greenish ring might form just gradually throughout thin Layer.

(b)Salkowski's test - Crude extract was mixed with 2ml of chloroform. Then 2ml of concentrated H₂SO₄ was added carefully and shaken gently. A reddish brown colour indicated the presence of steroidal ring, i.e., glycone portion of the glycoside.

9-Test for phenols and tannins

(a) Crude extract was mixed with 2ml of 2% solution of FeCl₃. A blue-green or black coloration indicated the presence of phenols and tannins.

(b)Tannins – The substance mixed with lead acetate solution. Formation of white precipitate indicates the presence of Tannins.

10- Fixed Oils and Fats

(a)Spot Test – A small quantity of extract was pressed between two filter papers. oil stain on the paper indicates the presence of fixed oil.

11-Test for gum and Mucilages-

The extract is dissolved in 10 ml of distilled water and to this 2 ml of absolute alcohol is added with constant stirring. White or cloudy precipitate indicates the presence of Gums and Mucilages.⁽¹⁵⁾

ANTIMICROBIALACTIVITY FOR SELECTED BACTERIA-

The filter paper disc diffusion method was used for screening the extract for antimicrobial activity. Standard size what's man filter paper disc (6.0mm diameter) were sterilized in an oven at 140°C for one hour, saturated with aqueous and oil plant extract such as fruit and leaf. The

discs were then placed on the surface of sterilized nutrient agar medium that had been inoculated with test bacteria (using bacterial suspensions) air dried to remove surface moisture. The thickness of the agar medium was kept equal in all the petriplates and the standard disc. Before incubation, the petriplates were placed for one hour in a cold room (5°C) to allow the diffusion of the compounds from the disc into the medium. Plates were incubated at 37°C for 20-24 hours after which the zone of inhibition or depressed growth could be easily measured. All the experiments were done in five replicates and the activity index was calculated for each of these. (Patanaik and Subramanyam)

RESULT AND DISCUSSION-

When we performed qualitative tests for phytochemicals in *A.racemosus* (Shatavari), various phytochemicals show to positive result in their specific tests. Though some are found in multiplicity while some in small amount. In this study, the phytochemical analysis of *A.racemosus* (Shatavari) root extract, showed positive results for saponins, tannins, terpenoids, glycosides, alkaloids, carbohydrates, steroids, coumarin, protein. and negative results for gum/mucilage and flavonoids. The root extracts contain different phytochemicals in biological activity that can be of valuable therapeutic index. In the present study, it was observed that the *A. racemosus* on which scientific studies have been conducted are validated in their uses by the various parts of Sarguja region in Chhattisgarh. The different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases. Phytochemical analysis of various extracts of *Asparagus racemosus* root used to study the presence of contained alkaloids, steroids, saponins, tannins and terpenoids and also have various medicinal values such as anti-inflammatory, anti-diabetic and analgesic activities and for central nervous system activity.

Table-1 Phytochemical present in aqueous and ethanolic extract of Asparagus racemosus

S.N.	Phytoconstituents	<i>Asparagus racemosus</i> aqueous extract	<i>Asparagus racemosus</i> ethanolic extract
1.	Alkaloid	–	+
2.	Carbohydrate	+	+
3.	Flavonoid	–	–
4.	Glycosides	++	++
5.	Gum/Mucilage	–	–
6.	Protein	–	+
7.	Phenol	++	++
8.	Saponins	+++	+++
9.	Tannins	++	++
10.	Terpenoids	++	+

(+, ++, +++ represent degree of intensity of colour change i.e. presence of phytochemical groups and the – represent no change of colour i.e. absence of phytochemical groups)

Table - 2:- Antibacterial activity for the root extract of Asparagus racemosus (Duration 24 hrs.)

S.No.	Isolated bacteria	Aqueous extract of A. racemosus	Alcoholic of A.racemosus
1.	E.coli met	–	–
2.	E.coli strain 1	+	+
3.	E.coli strain 2	+	+
4.	Staphylococcus strain 1	–	–
5.	Staphylococcus strain 2	–	–

(+ indicate presence and – indicate absence)

Antimicrobial activity - Antimicrobial activity for the roots of *Asparagus racemosus* against E.coli met, E.coli strain 1, E.coli strain 2, Staphylococcus strain 1, Staphylococcus strain 2 are summarized in table-2

Antimicrobial activity was carried out by well diffusion method using Nutrient agar against bacteria like E.coli met, E.coli strain 1, E.coli strain 2, Staphylococcus strain 1, Staphylococcus strain 2. The plant extract of *Asparagus racemosus* (roots) and had shown a strong antimicrobial activity against E.coli strain 1, E.coli strain 2.

These findings suggest that the root extracts have antimicrobial

properties and could be used for bio control of bacterial cultures. Antimicrobial activity was confirmed by the selected plant species and the results revealed that plant extracts varied in their property for inhibiting to the bacterial growth against the tested pathogens.

CONCLUSION-

The experimental plant *Asparagus racemosus* (Shatavari) studied here can be a potential source of useful drugs. Advanced studies are being conducted on these plants in order to identify, characterize and analyse the bioactive compounds. *Asparagus racemosus* (Shatavari) plant have been raw material for synthesis of many drug and they remain an important source of new therapeutic agent powder of Shatavari root can be given effectively to the chronic peptic ulcers patients along with other medication.

REFERENCES-

1. Hasler CM, Blumberg JB. Symposium on Phytochemicals: Biochemistry and Physiology. Journal of Nutrition 1999; 129: 756S-757S.
2. Gibson EL, Wardel J, Watts CJ. Fruit and Vegetable Consumption, Nutritional Knowledge and Beliefs in Mothers and Children. Appetite 1998; 31: 205-228.
3. Mathai K. Nutrition in the Adult Years. In Krause's Food, Nutrition, and Diet Therapy, 10th ed., ed. L.K. Mahan and S. Escott-Stump, 2000; 271:274-275.
4. Nostro A, Germanò MP, D'angelo V, Marino A, Cannatelli MA (2000) Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Lett Appl Microbiol 30: 379-384.
5. Krishnaiah D, Sarbatly R, Bono A (2007) Phytochemical antioxidants for health and medicine: A move towards nature. Biotechnol Mol Biol Rev 1: 97-104.
6. Wadood et.al. =, Biochem Anal. Biochem 2013 Vol. 2, Issue 4 1000144, Page 1
7. Raphael E. Phytochemical constituents of some leaves extract of Aloe vera and indica plant species. Glo Adv Res J Environ Sci Toxicol 2012; 1:14-17.
8. Kumari SPK, Sridevi V, Lakshmi MVVC. Studies on Phytochemical screening of aqueous extract collected from fertilizers affected two medicinal plants. J Chem Bio Phy Sci 2012; 2:1326-1332.
9. Kharat SS, Kumkar PB, Siddhesh RR, Sonawane KS. Qualitative phytochemical screening of *Gnidia glauca* (Fresen) Gilg. Plant extract. Int J Pharm Bio Sci 2013; 4:144-148.
10. Kavitha R, Premalakshmi V. Phytochemical analysis of ethanolic extract of leaves of *Clitoria ternatea* L. Int J Pharm Bio Sci 2013; 4:236-242.
11. Dasgupta S, Parmar A, Patel H. Preliminary phytochemical of *Kalanchoe Gastonis-bonnieri*. Int J Pharm Bio Sci 2013; 4:550-557.
12. RNS Yadav – Phytochemical analysis of some medicinal plants, Journal of Phytology 2011, 3 (12): 10-14
13. Ayoola GA, Coker HAB, Adesegun SA, Adepoju- Bello AA, Obaweya K, Ezennia EC et al. Phytochemical Screening and antioxidant activities of some Selected Medicinal Plant used for malaria therapy in Southwestern Nigeria.
14. Odebiyi OO, Sofowora EA. Phytochemical screening of Nigerian medicinal plant II. Lloydia 1978; 41:234-246
15. Whistler R.L. and J.N. BeMiller, 'Industrial Gums ; Polysaccharides and their Derivatives Academic Press, London, 1993