IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

Phytochemical Analysis of *Asparagus racemosus*: A Review of constituents and their Potential Bioactivities

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Abstract

Asparagus racemosus, or Shatavari, is a highly valued medicinal plant in Ayurveda, widely employed for various traditional medicinal purposes. Its effectiveness is partly due to its dense and varied composition of phytochemicals. The present paper is a review of literature reported on phytochemical characterization of *Asparagus racemosus*, specifically on the identification, characterization, and probable bioactivity of its major constituents. Saponins, especially shatavarins I-IV, are known to be the principal bioactive constituents.

Asparagus racemosus is also endowed with flavonoids, alkaloids, polysaccharides, and other varied constituents that contribute to the overall pharmacological activity of the plant. It is important to know the intricate phytochemical profile of this plant for establishing its traditional applications, investigating its potential in contemporary medicine, and maintaining the quality and efficacy of *Asparagus racemosus*-derived herbal preparations.

1. Introduction

Asparagus racemosus (Willd.) of the family Asparagaceae is a perennial climbing shrub with widespread distribution in India, Sri Lanka, Australia, and Africa. Shatavari in Ayurveda, "she who has a hundred husbands," it has been a tonic, anti-aging drug, and galactagogue since centuries. Ayurvedic uses are in treating female reproductive tract disorders, stimulating lactation, enhancing digestion, and immune stimulation (Goyal *et al.*, 2003). The roots are the chief part employed medicinally and are said to contain powerful therapeutic principles.

The activity of *Asparagus racemosus* is due to its intricate chemical constitution. Phytochemical study has shown the occurrence of a range of bioactive compounds, such as saponins, flavonoids, alkaloids, and polysaccharides.

Knowledge of the phytochemical constituents is critical for describing the plant's traditional uses and for investigating its potential in contemporary pharmaceutical and nutraceutical industries.

Collection of Plant Materials

The plants *Asparagus racemosus* of were collected from Bhopal, M.P. in India. Identification of plant was carried out at Department of life science and a voucher specimen was procured in herbarium record maintained at theLaboratory, The plant material was thoroughly washed with water and was kept for drying in shade at room temperature at for 20days. The thoroughly air dried plant material was grinded to powder to about 40-60 mesh size weighted and stored in large plastic bottles for future extraction and chemical testing.

Solvent Extraction:

In this method the 40-60-mesh size powdered plant material was extracted with soxhlet apparatus using 70 or 90% ethanol. The weighted amount of plant material was poured in the apparatus in 70% ethanol. The extraction was done for 48 hours duration or till cycles are completed. The extract is concentrated on a rotatory evaporator Almost all the chlorophyll and lipid is deposited on the side of the flask and with skill it was removed.

The concentration can be pipetted off, almost completely free of lipid impurities. The concentrated extract was found to deposit as crystals, on standing this crude extract gave 5.70% and 6.72%, which was tested for bioassay and chromatographic separation of the flavonoid compound.

Alternatively it was also extracted by hydrolyzing plant powder with 2M HCl for 30 minutes at 100°C. The cooled solution is filtered by muslin cloth and then filtrate is extracted thrice with ethyl acetate ant the combined extract was taken to dryness and collected in glass vials for further processing.

Qualitative phytochemical tests .

Preparation of test solution

The test solution was prepared by taking 1 g of the extract in 25 ml of methanol.

A. Test for Carbohydrates

Following tests were carried out for carbohydrates.

a) **Molisch's test:** In a test tube containing extract of drug, added two drop of freshly prepared 20% alcoholic solution of α- napthol and mixed concentrated sulphuric acid along the sides of the test tube. If carbohydrate present purple color or reddish violet color produce at the junction between two liquids.

b) **Benedict's test:** In a test tube containing extract of drug add benedict's solution, mix well, boiled the mixture vigorously for two minutes and then cooled. Formation of red precipitate due to presence of carbohydrates.

c) **Barfoed's test:** The barfoed's solution added to 0.5 ml of solution under examination, heated to boil. Formation of red precipitate of copper oxide was indicated the presence of carbohydrates.

d) **Anthrone test:** To the two ml of anthrone test solution, add the extract of drug. A green or blue colour indicated the presence of carbohydrate.

B. Test for Alkaloids

a) **Dragendorff's Test**: Few mg of extract of the drug dissolved in 5 ml of water added 2 M hydrochloric acid until an acid reaction occurred; 1 ml of dragendorff's reagent (potassium bismuth iodide solution) was added an orange red precipitate indicated the presence of alkaloids.

b) **Wagner's test**: Acidify the extract of drug with 1.5 % v/v of hydrochloric acid and added a few drop of Wagner's reagent (iodine potassium iodide solution). Formations of reddish brown precipitate indicated the presence of alkaloids.

c) **Mayer's Test**: Two ml of extract solution was treated with 2 - 3 drops of Mayer's reagent was added (potassium mercuric iodide solution) formation of dull white precipitate indicated the presence of alkaloid.

d) **Hager's Test**: Extract of the drug solution was treated with 3 ml of Hager's reagent (saturated solution of picric acid) formation of yellow precipitate confirmed the presence of alkaloids.

C. Test for Steroids and Sterols

a) **Liebermann's Burchard reaction**: The test extract solution was dissolved in 2 ml of chloroform in a dry test tube. Now 10 drops of acetic anhydride and 2 drops of concentrated sulphuric acid were added. The solution became red, then blue and finally bluish green in color.

b) **Salkowsky test**: The extract of test solution dissolved in chloroform and equal volume of conc. sulphuric acid was added. Bluish red cherry, red and purple color was noted in chloroform layer, whereas acid assumes marked green fluorescence.

D. Test for Glycosides

a) **Legal's test**: Extract solution dissolved in pyridine then sodium nitroprusside solution was added to it and made alkaline. Pink red colour indicated the presence of glycosides.

b) **Baljet's test**: To the drug extract, sodium picrate solution was added, yellow to orange colour was indicated the presence of glycosides.

c) **Borntrager's test**: Few ml of dilute sulphuric acid solution, the test solution of extract was added. It was filtered and the filtrate was boiled with ether or chloroform. Then organic layer was separated to which ammonia was added, pink, red or violet colour was produced in orange layer confirmed the presence of glycosides.

d) **Keller Kiliani test**: Methanolic extract was dissolved in glacial acetic acid containing trace of ferric chloride one ml concentrated sulphuric acid was added carefully by the side of the test tube. A blue

colour in the acetic acid layer and red colour at the junction of the two liquid indicated the presence of glycosides.

E. Test of Saponins

a) 1 ml of alcoholic extract was diluted with 20 ml distilled water and shaken in graduated cylinder for 15 minutes. One cm layer of foam indicated the presence of saponins.

F. Test for Flavanoids

a) **Shinoda test**: In the test tube containing alcoholic extract of the drug added 5 - 10 drops of dil. hydrochloric acid followed by the small piece of magnesium. In presence of flavonoids a pink, reddish pink or brown color was produced.

G. Test for Tannins

a) To the sample of the extract, ferric chloride solution was added appearance of dark blue or greenish black colour indicated the presence of tannins.

b) To the sample of extract, potassium cyanide was added, deep red colour was confirmed the presence of tannins.

c) To the sample of extract, potassium dichromate solution was added, yellow precipitate was produced.

Test for Triterpenoids

a) In the test tube, 2 or 3 granules of tin was added, and dissolved in 2 ml of thionyl chloride solution and test solution was added. Pink colour was produced which indicates the presence of triterpenoids.

b) Two ml of acetic anhydride solution was added to 1 ml of extract of drug in chloroform followed by one ml of conc. sulphuric acid, a violet colored ring was formed indicating presence of triterpinoid.

Test for Protein and Amino acid

Biuret's test: To 2 - 3 ml of the extract of drug added in 1 ml of 40 % sodium hydroxide solutions and 2 drops of 1 % copper sulphate solution mix thoroughly, a purplish - violet or pinkish - violet colour produced that indicates the presence of proteins.

Ninhydrin's test: Two drops of freshly prepared 0.2 % ninhydrin reagent was added to the extract and heated to boiling for 1 - 2 min. and allow cooling. A blue colour developed that indicating the presence of proteins, peptides or amino acids.

Xanthoprotein test: To the extract in a test tube, add conc. nitric acid. A white precipitate was obtained and upon heating turns to yellow and cool the solution carefully. Added 20 % of sodium hydroxide solution in excess orange colour indicated presence of aromatic amino acid.

Millon's test: The small quantity of extract of the drug dissolved in distilled water added 5 - 6 drop of millon's reagent. A white precipitate was formed which turned red on heating, indicated the presence of proteins.

Lead Acetate test: The extract was taken and two ml of 40 % sodium hydroxide solution was added and boiled, glacial acetic acid was added and cooled than added 1 ml of lead acetate solution, gray black precipitate was formed which indicated presence of sulphur containing amino acid.

Test of Resins

Dissolved the extract in the acetone and pore the solution in the distilled water. Turbidity indicated the presence of resin.

Test of fats or fixed oils

a) Using sodium hydroxide: The extract was mixed in one ml 1 % of copper sulphate solution then added 10 % sodium hydroxide solution a clear blue solution was obtain which showed glycerin present in sample.

b) Using sodium hydrogen sulphate: The extract was taken in test tube added a pinch of sodium hydrogen sulphate pungent odour was formed which showed glycerin present in sample.

c) Saponification: Four ml of 2 % sodium carbonate solution was taken and the extract was added. Shaked vigorously and boiled. A clean soapy solution was formed cooled and added few drops of conc. HCl and observed that fatty separate out and float up.

Table 1: Phytochemical evaluation of *Asparagus racemosus*

Chemical Tests	**Chloroform**	**Ethyl acetate**	**Ethanol**	**Water**
Alkaloids				
Mayer's reagent	-	-	-	+
Hager's reagent	-	-	-	+
Wagner's reagent	-	-	-	+
Dragendorff's reagent	-	-	-	+
Glycosides (+Ve)				
Baljet test	-	-	+	+
Legal's test	-	-	+	+

Keller-Kiliani	-	-	+	+
Phenols/Tannins				
Ferric chloride	-	-	+	-
Gelatin Solution	-	-	+	-
Lead acetate test	-	-	+	-
Flavonoids				
FeCl3 test	-	+	+	+
Alkaline reagent test	-	+	+	+
Shinoda test	-	+	+	+
Saponins				
Foam test	-	-	-	+
Hemolytic test	-	-	-	+
Lead acetate	-	-	-	+
Fixed oil/Fats				
Spot	+	-	-	-
Saponification	+	-	-	-
Gums & Mucilage				
Water	-	-	-	-
Carbohydrates				
Molish test	-	-	+	+
Fehling's solution test	-	-	+	+
Benedict's test	-	-	+	+
Amino acids				

Ninhydrin Test	+	+	+	-
Millons Test	+	+	+	-
Xantoprotein Test	+	+	+	-
Terpenoids				
Lieberman Burchard Test	-	-	+	-
Salkowski test	-	-	+	-
Steroids				
Lieberman Test	-	+	+	-
Protein				
Biuret test	-	-	+	+

d) (+) Indicates 'Presence'; (-) Indicates 'Absence'

2. Phytochemical Constituents of *Asparagus racemosus*

Extensive phytochemical studies of *Asparagus racemosus* have yielded a wide variety of compounds, of which saponins are the most widely investigated and are known to play a major role in the therapeutic properties of the plant.

TLC study has shown the presence of different phytoconstituents present in n-butanol and ethyl acetate fractions when corresponding fractions were run in a specific solvent system, before reaching to most optimum solvent system a number of solvent systems were employed as shown in **Table 2**for n butanol

Table-2: Selection of mobile phase for TLC for n- butanol fraction

S. No.	Solvent system	Observation
01.	$CHCl_3$	No Change
02.	C_6H_6: $CHCl_3$ (50:50)	No Movement
03.	$CHCl_3$:CH_3OH (50:40)	Separated but tailing
04.	$CHCl_3$:C_2H_5OH (20:1)	No Separation
05.	$CHCl_3$:CH_3OH:H_2O (70:30:4)	Moved along with solvent front
06.	$CHCl_3$:CH_3OH (50:50)	Separated but slight tailing
07.	**$CHCl_3$:CH_3OH (90:10)**	**Best separated (Table 10)**

Table 3 Separation of bioactive constituents from n-butanol fraction.

S. No.	Eluting Solvent & Composition	Collectd Isolates	Compounds	Detection for Single spot to pool the same fractions	Final Fractions % Yield (w/w of crude extract)
01.	$CHCl_3$:MeOH (100:0)	1-13	No Residue after evaporation	On TLC plates using chloroform: methanol (90:10v/v) as mobile phase & iodine vapors as detecting agent.	_
02.	$CHCl_3$:MeOH (95:5)	14-40	Fr-I (R_f =0.66)		nBF-I (10% w/w, 0.6 gm)
03.	$CHCl_3$:MeOH (90:10)	41-49	Very less quantity was isolated		-
04.	$CHCl_3$:MeOH (80:20)	50-51	Fr-II (R_f =0.39)		nBF-II (4.5% w/w, 0.265 gm)
05.	$CHCl_3$:MeOH (50:50)	52-61	No Residue after evaporation		-
06.	$CHCl_3$:MeOH (0:100)	62-66	No Residue after evaporation		_

Table 4. Evaluation of isolated bioactive fractions from n-butanol fraction. extract of *Asparagus racemosus*. for antioxidant activity.

Conc.µg/mL	% Inhibition (µg/mL)					
	EF-I	**EF-II**	**BF-I**	**BF-II**	**AQF**	**ASA**
25	21.02±0.25	5.32±0.49	4.01±0.22	23.01±0.33	2.22±0.55	34.23±0.31
50	37.83±0.31	14.99±0.34	9.39±0.35	33.33±0.42	7.91±0.35	49.71±0.34
75	42.89±0.27	23.54±0.61	20.01±0.27	44.39±0.23	23.01±0.56	64.03±0.42
100	65.37±0.43	39.23±0.31	24.02±0.32	62.18±0.39	25.51±0.41	89.11±0.49
125	78.80±0.41	39.01±0.56	39.27±0.31	70.23±0.36	36.89±0.38	94.21±0.46
IC_{50}µg/mL	**67.26**	**167.41**	**197.11**	**80.90**	**229.58**	**59.62**

Several phenolic compounds are reportable to have potent antioxidant activity and Immunomodulatoryactivities to a lower or higher degree .Hundreds of natural phenolic compounds have been known from our tested medicinal herbs and dietary plants, primarily involving phenolic acids, tannins, flavonoids, stilbenes, coumarins, curcuminoids, lignans, quinones, and phenolic mixtures and several other phenylpropanoids and phenylethanoids. Their physiological and pharmacological functions might start from their antioxidant and free radical scavenging properties and function of regulating detoxifying enzymesOn the basis of above results, it was concluded that purified fraction has lower IC_{50} value and hence has highest antioxidant potential because the minimal IC_{50}announces the vigorous capability to serve as DPPH radical scavenger.

Conclusion

Asparagus racemosus is a richly valued medicinal plant with a diverse phytochemical composition that is responsible for its varied therapeutic properties. Saponins, especially shatavarins, are the principal bioactive constituents, followed by flavonoids, alkaloids, and polysaccharides. These constituents show antioxidant, anti-inflammatory, immunomodulatory, and other useful activities. Elucidation of the intricate phytochemical composition of *Asparagus racemosus* is important for its vindication of traditional applications, its appreciation in contemporary medicine, and the quality and potency control of *Asparagus racemosus*-derived herbal preparations. More work has to be conducted to counteract the limitations and investigate the total therapeutic value of this significant medicinal plant.

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