RESEARCH ARTICLE

Phytochemical Screening of *Asparagus racemosus* Willd

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ABSTRACT

Asparagus racemosus, popularly referred to as Shatavari in both Hindi and Sanskrit, is considered one of the greatest important therapeutic plants around. The use of *A. racemosus* as a galactotgogue and for preventing and treating stomach ulcers and dyspepsia is suggested in Ayurvedic writings. Additionally, it helps with inflammation, neurological disorders, liver problems, and even some viral infections. Phytochemical analysis of the extracts in this research indicated the presence of a variety of chemicals in extracts. According to the findings of a research that investigated the phytochemical properties of *A. racemosus*, this plant has the potential to be used in the development of novel medicines.

Keywords: Phytochemical, Screening, *Asparagus racemosus* willd.

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INTRODUCTION

As a subcontinent, India has a vast amount of information about local medical practices. Alternative medicine systems like homeopathy provide comprehensive therapeutic treatments for many health conditions. Over 80,000 out of the 250,000 species of higher plants found on Earth possess medicinal properties. India is acknowledged as one of 12 global biodiversity hubs, with a remarkable collection of over 45,000 distinct plant species. A great deal of diversity can be found across India's 426 biomes, 16 distinct agro-climatic zones, 10 vegetation zones, and 25 biotic provinces. Approximately 15,000 to 20,000 of these plants have therapeutic qualities. However, traditional societies only use a limited number of species, namely 7000 to 7500, because of their believed healing properties. Traditional Indian medicine operates *via* two separate paths throughout society. A prominent example is the non-codified medical system, widely practiced by indigenous communities in the rural and tribal regions of India.[1-3]

Homemakers, traditional birth attendants, bone setters, acupressure specialists, eye care professionals, snakebite healers, and Vaidhyas traditional herbalists all carry on these practices from generation to generation. Thus, these local health practices serve as a substitute for a community-backed, village-level healthcare system that operates in tandem with the government-funded system. The classical or academic health care system is the second tier of the conventional system. Regional manuscripts consist of structured and regulated medical information and extensive philosophical and theoretical interpretations. The treatises include a wide array of medicinal disciplines, including Ayurveda, Siddha, Unani, Yoga, Naturopathy, and Amchi. Indian systems of medicine (ISM) comprise medical systems that originated outside of India but were later integrated within the nation.[4]

Ayurveda primarily utilizes botanical therapies, using indigenous botanicals despite the effectiveness of modern treatments. While modern medicine relies on about 30 species, Ayurvedic medicine utilizes a vast array of 700 species. Similarly, Unani, Siddha, and Amchi traditions use 700, and 600 species, respectively. Plants in their whole or in its component parts—the stem, the leaves, the seeds, the flowers, and the bark—are the sources of pharmaceuticals. Exudates (such as gum, resins, and latex) produced by plants are an ingredient in some pharmaceuticals. Many medicinal compounds originating from plants have been included in the allopathic medical system, and botanical medicines remain an essential component of the current pharmacopeia. Moreover, plants provide crucial chemical compounds that are necessary for the production of contemporary medications. Plant-derived medications make up a significant share of the worldwide market and serve as a huge reservoir of pioneering drugs. Modern medicine continues to use traditional medicinal methods in several areas. A growing global population is increasingly acknowledging Ayurveda as a holistic approach to treatment. While Ayurveda is currently practiced in Nepal, Bhutan, Sri Lanka, Bangladesh, and Pakistan, it appears to have inspired the ancient medical systems of Thailand, Tibet, and Mongolia. The use of phytomedicines is on the rise in Western Europe.[5,6]

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At the National Institutes of Health in Bethesda, the United States government has established the Office of Alternative Medicine. This initiative aims to investigate the possibility of integrating beneficial therapies with contemporary pharmaceuticals. This institute supports alternative medicine by engaging in both basic and practical research in traditional medical systems, including Chinese medicine, Ayurvedic medicine, and others. Ayurveda is a comprehensive medical system that utilizes a wide range of plant species, estimated to be between 35,000 and 70,000, in order to provide more than 8000 plant-based cures. It is used in India, Sri Lanka, and Southeast Asia. China has shown the utmost effectiveness in implementing traditional medicine into the healthcare industry.[7,8]

MATERIAL AND METHODS

Collection and Authentication

A botanical garden was the source of the *Asparagus racemosus* plant (Figure 1). Botanists recognized and authenticated the plant *A. racemosus*, which is a member of the Asparagaceae family.

Preparation of Plant Extract

The seeds of *A. racemosus* were acquired from a medicinal plant garden. An eminent botanist has verified the authenticity of a plant. A voucher specimen of the plant's seeds was extracted and desiccated in a distinct oven set at a temperature of 45ºC. The motorized grinder pulverized the desiccated seeds into a fine powder. Using a soxhlet apparatus, a mixture of ethanol and water (500 mL) was used as the solvent to extract the seed powder (52 g). The filtrate obtained was concentrated using normal-pressure evaporation in a water bath.[9,10]

Phytochemical Screening

For phytochemical analysis, the extract adhered to conventional procedures. Many compounds were examined in the extract, including flavonoids, alkaloid compounds, phenols, anthocyanins, terpenoids, tannins, phytosterols, carbs, proteins, and amino acids. Crude extracts are subjected to qualitative analysis to determine the presence of phytochemicals. *A. racemosus* acts as a reservoir for many phytochemicals.[11]

Test for alkaloids

After reconstituting the extracts, a diluted hydrochloric acid solution was added, mixed thoroughly, and filtered. The extract is tested by Mayer's, Wagner's, Hager's, Dragendorff's tests.[12]

Test for flavonoids

Flavonoid is tested by ferric chloride, zinc-hydrochloric acid reduction, Shinoda, alkaline reagent and lead acetate solution test.[13]

Test for triterpenoids

Triterpenoids are tested by the Salkowaski, Liebermann-Burchard test.

Test for sterols

Sterols are tested by Salkowaski, Liebermann-Burchard and sulphur test.

Figure 1: Seeds and plant of *A. racemosus*

Figure 2: Whole plant and leaves of *Asparagus racemosus* Willd.

Test for quinones

Quinones are tested by potassium iodide test.

Test for saponins

Saponins are tested by foam test.[14]

Test for glycosides

Glycosides are tested by Baljet, Keller-Killiani, Raymond, Legals test and bromine water test.

Test for other phytoconstituents

The following solutions are used for tannin testing: 5% ferric chloride and 10% lead acetate; foam for saponin testing; 10% sodium hydroxide for coumarins; Molisch for carbohydrates; and a small amount of a 5% calcium chloride solution for organic acids.

RESULT AND DISCUSSION

Macroscopy

The leaves (Figure 2), sometimes known as cladodes, resemble pine needles in their tiny and uniform size. The blooms are white and adorned with little spikes.[15]

Macroscopic examination of the leaves was carried out. Under the microscope, several structures were observed, including epidermis, vascular bundles, xylem, sclerenchyma, scale, stomatal edge, stomatal pore, and mesophyll tissues.

Microscopy

Plant exhibits an ascending, slender, cylindrical stem that supports cladodes or phyllodades, which are finger-shaped, green, photosynthetic structures, in addition to scale-like, thin, membranous leaves. When observed in a transverse section, the phyllodes manifest as lateral protrusions arranged in three to four angles. The phyllodade is linear in shape and has a

thickness of approximately 400 µm. The structure is composed of radially elongated, thick, and broad epidermal cells, each of which has a unique cuticle. The thickness of epidermal cells is 40 µm. Two to three layers of circular, chlorophyll-containing palisade cells comprise the epidermis, which are readily apparent. The palisade zone is made up of one or two layers of parenchyma cells with thin walls.[16] A vascular system consists of a thick sheath of fibers encircling two tiny vascular components. A vascular filament comprises two or more narrow, angular xylem segments and a dense assembly of phloem components. The bundle sheath fibers have thick walls impregnated with lignin and show a restricted central hollow. The xylem components have a diameter of 30 µm. A diameter of 220 µm is measured for the fibrovascular system. There are certain anatomical locations that have stomata that are subcutaneously located. The stoma is adorned with two conical stomatal projections situated on its inner and outer surfaces.

Scale leaf

The scale leaf has a 70 µm thickness and is spindle-shaped in the middle. Its three cell layers comprise a central nucleus of seven to eight cells with robust walls. The scale's margin is uniseriate, whereas the cell itself is spindle-shaped.

Powder Microscopy

The application of powder microscopy identified lignified fibers, palisade cells in the epidermis, and stomata in the epidermis. The substance may utilize these characteristics for interspecific identification.[17]

Organoleptic characters

The substance exhibits a light greenish hue, imparting a visually distinctive appearance. It has a characteristic odor to its smell and a noticeably bitter flavor to its taste. These sensory attributes collectively contribute to its identification and recognition within various contexts.

Microscopical characters

Important diagnostic features such as lignified fibers, epidermal palisade cells, and stomata were found in the leaf powder during microscopic analysis.

Physicochemical Parameters

The overall ash content often comprises carbonate, phosphate, and silicate compounds. The amount of ash that was measured was found to be 10.47 ± 1.12% w/w. Pollution is indicated by the presence of acid-insoluble ash, which implies the presence of siliceous particles like dirt and sand. A value of 1.34 ± 0.38% w/w was found. The ash content that could be dissolved in water was found to be 5.20 ± 0.45% w/w. The oxides are transformed into sulfates by treating them with diluted sulfuric acid, resulting in sulfated ash. The results showed a value of 6.62 ± 0.51% w/w. Predicting component properties is made easier with the use of the extractive value, a useful metric. The soluble extractives in alcohol were found to be 11.42 ± 0.27% w/w, while in water, they were 38.86 ± 0.47% w/w. The results showed that the ether insoluble volatile extractive percentage was 11.23 ± 0.73% w/w, whereas the ether insoluble non-volatile extractive percentage was 21.74 ± 0.35% w/w. Because of its high extractive value and solubility in both alcohol and water, it is likely that there are many polar molecules present. These constants would be useful for future research in identifying and standardizing the plant. The measured crude fiber content was 51.32 ± 1.42% w/w, and it is used as a criterion to differentiate similar drugs. The loss on drying test measures the amount of any volatile chemical that can be removed under specific conditions. The measured value was 04.68 ± 0.44% w/w, with an estimated swelling index of 3.63 ± 0.76 mL/gm and a foaming index below 100. The evaluated physicochemical characteristics are displayed in Table 1, and the measured values of the corresponding physicochemical constants are shown.

Table 1: Physicochemical constants

Constant	*Parameter*	*Results in w/w*
Ash values	Water soluble	5.62 ± 0.35
	Total ash	11.31 ± 1.12
	Acid soluble	1.45 ± 0.28
	Sulphated	7.15 ± 0.48
Extractive values	Ethanol soluble	12.33 ± 0.27
	Ether insoluble non-volatile	23.48 ± 0.26
	Water soluble	41.97 ± 0.35
	Ether insoluble volatile	12.13 ± 0.65
Others	LoD	5.05 ± 0.32
	Crude fibre content	55.43 ± 1.22
	Swelling index	3.91 ± 0.65
	Foaming index	Less than 100

Pharmacognostic tests play a crucial role in confirming that the plant components are genuine. By analyzing its morphological and anatomical features and carrying out the physicochemical tests suggested by the WHO, the botanical identification of the leaf was confirmed. The standardization process might provide valuable information on the botanical identity of *A. racemosus* leaves. This information could be important in verifying the authenticity of the plant and differentiating leaves from counterfeit and adulterated ones. Main factors used to assess quality and purity of powdered medication are physicochemical criteria. The presence of soil particles, minerals, and other impurities in a drug may be determined by analyzing its ash content. As ash values are used to distinguish substandard and depleted medicines, they serve as a vital parameter for evaluating purity of raw drugs. A high ash value suggests adulteration, contamination, or replacement.

Heavy metal and inorganic elements analysis

The findings of the qualitative analysis of inorganic element estimation are presented in Table 2.

Quantitative estimation of heavy metals by ICP-OES method

Heavy metals, such as arsenic, lead, cadmium, and mercury, in pulverized leaves of *A. racemosus* Willd were quantified using the ICP-OES method. Results are presented in Table 3. The concentration of heavy metals in the leaves complies with the criteria set by the World Health Organization, as shown

by the aforementioned observation. Hence, consuming the leaves internally is safe. The qualitative research focused on heavy metals and inorganic elements. While the beneficial components and lack of pesticide residue are noteworthy, quantitative investigation of heavy metals and residue of pesticides revealed negligible amounts of heavy metals. The thorough pharmacognostical investigations performed on the leaves of *A. racemosus* provide useful insights for identifying particular drugs and distinguishing the plant from counterfeit and alternative alternatives.

Table 2: Determination of inorganic components

Components	*Comment*
Al	Present
As	Absent
Ca	Present
CO_3	Absent
Cl	Present
Cu	Present
Fe	Absent
Pb	Absent
Mg	Present
Hg	Absent
PO_4	Present
SO_4	Present

Table 3: Determination of heavy metals

S. No.	*Element*	*Concentration (ppm)*	*Maximum allowable limit*
1	Hg	0.0023	Not exceeding 0.5 ppm
2	As	0.0078	Not exceeding 5.0 ppm
3	Pb	Not found	Not exceeding 10 ppm
4	Cd	0.0029	Not exceeding 0.3 ppm

Table 4: Yield of extracts of leaves

S. No.	*Extract*	*Extraction method*	*Color*	*Yield percentage (%)*
1	n-Hexane	Continuous soxhlet extraction	Yellow	03.8
2	Chloroform		Green	05.02
3	Ethyl Acetate		Brown	04.20
4	Ethanol		Brown	04.10

Extraction

India has mostly relied on oral transmission, without a defined inventory, to convey its traditional knowledge about medicinal plants. In order to achieve thorough execution, methodical supervision, and precise record-keeping, it is necessary to perform particular actions.[18] Herbal medicines are susceptible to contamination, deterioration, and variations in composition since they are made using plant-based ingredients. Therefore, scientists must establish plants' effectiveness and authenticate their genuineness before undertaking clinical research. Various analytical methods have been developed to control the quality of medications obtained from botanical sources. Consequently, biochemical screening alone is inadequate for determining quality characteristics and comprehending the therapeutic dynamics of medicinal plants; a phytochemical study is needed. Table 4 outlines the %yield of extracted *A. racemosus* Willd. leaves obtained during successive extraction.

Qualitative phytochemical analysis

Ainitial qualitative phytochemical analysis was performed using several chemical reagents to identify specific kind and amounts of phytoconstituents in each extract and powder. Flavonoids were detected in the n-hexane extract. The chloroform extract was found to include phenolic chemicals, flavonoids, saponins, and steroids. Proteins, tannins, terpenoids, flavonoids, and phenolic substances were discovered to be present in the ethyl acetate extract.[19] A variety of compounds were identified in the ethanolic extract, including proteins, saponins, carbs, steroids, flavonoids, phenols, and tannins. The chemical makeup of the leaf powder and different extracts was examined, and the results are shown in Table 5 in a succinct manner.

Table 5: Primary phytochemical screening

Test	*Powdered leaf*	*Ethanol*	*Chloroform*	*n-Hexane*	*Ethyl acetate*
Carbohydrates	No	Yes	No	No	No
Steroids	Yes	Yes	No	No	No
Flavanoids	Yes	Yes	Yes	Yes	Yes
Volatile oils	No	No	No	No	No
Saponins	Yes	Yes	Yes	No	Yes
No glycosides	No	No	No	No	No
Phenolic compounds	Yes	Yes	Yes	No	Yes
No proteins	No	No	No	No	No
Alkaloids	No	No	No	No	No
Tannins	Yes	No	No	No	No
Quinones	No	No	No	No	No
Terpenoids	Yes	No	No	No	No

Quantitative estimation of phytoconstituents

The chloroform, ethyl acetate, and ethanol extracts underwent quantitative phytochemical analysis. A total phenolic content of 40.31 ± 0.49, 60.85 ± 0.29, and 73.78 ± 0.53 µg/mL %w/w were found through measurement. The tannin content was found to be 14.73 ± 0.37, 16.58 ± 0.45, and 19.34 ± 0.33 µg/mL %w/w, according to the tested results. The total flavonoid concentration, calculated as a percentage by weight, was found to be 4.67 ± 0.56, 7.98 ± 0.48, and 36.44 ± 0.62 µg/mL. *A. racemosus* Willd. leaves were ground into a coarse powder and then tested for several kinds of phytoconstituents. In a soxhlet system, solvents with increasing polarity were used to do this, including n-hexane, chloroform, ethyl acetate, and ethanol. The series of extraction assays revealed the powdered metabolites' solubility and polarity properties. Each of the following extracts had a different percentage yield: n-hexane (3.5% w/w), chloroform (4.89% w/w), ethyl acetate (3.9% w/w), and ethanol (4.35% w/w). Among the many extracts tested, chloroform showed the most promising extraction yield. Tables 6, 7, and 8 show the findings of the quantitative estimation of phytoconstituents, which include tannin, flavonoid, and phenolic compounds.

Table 6: Estimation of total tannin content

Standard (ng/mL)	*Absorbance at 765 nm*
5000	0.11
10000	0.18
15000	0.24
20000	0.34
25000	0.41
$CHCl_3$	0.22 ± 0.02
Et. Acetate	0.37 ± 0.04
Et-OH	0.40 ± 0.05

Table 7: Estimation of total flavanoid content

Standard (ng/mL)	*Absorbance (765 nm)*
10000	0.1
20000	0.15
30000	0.22
40000	0.29
50000	0.37
$CHCl_3$	0.18 ± 0.02
Et. Acetate	0.21 ± 0.03
Et-OH	0.31 ± 0.02

Table 8: Estimation of total phenolic content

Standard (ng/mL)	*Absorbance at 765 nm*
10000	0.12
20000	0.16
30000	0.19
40000	0.23
50000	0.30
$CHCl_3$	0.13 ± 0.03
Et. Acetate	0.23 ± 0.04
Et-OH	0.24 ± 0.04

- *Total tannin content*

Total tannin contents were determined using standard procedures and reported in Table 6.

- *Total flavanoid content*

Total flavanoid contents were studied and reported in Table 7.

- *Total phenolic content*

Total phenolic contents were determined and results are shown in Table 8.

CONCLUSION

The introduction of innovative, eco-friendly herbal drugs that are effective and biologically safe is of the utmost importance. Medicinal plants frequently comprise a multitude of phytochemical compounds that are critical for various activities. The phytochemical profile of the plant being studied can be better understood with this information.

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