

PHARMACOGNOSTICAL, PHYTOCHEMICAL AND ANTIBACTERIAL ACTIVITY OF
ASPARAGUS RECEMOSUS

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ABSTRACT

This study evaluated the physicochemical properties, phytochemical content, heavy metal safety, and antimicrobial activity of *Asparagus racemosus* leaf extracts. The analysis revealed significant ash values and extractive yields, indicating the presence of valuable bioactive compounds. Quantitative estimation showed notable amounts of phenolics, flavonoids, and tannins, particularly in the ethanol extract. Heavy metal concentrations were found to be within safe limits. Chromatographic techniques including TLC and HPLC confirmed the complexity of the extracts. Antibacterial tests demonstrated moderate activity against common pathogens. These results validate the traditional use of *Asparagus racemosus* and support its potential for further pharmacological applications.

1. INTRODUCTION

Asparagus racemosus, commonly known as Shatavari, is a well-known medicinal plant widely used in traditional Ayurvedic medicine. It is valued for its diverse therapeutic properties, including adaptogenic, immunomodulatory, and antioxidant effects. The pharmacognostical study of *Asparagus racemosus* involves the detailed examination of its macroscopic and microscopic characteristics, which helps in authenticating the plant material and ensuring its quality and purity.^[1]

Phytochemical investigation reveals the presence of various bioactive secondary metabolites such as saponins, flavonoids, alkaloids, tannins, and phenolic compounds. These constituents contribute to the plant's pharmacological activities and are of interest for their potential health benefits. *Asparagus racemosus* exhibits notable antibacterial activity against a range of pathogenic microorganisms, making it a promising candidate for developing natural antimicrobial agents. Evaluating its antibacterial potential helps in validating its traditional use and exploring alternative treatments against resistant bacterial strains.^[2]

2. MATERIAL AND METHOD**2.1 Collection of plant material**

The leaves of *Paederia foetida* was collected from CCS University, Meerut, U.P. India. Herbarium was deposited in Department of Botany, CCS University, Meerut, U.P. India. The leaf was washed with tap water, then surface sterilized with 10% sodium hypochlorite solution to prevent contamination of any microbes. The sample rinsed with distilled water and allowed to 50°C and then ground into powder using electric blender.^[3]

2.2 Physicochemical constant

The shade-dried and powdered leaves of *Paederia foetida* were analyzed to determine key physicochemical parameters, following WHO guidelines for quality control of medicinal plant materials. These tests help assess the purity, identity, and quality of the crude drug.^[4]

2.3 Ash values

Ash values are crucial analytical indicators used to assess the quality, identity, and purity of powdered crude drugs. They help detect contamination or adulteration by measuring the total inorganic residue, which may include natural minerals, soil, dust, or added impurities.^[5]

2.4 Total ash

Total ash refers to the total amount of inorganic residue left after complete combustion of plant material. It includes both physiological ash (from the plant's natural minerals) and non-physiological ash (from external contaminants like soil or dust). This measurement is essential for evaluating the purity and quality of crude drugs.^[6]

2.5 Water soluble ash

Water-Soluble Ash is determined by heating total ash with purified water, filtering, and incinerating the insoluble residue. The difference between total ash and the remaining insoluble ash gives the water-soluble ash, expressed as a percentage of the air-dried plant sample. This helps assess the amount of inorganic components that are water-soluble, indicating the presence of natural water-soluble salts and possible impurities.^[7]

2.6 Acid insoluble ash

Acid-Insoluble Ash is the portion of total ash that remains undissolved after boiling with dilute hydrochloric acid. It mainly indicates the presence of silica, such as sand or siliceous matter, and helps detect contamination with earthy materials in plant drugs.^[8]

2.7 Sulphate ash

Sulphated ash is determined by heating a powdered sample in a silica crucible, moistening the residue with concentrated sulfuric acid, and igniting it at high temperature until all carbon is removed. This process is repeated until a constant weight is achieved, ensuring accurate measurement of inorganic content.^[9]

2.8 Determination of extractive value

Extractive values help assess the phytoconstituents in plant materials by measuring the amount of active compounds extracted using specific solvents. They provide insight into the chemical nature and concentration of these compounds, revealing their solubility and aiding in evaluating the quality of crude drugs.^[10]

2.9 Determination of soluble extractive value

To determine the water-soluble extractive value, 5 grams of finely powdered leaf material is soaked in 100 ml of chloroform water (95 ml distilled water and 5 ml chloroform) in a tightly closed container for 24 hours. The mixture is shaken periodically during the first six hours, then left undisturbed for 18 hours. After maceration, the solution is filtered quickly to prevent solvent loss. A 25 ml portion of the clear filtrate is then dried on a pre-weighed flat-bottomed plate to calculate the extractive value.^[11]

$$\text{Water-soluble extractive (\%)} = (\text{Weight of dried extract} / \text{Weight of sample}) \times 100$$

2.10 Examination of alcohol soluble extract

Five grams of crushed plant material was soaked in 100 ml of 90% ethanol in a sealed container. The mixture was shaken for six hours, then left to stand for 12 hours,

followed by 18 hours of rest. After maceration, it was quickly filtered to prevent solvent loss. A 25 ml portion of the filtrate was evaporated to dryness in a pre-weighed flat-bottomed dish, then dried in a hot air oven at 105°C for one hour. The dish was cooled in a desiccator and weighed, repeating drying and weighing until a constant weight was obtained. The alcohol-soluble extractive value was then calculated.^[12]

2.11 Examination of fixed oil content

Powdered plant material was placed in a thimble and subjected to Soxhlet extraction using petroleum ether (boiling point 40–60°C) for six hours to remove ether-soluble compounds. The petroleum ether extract was collected in a weighed evaporation plate and evaporated to dryness. The residue was then heated at 105°C in a hot air oven until constant weight to remove any residual solvent or moisture.^[13]

2.12 Examination of volatile ether soluble extract

Two grams of plant powder were continuously extracted with anhydrous diethyl ether for twenty hours. The ether extract was transferred to a pre-weighed porcelain plate and allowed to evaporate at room temperature. The residue was then placed over phosphorus pentoxide for 18 hours to remove moisture and residual ether. Finally, the sample was dried in a hot air oven at 105°C until constant weight, noting weight loss due to volatile components.^[14]

2.13 Loss on drying

A precise amount of the sample was weighed and placed in a pre-weighed, ignited, and cooled silica crucible. The sample was spread evenly to ensure uniform heating, and the crucible with its lid was weighed to record the initial weight. It was then heated in a hot air oven at 105°C until a constant weight was achieved, indicating complete moisture removal. After heating, the crucible was covered and cooled in a desiccator to avoid moisture absorption.^[15]

2.14 Determination of foaming index

The foaming ability of the plant extract was tested by boiling 1 g of powdered material in water, filtering, diluting, and shaking different volumes in test tubes. Foam height after 15 minutes indicated the presence of saponins, and the foaming index was calculated based on foam measurements.^[16]

2.14 Examination of swelling index

The swelling index measures the volume occupied by 1 gram of plant powder after soaking in water. The powdered sample is mixed with 25 ml water in a graduated cylinder, shaken periodically, and left to swell for four hours. The final volume shows the swelling capacity, indicating the presence of mucilage or gums.^[17]

2.15 Examination on crude fiber content

The powdered plant sample is first defatted with ether to remove fats. The residue is then heated with 1.25%

sulfuric acid for 30 minutes, filtered, and washed. Next, it is refluxed with 1.25% sodium hydroxide for 30 minutes, filtered again, and washed until neutral pH is reached.^[18]

2.16 Quantitative Examination of Phytoconstituents

Total Phenolic content

The total phenolic content of the extracts was measured using the Folin-Ciocalteu method. Samples were mixed with sodium carbonate and diluted Folin-Ciocalteu reagent, incubated at 40°C for 30 minutes, and their absorbance was read at 765 nm. Results were expressed as mg of gallic acid equivalents per gram of extract (mg GAE/g), using gallic acid as the standard.^[19]

Determination of Total Flavonoid Content

Flavonoid content was measured colorimetrically using quercetin as a standard. Extracts dissolved in DMSO were mixed with 2% aluminum chloride, incubated for 10 minutes at room temperature, and absorbance was read at 435 nm. Results were expressed as mg quercetin equivalents per gram of extract (mg QRT/g).^[20]

For total tannin content, 0.1 g of extract was boiled with distilled water, filtered, and diluted to 500 ml. Aliquots of 0.5 ml were reacted and absorbance measured at 510 nm. Tannin content was expressed as mg tannic acid equivalents per gram of extract (mg TAE/g).^[21]

HPTLC

High-Performance Thin-Layer Chromatography (HPTLC) is an advanced technique used to separate and analyze complex mixtures. A small amount of sample is applied as a spot or band on a silica gel plate, which is then developed in a solvent chamber. As the solvent moves up the plate, components separate based on their affinity for the stationary and mobile phases. After drying, the plate is examined under UV light or with reagents, and a densitometer scans the spots to provide qualitative and quantitative data.^[22]

HPLC

High-Performance Liquid Chromatography (HPLC) is a precise technique for separating, identifying, and measuring components in a mixture. The sample, dissolved in a solvent, is injected into a high-pressure system where it passes through a column filled with a stationary phase. Components separate based on their interactions with this phase. A detector records each compound as it exits the column, producing a chromatogram. The retention time and peak area help identify and quantify the substances in the sample.^[23]

2.17 Antibacterial activity

Test Organisms

The study involved testing various microorganisms, including Gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*), and the fungus *Candida utilis*. All cultures were revived on

nutrient agar to confirm viability. Identification used standard microbiological methods such as colony morphology, microscopic examination, and biochemical tests. Stock cultures were stored on nutrient agar slants at 4°C, and before testing antibacterial activity, they were incubated in nutrient broth at 37°C.^[24]

Antimicrobial Activity: Agar Well Diffusion Assay

The antibacterial and antifungal activities of the plant extracts were evaluated using the agar well diffusion method, following the procedure described by Perez et al. (1990).^[25]

Media Preparation

Nutrient Agar (HiMedia) was used for bacterial culture, while Sabouraud's Dextrose Agar (HiMedia) was employed for fungal growth, both prepared according to the manufacturer's instructions.^[26]

Inoculation and Assay Procedure

After the agar solidified in sterile glass plates, the test microorganisms were evenly spread onto the surface under sterile conditions. Wells about 5 mm in diameter were made using a sterile cork borer, and each well was filled with a measured amount of plant extract. Standard antibiotics served as positive controls, while a negative control containing only the solvent (without plant extract or inoculum) ensured sterility and baseline reference. Plates with bacterial cultures were incubated at 37°C for 24 hours, and fungal cultures were grown under their optimal conditions.^[27]

3. RESULTS AND DISCUSSION

3.1 Physicochemical parameters

Table 1: Results of Physicochemical contents examination.

S. no.	Parameters	Percentage w/w
Ash value		
1	Total ash	7.03±0.11
2	Water soluble ash	6.01±1.24
3	Acid soluble ash	2.65±0.78
4	Sulphate ash	7.09±0.53
Extractive value		
5	Ethanol solubility extractive	13.23±1.48
6	Water soluble extractive	40.78±0.47
7	Ether insoluble non-volatile	22.85±0.29
8	Ether insoluble volatile	12.35±0.35
9	Loss on drying	6.24±0.22
10	Crude fiber content	50.34±0.28
11	Foaming index	Less than 100

3.2 Percentage yield

Table 2: Percentage yield of leaves extract of *Asparagus racemosus*.

S. No	Extract	Method of extraction	Physical nature	Colour	Percentage yield(w/w)
1	Chloroform	Extraction by Soxhlet apparatus	Semisolid	Green	4.92%
2	Ethanol	Extraction by Soxhlet apparatus	Semisolid	Brown	3.6%
3	Ethyl acetate	Extraction by Soxhlet apparatus	Semisolid	Yellowish brown	4.0%

3.3 Quantitative examination of phytoconstituents

Table 3: examination of total content.

S. No.	Concentration of standard solution	Absorbance (760 nm)
1	5	0.12
2	10	0.18
3	15	0.24
4	20	0.32
5	25	0.39
6	Chloroform	0.22±0.020
7	Ethyl Acetate	0.36±0.040
8	Ethanol	0.39±0.047

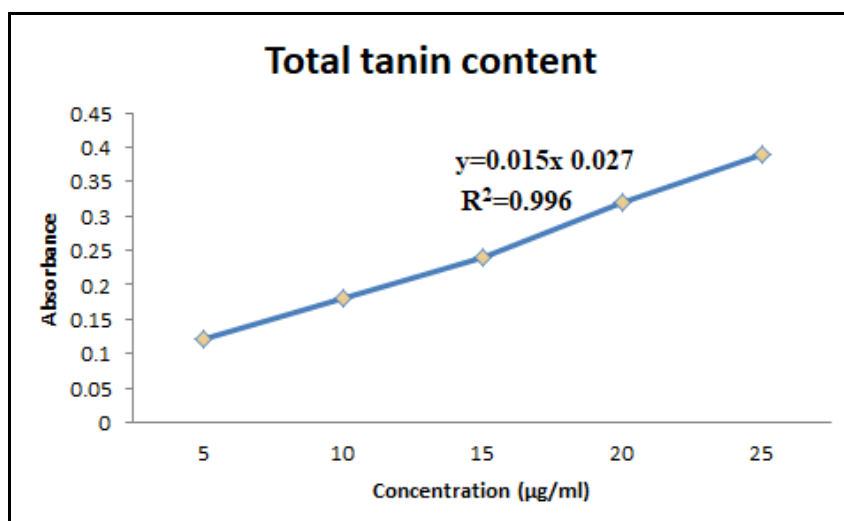


Figure 1: Standard calibration curve of total tannin content.

3.4 Total flavonoid content

Table 4: Examination of total flavonoid content.

S. No.	Concentration of standard solution	Absorbance (760 nm)
1	10	0.10
2	20	0.17
3	30	0.22
4	40	0.29
5	50	0.37
6	Chloroform	0.19±0.023
7	Ethyl Acetate	0.21±0.042
8	Ethanol	0.34±0.042

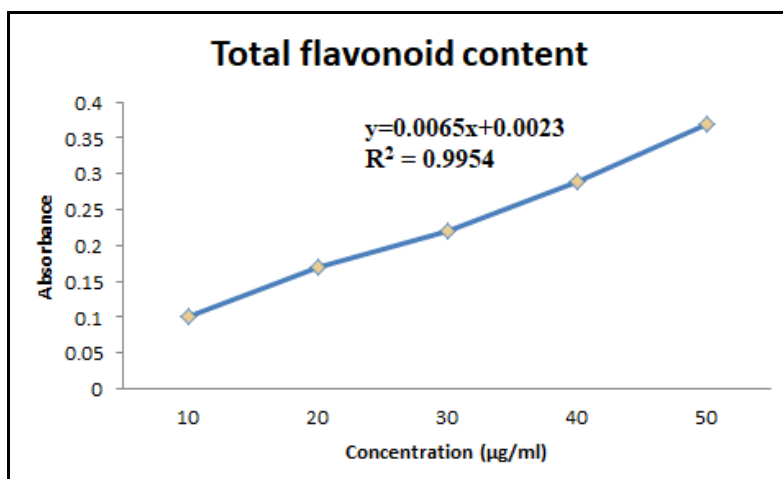


Figure 2: Standard calibration curve of total flavonoid content.

3.5 Total phenolic content

Table 5: Examination of Total phenolic content.

S. No.	Concentration of standard solution	Absorbance (760 nm)
1	15	0.12
2	30	0.17
3	45	0.22
4	55	0.27
5	65	0.33
6	Chloroform	0.16±0.024
7	Ethyl Acetate	0.23±0.012
8	Ethanol	0.29±0.029

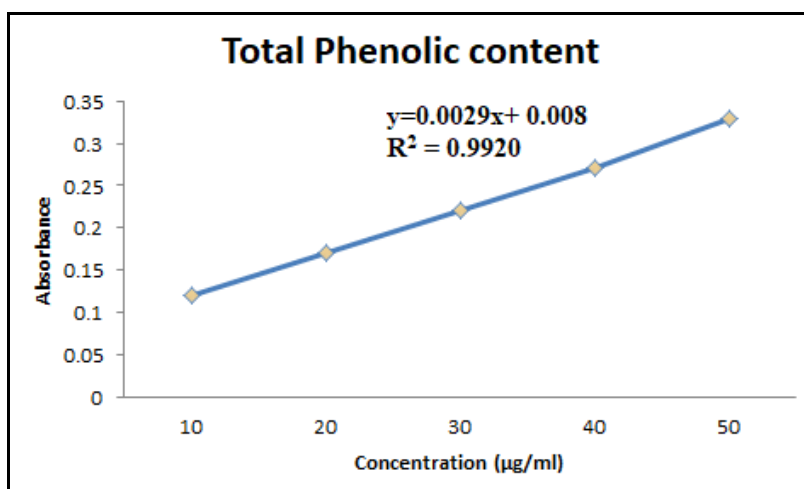


Figure 3: Standard calibration curve of total phenolic content.

3.6 TLC of extract

Table 6: Thin layer chromatography examination.

S. No.	Extract	Solvent	No. of spots	Rt value
1	Ethanol	Ethyl acetate: chloroform (6:4)	4	0.30 0.32 1033
2	Ethyl acetate	Ethyl acetate: chloroform (6:4)	4	0.47 0.51 0.54
3	Chloroform	Ethyl acetate: chloroform: Ethanol (5:3:2)	3	0.38 0.40 0.47

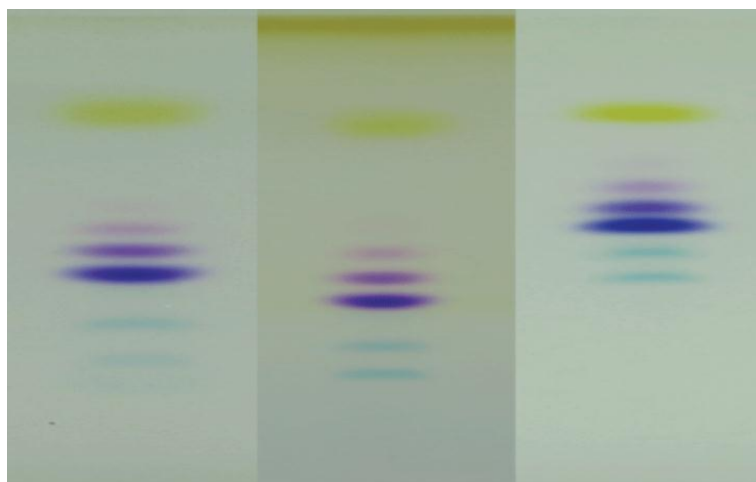


Figure 4: TLC of extract with Ethanol, Ethyl acetate and Chloroform.

3.7 HPLC Examination

Table 7: HPLC profile of ethyl acetate extracts.

S. No.	Extract	Solvent	No. of spots	Rt value
1	Leave extracts	Toluene:Ethyl acetate: Methanol:Formicacid m(6::3:1:0.1)	10	11,23,28,33,34,39, 64,71,74,76

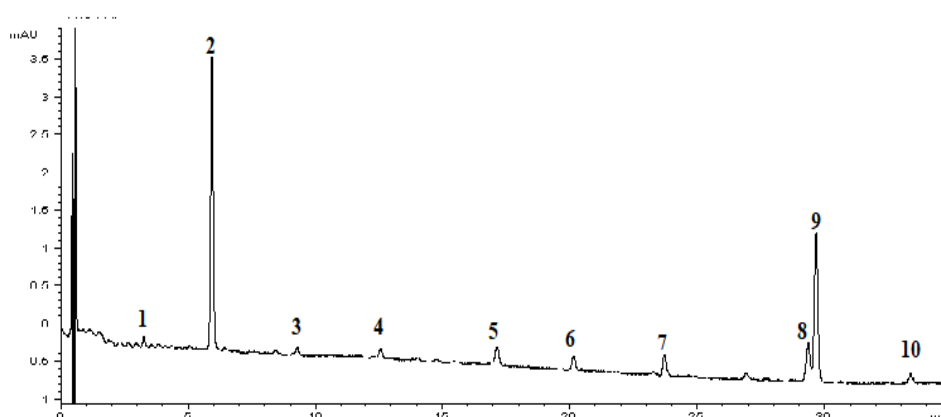


Figure 5: HPLC chromatograph of ethyl acetate of extract of *Asparagus racemosus*.

3.8 Antibacterial activity of *Asparagus racemosus* leaves extract

Table 8: Results of Antibacterial activity of *Asparagus racemosus* leaves extract on *Bacillus subtilis*, *Staphylococcus aureus*, *E. coli* and *Pseudomonas aeruginosa*.

Zone of inhibition (nm)					
Extract	Concentration (µg/ml)	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>E. coli</i>	<i>Pseudomonas aeruginosa</i>
Methanol	30	20	16	17	15
	60	23	19	20	18
	100	24	21	21	22
	Control	0	0	0	0
Chloroform	30	15	13	13	11
	60	17	17	15	17
	100	20	18	17	19
	Control	0	0	0	0
Ethyl acetate	30	11	13	7	6
	60	13	16	8	8
	100	14	20	9	11
	Control	0	0	0	0

DISCUSSION

The physicochemical analysis of *Asparagus racemosus* leaves revealed important quality indicators. The ash values indicate the total mineral content, with total ash at 7.03%, reflecting moderate inorganic residue after incineration. Water soluble ash (6.01%) and acid soluble ash (2.65%) further specify the soluble mineral fraction, relevant for assessing purity and adulteration. Extractive values showed that water soluble extractives (40.78%) are significantly higher than ethanol soluble extractives (13.23%), indicating the predominance of polar constituents. The crude fiber content was notably high (50.34%), consistent with the plant's fibrous nature. The foaming index being less than 100 suggests a low presence of saponins or foaming agents. Overall, these parameters confirm the quality and chemical nature of the plant material and serve as benchmarks for standardization.

CONCLUSION

The physicochemical evaluation of *Asparagus racemosus* leaves confirms the presence of significant bioactive compounds and good quality raw material. The ash values and extractive percentages indicate the plant's mineral and phytochemical richness. The high crude fiber content supports its traditional use, while the low foaming index suggests limited saponin content. Heavy metal analysis showed safe levels within permissible limits, ensuring safety for use. Overall, the findings provide a strong foundation for further pharmacological and quality control studies of this medicinal plant.

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