RESEARCH ARTICLE

Pharmacognostic, Phytochemical, and Antioxidant Investigations of *Asparagus curillus* Buch.-Ham. Ex Roxb: Ethnomedicinal Species of the Lower Himalayas

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ABSTRACT

Asparagus curillus Buch.-Ham. ex Roxb., a lesser-known yet therapeutically significant ethnomedicinal species of the Lower Himalayas, holds substantial potential for pharmacological applications. Current study aims to explore the pharmacognostic, phyto-chemical and anti-oxidant properties of *A. curillus* to validate its traditional uses and uncover new therapeutic potentials. The pharmacognostic evaluation involved detailed examination of the anatomical (histological) characteristics of the roots, providing essential diagnostic features for its identificastion and quality control. Phyto-chemical investigation shown the presence of various bioactive phyto-constituents, including flavonoids, alkaloids, and saponins, which are recognized for their therapeutic properties. The antioxidant activity was assessed using standard assays, confirming the plant's capacity to neutralize free radicals, thus supporting its use in managing oxidative stress-related conditions and induced disorders. The findings of present investigation contribute to the developing form of evidence on *A. curillus* and underscore its significance as a valuable resource for developing novel therapeutic agents.

Keywords: Uttarakhand Himalaya, Ethnomedicine, *Asparagus*, Rutin, DPPH assay

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INTRODUCTION

The Himalayas, a mountain region in Asia that mostly covers India, Nepal, China, Tibet, and Pakistan, is as well recognized as a treasure house of the valuable medicinal plants, with around 8000 identified species, 1748 of which have medicinal properties.[1] The medicinal plant diversity in the lower Himalayan region is extensive and is extensively utilized by the indigenous communities for their healthcare needs.[2] This area is well-known for its deep forest as well as its cultivation of a variety of crops and medicinal.[3, 4] Certain species are native to the area, but many more are introduced.[5] The majority of people living in rural areas of China, Nepal, Tibet, and India mostly rely on traditional medical practices.[6] The genus *Asparagus* Tourn. ex L. comprises 210 accepted species worldwide. Among these, approximately 22 species are recorded in India.[7] The five medicinally important species—*Asparagus adscendens*, *A. capitatus* subsp. *gracilis*, *A. curillus*, *A. filicinus* and *A. racemosus*, are found in the Uttarakhand Himalaya region.[4] Notably, *A. racemosus* is recognized as both an ornamental and medicinal plant, valued in ancient systems of the medication as well as in modern systems.[7] *A. curillus* Buch.-Ham. ex Roxb, is a much-branched, subscandent undershrub. The roots measure 7-18 inches in length, are light brown in color, odorless, and have a sweetish taste.[8, 9] It grows on sub-tropical Himalaya in open Oak Rhodendron forests (Grahwal- Kumaon to Nepal) between altitudes from 600-1300 m.[8-10] The roots are used as bitter tonic.[10], aphrodisiac, appetiser, stimulant, and anti-inflammatory, galactogogue[11] and diuretic.[11] Its ripe fruits are believed to induce abortion, and its tuberous roots are used to treat dysuria, diabetes, and dysentery when combined with honey.[12] Ethno-medicinally, roots are used in Gonorrhoea, piles, cough, as rejuvenating tonic, stomachache agent, in urinary disorder, dysentery and diabetes mellitus in Uttarakhand Himalaya.[9, 13] Roots are used in folk medicines to cure diabetes; 10g powder of dried roots mixed with the powder of Methi seeds (*Trigonella foenum-graecum*) in equal quantities is given once daily for a month. [9, 13] It contains phytoconstituents such as steroidal saponin (asparasides, curillins, curillosides and sarsasapogenin), spirostanol glycosides and oligofurostanosides and oligospirostanosides as active constituents.[14, 15] It has been less explored for its pharmacological potential; to date, only anticancer and molluscicidal activity has been reported. [9, 16-18] The present study establishes comprehensive standardization parameters for the accurate identification and evaluation of *A. curillus*. This includes pharmacognostic analysis, which examines the plant's physical and structural characteristics through macroscopic and microscopic techniques. The study also includes detailed phytochemical investigation to recognize and quantify the various bioactive compounds present in the plant, employing methods such as qualitative and quantitative tests. Additionally, the

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Figure 1: (a) Roots of *A. curillus* (b) Leave and stems *A. curillus*

research evaluates *in vitro* anti-oxidant action of *A. curillus* roots, using DPPH assays to assess its potential to neutralize free radicals and counteract oxidative stress. These standardization parameters are crucial for ensuring reliable identification and understanding the therapeutic potential of the plant.

MATERIAL AND METHODS

Collection and Identification of Plant Material

The plant *A. Curillus* was collected from the Nainbag region of Tehri District, Uttarakhand State, India. The collected plant sample was authenticated from the Botanical Survey of India (BSI), Dehradun with the Accession number 753. The specimen of the plant sample was also submitted at the BSI herbarium for the future records.

Processing of the Plant Material

The freshly harvested roots were thoroughly rinsed with ample tap water to eliminate any impurities. After cleaning, some roots were reserved for microscopic examination, while the rest were placed in a Hot Air Oven to dry at 50°C for 48 hours, with periodic turning to ensure even drying. The dried roots were then coarsely powdered for further analysis.

Microscopic Characterization

The fresh root was processed to prepare and examine transverse sections following established protocols.[19, 20] The root was tightly positioned between the left hand's thumb and fingers, while thin sections were meticulously cut using a sharp razor blade in right hand. The sections were first collected in watch glass containing purified water, and then transferred to a different watch glass with a glycerol (30%) in ethyl alcohol solution. A root section was placed on the glass slide and treated with various stains, such as safranin, phloroglucinol- hydrochloric acid (HCl) and iodine. Afterward the staining, the slide was mounted using glycerine and covered with the cover slip. The observations were conducted at different magnifications (4x, 10x, 40x, and 100x) with a compound microscope and pictures were taken using a camera. [21]

Determination of Foreign Matter

A thin layer of 250 g of roots was placed on a white background for visual inspection and sorting of foreign matter (FM). Each component of the sorted FM was weighed to within 0.05 g. Next, for each group, the content in grams for every 100 grams of the air-dried sample was determined.[20] Each part of the sorted FM was weighed with a precision of 0.05 g. The weight in grams for every 100 grams of the air dried sample was then calculated for each group

% FM = (Weight of shorted FM / Weight of sample drug) × 100

Determination of the Moisture Content

A classical laboratory technique for determining the moisture levels in semi-solid or solid sample was the loss on drying (LOD) test. This test is considered to measure the quantity of volatile matter and water in the sample when it is dried in defined conditions. To perform this test, 3 grams of the drug were placed in a tarred china dish (TCH). After that, the TCH was dried at 110° C in a hot air oven. After drying, the dish was allowed to cool in the desiccator (at room temperature). The sample weight was noted at 5-minute intervals until two consecutive readings show a constant weight. The weight loss was noted as the content of moisture in the drug sample, and the LOD was determined with the following formula.[20, 22]

LOD (%) = (Weight loss / Weight of drug) X 100

Determination of the Ash Value

The estimation of ash values helps assess the quality and purity of a crude drug. Ash contains the inorganic compounds such as carbonates, phosphates, silicates, calcium potassium and magnesium. These compounds removed through acid treatment, and the remaining acid-insoluble ash values are then measured.

Total Ash Value

The quantity of crude drug left over afterward ignition is measured by total ash, which takes into account both "physiological ash" from the plants tissue itself and "non-physiological ash" from foreign materials like soil and sand that stick on the plant's outer surface.

Two grams of the root powder were added to a previously dried and tarred crucible in order to compute the total amount of ash. The crucible was placed for six hours at 500–600°C in a muffle furnace (MF). The MF was ignited, turned off and given time to cool. The crucible was taken out and allowed to cool further in the desiccator before being weighed. [20, 22]

The following calculation was then calculated to measure the overall ash percentage.

Total Ash (%) = (weight of ash / weight of drug) x 100

Water Soluble Ash Value

The water-soluble ash (WSA) content was calculated by measuring the difference in weight between the total ash and residue remaining after the total ash has been treated with water. Twenty-five milliliters of water was added to the crucible containing the total ash and the mixture was heated for five minutes to determine the quantity of WSA. After that, the insoluble material was collected onto Whatman filter paper and given a hot water wash. The filter paper, after being placed back into the original crucible, was burned for fifteen minutes at a maximum

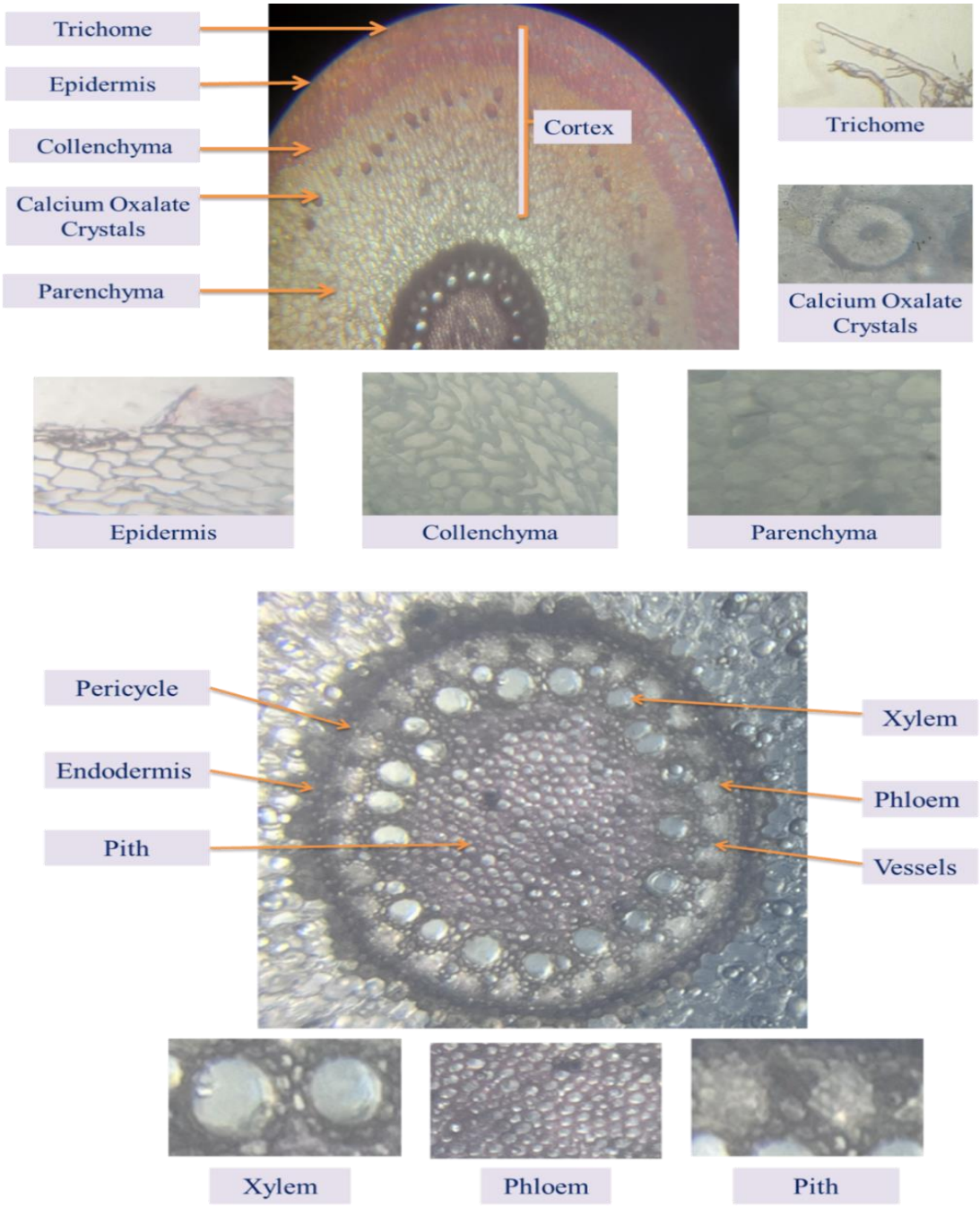

Figure 2: Microscopy (Transverse Section) of *Asparagus curillus* root.

temperature of 450°C. To measure the water soluble ash, subtract the weight of the residue from the weight of the total ash. [20, 22]

The given formula was then taken to measure the proportion of WSA.

WSA (%) = (Weight of WSA / Weight of drug) × 100

Acid Insoluble Ash Value

Acid insoluble ash (AIA) is the residue that persists after the remaining insoluble matter is ignited and the produced total ash is boiled with diluted HCl. This measurement shows the amount of silica, particularly in the form of siliceous earth and sand. To determine AIA, the total ash was heated with twenty five milliliters of diluted HCl for five minutes. After gathering the insoluble residue on Whatman filter paper, the filtrate was carefully washed with hot water till its pH reached neutral. Then the Whatman filter paper was placed in a crucible & ignited in a MF at 500 to 600° C for six hours. After turning off the MF, allowing it to cool, removed the crucible from it, further cooled using a desiccator, and the obtained ash was weighed.[20, 22]

AIA (%) = (weight of AIA / weight of drug) x 100

Sulphated Ash Value

The Root powder was boiled with twenty five milliliter of dilute sulfuric acid for five minutes. After that, the insoluble debris was separated on Whatman filter paper then treated with hot water up until the wash water was

Table 1: Standardization Parameters of *Asparagus curillus* root

Parameters	Values % (W/W)
Foreign Matter	Absent
Moisture Content	4.4%
Total Ash Value	10.21 %
Acid Insoluble Ash (AIA)	3.6 %
Water soluble Ash (WSA)	5.3%
Sulfated Ash	6.6 %
Foaming Index (FI)	< 100
Crude Fiber Content	9.53%

neutralized. This filter paper was placed back in the original crucible and heated in a MF at 500-600° C for six hours. After that allow cooling and then the weight of crucible was taken. The estimation of sulfated ash was measured using the given formula.[20, 22]

Sulphated ash (%) = (weight of sulphated ash / weight of drug) x 100

Determination of Foaming Index

The foaming index (FI) measures the foam height formed by 1g of crude drug sample under definite conditions. Various medicinal plants and their parts contain saponins, which give persistent foam when their decoction (aqueous) is shaken. The ability to produce foam of these extracts is quantified by the foaming index.

To determine this, one g of plant sample was ground into a coarse powder, accurately weighed, and placed in a five hundred milliliters conical flask containing hundred milliliters of boiling water. The mixture was boiled at 100°C for thirty minutes, then cooled & filtered into a 100 ml volumetric flask, which was topped up with water. The decoction was then divided into ten test tubes (stoppered) in successive portions of 1 ml, 2 ml, 3 ml, 4 ml, 5 ml, 6 ml, 7 ml, 8 ml, 9 ml, and 10 ml, with each tube's volume adjusted up to 10 ml with water. The test tubes were shaken longitudinally for fifteen seconds at a rate of two shakes each second and left to stand for fifteen minutes before measuring the foam height.[20] The FI was then determine by using the given formula.

FI = 1000/a

Where *a* = the amount of decoction in milliliters that was used to prepare the dilution in the tube where foaming up to a height of one centimeter is seen.

Determination Crude Fiber Content

Weighed 5 grams of the drug accurately and mixed it with 125 milliliters of nitric acid solution (10% v/v). This mixture was boiled for thirty seconds while stirring continuously. Filtered the mixture through a hardened filter paper then washed the residue with boiling water until the wash water was free of acid. Transferred the residue back into the glass flask using 125 ml of sodium hydroxide solution (2.5% v/v), then boiled it with continuous stirring for about thirty seconds after boiling began. Quickly filtered the liquid through a pre-weighed filter paper and boiling water was used to wash the residue until the pH of the wash water was neutral. Then the residue was dried at 110° C to a constant weight. Then dried residue was incinerated to a constant weight. The difference between the weights of the residue before and after incineration represented the crude fiber content, which was then expressed as a proportion of the original weight of the crude drug sample.[24]

Table 2: The extractive value of *Asparagus curillus* root

Solvent	Extractive Value (% w/w)	
	Cold Maceration	Hot Extraction
Petroleum Ether	2.52 ± 0.02	5.2 ± 0.04
Chloroform	3.92 ± 0.011	6.7 ±0.024
Ethanol	8.86 ± 0.011	12.2 ±0.021
Water	7.2 ± 0.025	10.2 ±0.034

Determination of Extractive Values

The extractive value (EV) refers to the weight of the residue obtained after extracting a crude drug with a specific solvent. This residue encompasses various phytoconstituents such as tannins, glycosides, alkaloids and triterpenoids. The nature of the menstruum employed and the crude drugs both affect the phyto-constituents' composition in the solvent. EV was determined using two methods.[20, 22, 24]

Method 1: By Cold Maceration (Cold Extractive Value)

Four grams of air-dried, coarsely powdered root was macerated with hundred milliliters of the specified menstruum (Water, Ethanol, Chloroform and Petroleum ether) strength (concentration) in the closed flask for twenty four hours. For the first six hours, the mixture was shaken constantly; for the next eighteen hours, it was left undisturbed. This mixture was filtered quickly, taking care to prevent menstruum loss. 25 ml of this filtrate was evaporated to dryness in the pre-weighed shallow dish with a flat bottom, and then dry it at 105° C until it reaches a constant weight. Record the weight. The proportion of the EV was estimated relative to the weight of the air dried crude drug.

% EV = (Yield of extract / weight of crude drug) × 100

Method 2: By Hot Extraction (Hot extractive value)

Four grams of air dried, coarsely powdered root was exactly weighed and place it in a stoppered glass conical flask. Hundred milliliters of the menstruum (Water, Ethanol, Chloroform and Petroleum ether) was added in conical flask and then weigh the flask to get the total weight. After shaking the mixture, it was allowed to stand for an hour. The conical flask was placed on a reflux condenser, which was used to gradually boil it for an hour. After cooling, the flask was weighed again and adjusted the total weight to the original by adding the specified menstruum. After shaking the mixture, it was quickly filtered through a dry filter. Twenty five milliliters of the filtrate was transfer to a pre-weighed flat bottom glass dish and evaporated it to desiccation using the water bath apparatus. The residue was then dried at 105° C for six hours and then cools using the desiccator for thirty minutes. The weight was measured immediately. The proportion of extractive value was estimated relative to the

Table 3: Characteristics of Extracts

S. No	Name of Extract	Colour of Extract	Consistency of Extract	Percentage Yield (w/w)
1	Petroleum Ether Extract (ACRPE)	Light Brownish	Sticky	1.56%
2	Chloroform Extract (ACRC)	Brownish black	Viscous, Sticky	3..65 %
3	Ethanol Extract (ACRE)	Dark brown	Viscous	6.31 %
4	Aqueous Extract (ACRW)	Brownish black	Sticky	6.76%

weight of the air dried crude drug. Specifications for hot extraction are detailed in Table 6.2.

% Hot EV = (extract's Yield / crude drug weight) × 100

Preparation of Extracts

The previously dried & coarsely powdered roots was used for successive extraction with the menstruums such as Petroleum ether (PE), Chloroform (C), and Ethanol (E) by continuous soxhlation and aqueous (W) extract by decoction method.[4] The extracts of root were dried by using vacuum rotatory evaporator (Equitron, India) and stored the extracts at 4 °C for further use.[22]

Qualitative Phytochemical Analysis

All four extracts were examined to the preliminary phyto-chemical analysis for the detection of different phyto-constituents for example glycosides, alkaloids, saponins, phenolic compounds & tannins, steroids, flavonoids, triterpenoids, carbohydrates, proteins & amino acids, fixed oils and fats.[19, 25, 26]

Quantitative Phytochemical Analysis

Total Phenolic Content

The milligram of Gallic acid (GA) equivalents per gram of extract was used to express the total phenolic content (TPC). An extract stock solution (1 mg/ml) in methanol was prepared. A appropriate amount of the extract was taken out from the stock solution and added to a twenty-five milliliter volumetric flask along with 1.5 milliliters of Folin-Ciocalteu reagent and ten milliliters of water. After letting the mixture sit for five minutes, four milliliters of a twenty percent solution of sodium carbonate was added to it and double distilled water was used to make up the volume to twenty-five milliliter. After thirty minutes of storage, the mixture's absorbance at 765 nm was measured. The proportion of total phenolics was estimated from calibration curve of GA prepared by using the above method and calculated TPC as percentage of GA.[27]

Total Flavonoids Content

The aluminum chloride ($AgCl_3$) method was adopted to assess the total flavonoid content (TFC) of all extracts. After taking aliquots of the extract solutions, methanol was added to get the volume up to three milliliters. Subsequently, 2.8 milliliters of distilled water, 0.1 milliliter of potassium acetate (1 M), and 0.1 milliliter of $AgCl_3$ (10%) were added. After giving the test solution a vigorous shake and leaving it remain for thirty minutes, the absorbance at 415 nm was taken. At 415 nm, the standard calibration plot was produced using rutin concentrations that were known. The calibration plot was used to calculate the TFC in the test samples of plant, which were then represented as mg rutin equivalent per gram of sample.[28]

Evaluation Antioxidant Activity

Using a DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay, the anti-oxidant capacity of petroleum ether, chloroform, ethanol, and water extract of the root was assessed. Following this procedure, stock solutions were made by dissolving fifty milligrams of the extracts and ten milligrams of rutin (used as a reference) in one milliliter of methanol. After then, various concentrations were obtained by periodically diluting these stock solutions. The final volume was adjusted to four milliliters with methanol after mixing ten microliter of each test sample (at all concentrations) with one milliliter of DPPH radical solution, which was made by dissolving Ninety µM DPPH in one liter of methanol. Ten after giving the combinations a good shake, they were allowed to stand, at room temperature for sixty minutes in the dark. As a control, a DPPH solution in methanol was employed. The solutions were then measured at 517 nm for absorbance by an Epoch Microplate Reader (BioTek, USA).[21]

The DPPH radical scavenging power was determined by applying the following formula.

% Radical scavenging capacity = $[(A_0 - A_1) / A_0 \times 100]$

where A_0 represents the control's absorbance and A_1 the sample's absorbance.

Table 4: Phytochemical Analysis of extracts of *Asparagus curillus*

Phytoconsituents	ACRPE	ACRC	ACRE	ACRW
Alkaloid	-	+	-	-
Glycoside	-	-	+	-
Phenolic	-	+	+	-
Steroid	+	-	-	-
Protein	-	-	-	+
Carbohydrate	-	-	-	+
Saponin	-	-	+	+
Coumarin	-	-	-	-
Flavonoid	-	-	+	-
Fat and Fixed oil	+	-	-	-

Table 5: Total phenolic and Flavonoid Content of extracts of *A. curillus*

S. No	Extract	Total phenolic Content (mg/gm equivalent to Gallic acid)	Total Flavonoid Content (mg/gm equivalent to Rutin)
1	Petroleum Ether Extract (ACRPE)	-	-
2	Chloroform Extract (ACRC)	-	27.15 ± 1.54
3	Ethanol Extract (ACRE)	24.56 ± 1.88	25.28 ± 2.42
4	Aqueous Extract (ACRW)	31.25 ± 2.35	-

RESULTS AND DISCUSSION

Microscopic Characterization

The transverse section of root show the epidermal layer was without intercellular spaces, thin walled, single layered and colourless. The outer layer has trichomes (root hairs), which are single-celled structures found on the surface of the root. These root hairs grow from small, thin walled cells, some of which are extended. The cortex, just below the surface, has more than 30 layers and is separated into two zones- the outer cortex, which has tightly packed, thick walled cells (Parenchyma), and the inner cortex, thin walled cells (Collenchyma) which has oval cells with spaces between them. In the inner cortex, there are also stone cells and calcium oxalate crystals. Below the cortex is the endodermis, a layer of thin walled cells, followed by the pericycle. The central part, or stele, contains xylem (vessels, tracheids, and parenchyma) and phloem, with the xylem vessels showing pitted patterns. The pith in the center consists of circular to oval cells, with some being slightly lignified (Figure 2).

Determination of physicochemical Parameter

Different parameter like foreign matter, moisture content, ash value (Total ash, acid insoluble ash, water soluble ash and sulphated ash), foaming index and crude fiber content (Table 1), and extractive value (Table 2) of *A. curillus* root was determined. The extracts of root in different solvents were prepared. The characteristics and yield of extracts is shown in Table 3.

Qualitative Phytochemical Analysis

The petroleum ether (ACRPE), chloroform (ACRC), ethanol (ACRE) and aqueous (ACRW) extracts Phytochemical Analysis of *A. curillus* showed the presence of major phyto-constituents for example flavonoids, phenolic, glycosides and saponins. In ACRPE, steroid, fats was only present. In ACRC, alkaloid, phenol was present. In ACRE, glycoside, saponin, phenolic compounds were present and lastly in ACRW protein, carbohydrate and saponin were present (Table 4)

Quantitative Phytochemical Analysis

Table 6: DPPH Radical Scavenging Activity of extracts and Rutine expressed as IC_{50} Value

S. No.	Name of Sample	Antioxidant Activity (IC_{50} mg/mL)
	Rutin (Reference/Standard)	0.58±0.14
	Petroleum Ether Extract	42.75±3.65
	Chloroform Extract	26.58±3.31
	Ethanol Extract	12.65±1.42
	Water Extract	16.58±2.35

Total Phenolic and Total Flavonoids Content

The TPC and TFC of different extracts of root were measured and expressed as equivalents to gallic acid and rutin, respectively. The petroleum ether extract didn't display any measurable total phenolic or flavonoid content. The chloroform extract also did not contain any measurable total phenolic content but had a TFC of 27.15 ± 1.54 mg/g equivalent to rutin. The ethanol extract contained 24.56 ± 1.88 mg/g of total phenolic content equivalent to gallic acid and 25.28 ± 2.42 mg/g of total flavonoid content equivalent to rutin. The water extract showed a total phenolic content of 31.25 ± 2.35 mg/g equivalent to gallic acid but did not have any measurable total flavonoid content (Table 5).

Antioxidant Activity

Different extract concentrations' capacities to scavenge DPPH radicals were evaluated, exposing unique antioxidant profiles. The concentration-dependent scavenging effect of rutin, a standard reference, ranged from 82.06% at 5 mg/mL to 18.25% at 0.15625 mg/mL. Significant antioxidant potential was shown by the ethanol extracts; values dropped from 78.25% at 50 mg/mL to 6.38% at 1.5625 mg/mL. The scavenging capacity of water extract was constant, ranging from 73.59% to 16.62% at different concentrations. Strong antioxidant activity was shown by chloroform extract, with values falling from 65.32% to 2.59%. In comparison to other samples, petroleum ether extract showed a reduced potential for scavenging. The present study demonstrates the diverse range of DPPH radical scavenging abilities exhibited by the evaluated extracts. Notably, ethanol extract and water extract have very strong antioxidant properties. All extracts exhibited concentration-dependent DPPH radical scavenging capacities. The IC_{50} value of root extracts and standard is given in Table 6.

DISCUSSION

To date, no comprehensive pharmacognostic characterization or study has been conducted on *A. curillus* to validate its purity, quality, and efficacy. Although the existing literature highlights its medicinal importance in traditional folk medicine, there remains a gap in standardized evaluation methods for this species. The current study aims to establish a benchmark for the future evaluation of *A. curillus* as a medicinal therapeutic agent. It also explores its potential as an alternative and substitute for the official species, *A. racemosus* (commonly known

as Shatavari), which is included in the Indian Pharmacopoeia and Ayurvedic texts. Previous research has made it clear that microscopy is essential for both the identification of medicinal plants or their derived products and the assurance of the quality of herbal substances.[29, 30] A parallel market of adulterated products has emerged in the current situation, where there is a considerable gap between the demand and supply of raw materials for herbal products.[31] Although each species of plant has distinctive characteristics, microscopy has been shown to be one of the most reliable and affordable methods for ensuring the quality of herbal products. To date, several attempts have been made to study the microscopy of various *Asparagus* species,[32-35] but none have focused on *A. curillus*, despite its medicinal importance being recognized.[10-17] This study offers the first complete and high-resolution microscopic characterization of the root of *A. curillus*. Present report on the transverse section (TS) of the root offers the distinguishing information to identify the anatomical microscopic features of *A. curillus*. The Pharmacognostic parameters are important for evaluating the quality and purity of natural crude drugs. The WHO (World Health Organization) recommends the assessment of crude drugs through the various methods like determining moisture content, foreign matter, ash values, and extractive values etc.[20] According to Santhosh Kumar et al. (2018), medicinal plants were found to be adulterated when collected from markets in South India. The plant sample used in our investigation was taken from a wild source, and no foreign matter was found in the sample.[36]. The existence of moisture in our samples, *A. curillus* root was within the range of 4.4%. The excessive water in medicinal plant materials can lead to microorganism growth, fungal or insect development, and degradation through hydrolysis.[20] Determining the ash values is essential for evaluating the purity and quality of crude pharmaceuticals. Inorganic substances such phosphates, carbonates, silicates, potassium, magnesium, and calcium are present in the ash. Three different ash values for medicinal plant materials were included in present analysis: total ash, acid insoluble ash, and water soluble ash.[20, 37] *A. curillus* exhibited total ash of 10.21%, with 5.3% water soluble ash and 3.6% acid insoluble ash. The weight of residue left over after a crude drugs is extracted using a solvent is measured by the extractive value. Extractive values vary based on factors like cultivation, collection, processing, and storage conditions. A low extractive value can indicate issues such as adulteration or improper processing, making it a crucial parameter for assessing the purity and quality of herbal substances. [20, 24 38] The extractive values for *A. curillus* roots show how much of the plant's product or material can be extracted using different solvents and methods. When using cold extraction, petroleum ether extracts 2.52% of the material, chloroform 3.92%, ethanol 8.86%, and water 7.2%. When using hot extraction, the amounts are higher as petroleum ether extracts 5.2%, chloroform 6.7%, ethanol 12.2%, and water 10.2%. This means that hot extraction generally gets more out of the plant compared to cold extraction in case of *A curillus* roots, and different solvents extract different amounts of material. The body uses oxygen to digest protein, fat, and carbs to produce energy. Owing to its reactive properties, oxygen can combine to form potentially harmful molecules known as reactive oxygen species (ROS), which are the primary source of the oxidative stress and it include peroxides, hydroxyl radicals, superoxides, and singlet oxygen.[39] Before free radicals and other radicals damage healthy cells, an antioxidant neutralizes and deactivates them. Among the well-known naturally occurring antioxidants include flavonoids, carotenoids, and other polyphenolics.[40] As the antioxidant activity of *A. curillus* extract is explored first time revealed that the ethanol and water extracts showed good action against DPPH when compare to standard drug (Rutin). Although the medicinal benefits and phytochemical characteristics of *A. curillus* have been mentioned in various sources,[10-17] no additional research has been conducted to scientifically verify these claims. Our study is a step toward exploring the importance of this underexplored yet therapeutically significant ethnomedicinal species of the Lower Himalayas.

CONCLUSION

This this study has provided a thorough pharmacognostic evaluation of *A. curillus*, including detailed microscopic characterization of its roots. We conducted comprehensive phytochemical screening and assessed its antioxidant activity, which further highlights its potential medicinal value. Despite these findings, scientific validation of the reported medicinal uses remains limited. Our research lays the groundwork for future studies aimed at confirming the therapeutic properties of this ethnomedicinal species. Further investigations are needed to validate the medicinal claims and to isolate and identify the pure constituents responsible for its biological actions.

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