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Pharmacognostic Evaluation and Standardization of Androsace globifera: Exploring Multifaceted Protocols and Parameters for Herbal Medicine Standardization

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ABSTRACT

Androsace globifera contains significant phytochemicals such as saponins and is utilized for treating liver and kidney diseases, amenorrhea, skin allergies, leucorrhoea, and as an abortifacient. Morphological studies reveal that the leaves are diverse in shape, ranging from speculating to elliptical. Organoleptic analysis indicates an astringent taste, aromatic odor, and brittle fracture, with the stem being straight and colored brownish-green. The flowers are pink with 12-15 blooms, five petals, seven sepals, a 1.5 mm style, and a 3 mm capsule. The powdered leaves and roots are greenish and brown, respectively, with an astringent and aromatic odor, and a bitter and acrid taste. Microscopic and physicochemical studies identify vascular bundles and upper and lower epidermal cells. The moisture content of roots and leaves is 2.5% and 3%, respectively. The total ash content of roots and leaves is 25% and 22.5%, acid-insoluble ash is 12.5% and 9%, and water-soluble ash is 10% and 8.9%. The extractive values for roots and leaves are as follows: water (0.8% and 1%), ethanol (4% and 2.25%), chloroform (8% and 8.5%), ethyl acetate (9% and 7%), and methanol (11% and 13%). Leaf constants include a stomatal number of 5, a stomatal index of 2.5-7, a vein islet number of 11-17, a vein termination number of 9-12, and a palisade ratio of 2:6. Fluorescent studies show the leaves and roots as light brown and dark brown, respectively. Histochemical analysis reveals the presence of lignified cellulose and cuticular cell walls, aleurone grains, calcium oxalate, fatty acids, resins, inulin, mucilage, tannins, and hydroxyl anthraquinones.

Keywords: Androsace globifera, Characteristics, Evaluation, Microscopy, Screening

1. INTRODUCTION

Androsace globifera is a plant known for its significant medicinal properties, primarily due to its phytochemical constituents such as saponins. Traditionally, it has been used to treat a variety of ailments including liver and kidney diseases, amenorrhea, skin allergies, leucorrhoea, and as an abortifacient[1]. The leaves of *Androsace globifera* are diverse in shape, ranging from speculate to elliptical, indicating adaptability and variability in its morphology. The plant produces pink flowers, typically in clusters, each with five petals and seven sepals. The stem is straight and has a brownish-green color. Organoleptic properties of the plant material reveal an astringent taste, an aromatic odor, and a brittle fracture. The powdered leaves and roots of the plant exhibit greenish and brown hues, respectively, with an astringent and aromatic odor, and a bitter and acrid taste[2]. Microscopic examination reveals vascular bundles and distinct upper and lower epidermal cells. Physicochemical studies indicate specific moisture and ash content for roots and leaves, along with extractive values for various solvents including water, ethanol, chloroform, ethyl acetate, and methanol. Leaf constants such as stomatal number, stomatal index, vein islet number, vein termination number, and palisade ratio are identified. Fluorescent studies show that the leaves appear light brown and the roots dark brown under fluorescent light.

Histochemical analysis reveals the presence of lignified cellulose and cuticular cell walls, aleurone grains, calcium oxalate, fatty acids, resins, inulin, mucilage, tannins, and hydroxyl anthraquinones. These compounds contribute to its medicinal properties and potential therapeutic applications. In summary, *Androsace globifera* is a plant with diverse morphological and chemical properties, making it valuable in traditional medicine for treating various health conditions. Its rich phytochemical profile supports its use in herbal remedies and underscores its importance in pharmacognosy[3].

The taxonomical classification of *Androsace globifera* places it within the kingdom Plantae, under the clades of angiosperms, eudicots, and asterids. It belongs to the order Ericales and the family Primulaceae. The genus is Androsace, and the specific species is *Androsace globifera*[4].

The leaves of *Androsace globifera* exhibit a notable diversity in their morphology, varying in shape from speculating to elliptical. This variability reflects the plant's adaptability to different environments. The leaves are a significant part of the plant's anatomy, playing a crucial role in photosynthesis and respiration. They have a greenish hue and, when examined in powdered form, retain this green coloration. Organoleptic analysis of the leaves reveals an astringent taste and an aromatic odor, contributing to the plant's overall sensory profile. Microscopic examination shows distinct upper and lower epidermal cells, which are essential for the leaf's protective and photosynthetic functions. Furthermore, the leaves contain vascular bundles that facilitate the transport of nutrients and water throughout the plant. Histochemical studies of the leaves indicate the presence of important compounds such as lignified cellulose, calcium oxalate, and various phytochemicals, which contribute to the plant's medicinal properties[5].

The stem of *Androsace globifera* is characterized by its straight and robust structure, providing essential support to the plant. It exhibits a brownish-green color, which may vary slightly depending on the environmental conditions and the age of the plant. The stem's rigidity and strength are vital for bearing the weight of the leaves and flowers, ensuring the plant remains upright and stable. Microscopic examination of the stem reveals a complex arrangement of vascular bundles, which are crucial for the transport of water, nutrients, and photosynthates between the roots and the leaves. These vascular bundles include both xylem and phloem tissues, facilitating efficient nutrient distribution throughout the plant. The stem's external texture is relatively smooth, although it can become slightly brittle, especially as the plant matures. This brittleness is an important characteristic observed in organoleptic studies, which also note the stem's straight growth pattern. Histochemical analysis of the stem indicates the presence of lignified cells, which contribute to its structural integrity and resilience[6].

The roots of *Androsace globifera* are a critical part of the plant's anatomy, playing a key role in its overall health and stability. These roots are typically brown and possess a robust structure that anchors the plant securely into the soil. They are integral to the plant's ability to absorb water and essential nutrients from the ground, which are vital for its growth and survival. Microscopic examination of the roots reveals the presence of vascular bundles that include both xylem and phloem tissues. These bundles are essential for the efficient transport of water, minerals, and nutrients from the soil to the rest of the plant. The roots also exhibit a well-developed system of root hairs, which increase the surface area for absorption and enhance the plant's ability to take up nutrients. Physicochemical studies show that the roots have a specific moisture content, which is crucial for maintaining their physiological functions. The roots also contain various ash components, which indicate the presence of mineral residues after the organic matter has been burned away. These components include total ash, acid-insoluble ash, and water-soluble ash, each reflecting different aspects of the root's mineral content. Histochemical analysis of the roots identifies several key compounds, including lignified cells, which provide structural support, and various phytochemicals that contribute to the plant's medicinal properties. These phytochemicals include saponins and other bioactive compounds that have been traditionally used for their therapeutic benefits[7].

The flowers of *Androsace globifera* are one of the most striking features of this plant, contributing to its aesthetic appeal and biological functions. These flowers are typically pink, appearing in clusters that can range from 12 to 15 blooms. Each flower consists of five petals, which are arranged in a radial pattern, enhancing the plant's visual appeal and facilitating pollination. Morphologically, the flowers are supported by a structure that includes seven sepals, which protect the petals and reproductive organs during the bud stage. The reproductive parts of the flower are well-defined, with a single style measuring approximately 1.5 mm in length, and a capsule that is about 3 mm long. This capsule eventually matures to release seeds, facilitating the propagation of the species. The pink coloration of the flowers not only attracts pollinators such as bees and butterflies but also plays a role in the plant's reproductive strategy by ensuring effective pollination. This interaction with pollinators is crucial for the plant's reproduction and the formation of viable seeds. From an organoleptic perspective, the flowers have a mild aromatic scent, which adds to their attractiveness for pollinators and humans alike. The structural integrity of the flowers is supported by the plant's robust stem, which holds the flower clusters upright, ensuring they are well-positioned to attract pollinators[8].

2. MATERIALS AND METHODS

Plant collection

Androsace globifera was collected from the apple orchards of Central Kashmir's Chadoora area, located in the Budgam district of Jammu and Kashmir. The plant's authenticity was confirmed by the Department of Botany at Madhyanchal Professional University in Bhopal, Madhya Pradesh. The collection took place in September, focusing on the aerial parts of the plant, including flowers, stems, and leaves. Upon collection, these parts were carefully cleaned with tap water to remove any surface dirt or contaminants, ensuring that the plant material was suitable for subsequent analysis and research.

Initially, the fresh weight of the collected plant was 5 kg. After the drying process, the weight was reduced to 2 kg, indicating the removal of moisture during the drying procedure. This dried and powdered form of *Androsace globifera* is now ready for various scientific studies and investigations into its chemical composition, medicinal properties, and potential applications in traditional medicine or pharmacology.

Pharmacognostic Studies

Pharmacognostic studies of *Androsace globifera* employed techniques such as the use of glycerin, formalin, chloral hydrate, and glycerin jellies stained with 1% safranine. These methods were aimed at understanding the anatomical or morphological characteristics of the plant. It was observed that these characteristics can vary significantly due to environmental differences and are not consistent across different plants of the same species. Variations in the diagnostic features of medicinal plants are influenced by environmental factors and can impact the potency of their chemical constituents, which in turn affects their pharmacological activities[9].

Histological examinations focusing on specific cell types are crucial for gaining valuable insights into the biological properties and therapeutic potential of medicinal plants. These studies provide important indications regarding the structural and functional aspects that contribute to the medicinal properties of *Androsace globifera* and similar plants.

Macroscopic and Organoleptic Description

Androsace globifera leaves and roots underwent macroscopic evaluation, which included organoleptic assessments such as color, odor, and taste (where applicable), appearance, texture, fracture, and shape of the plant material. These factors are moderately beneficial in the quality control of crude drugs and were assessed following the guidelines set by the World Health Organization (WHO).

To determine the dimensions of plant parts, a graduated scale was used to measure the length, width, and thickness accurately. The color of the plant material was observed under daylight conditions to note any variations or distinctive hues present. For odor evaluation, plant parts were gently crushed between the thumb and index finger to release their aroma, allowing for qualitative assessment. Taste evaluation applied only to specific parts of the plant where taste characteristics were distinctive and relevant. These macroscopic readings provide essential information about the physical attributes of *Androsace globifera*, contributing to its identification and quality assessment by established standards[10].

Microscopic characteristics

Androsace globifera's fresh roots and leaves were prepared for microscopic examination. Microscopic sections were cut using free-hand sectioning techniques to preserve the integrity of the tissue. Abundant temporary and permanent mounts of these sections were prepared and carefully scrutinized under a microscope.

Histochemical studies were conducted on the transverse sections of the leaves using staining reagents to highlight specific cellular components and structures. These studies provided insights into the distribution and composition of various compounds within the leaf tissues.

Photomicrographs of the microscopic sections were captured using a Binocular Zoom Light Microscope to document and analyze the detailed anatomical features of *Androsace globifera*. This approach allowed for a comprehensive examination of both the roots and leaves at a cellular level, contributing to a better understanding of their morphological characteristics and potential medicinal properties[11].

Powder drug characteristics

Preliminary examination of *Androsace globifera*'s powdered drug (from leaves and roots) involved testing with various chemical reagents to assess its chemical composition. The plant material, which was shade-dried and then ground to a fine powder passing through a sieve size of 10, underwent further analysis. Samples of the powdered drug were meticulously mounted onto aluminum stubs, followed by a coating with a thin layer of gold (30-40 nm thickness) and subsequent drying with CO_2. This preparation method ensured optimal sample conductivity and stability for examination under a scanning electron microscope (SEM)[12].

Leaf constants of *Androsace globifera*

Leaf constants, essential for characterizing *Androsace globifera*'s leaf anatomy, were determined through detailed microscopic analysis. Stomatal number, indicating the number of gas exchange pores on the leaf surface, along with the stomatal index, which quantifies stomatal density relative to total epidermal cells, were measured. Vein islet number, revealing vascular openings between veins, and vein termination number, indicating the branching complexity of the leaf's vascular system, were also assessed. Additionally, the palisade ratio, comparing the thickness of palisade mesophyll cells to total leaf thickness, provided insights into the leaf's photosynthetic capabilities. These parameters collectively offer a comprehensive view of *Androsace globifera*'s leaf morphology and physiological adaptations, crucial for understanding its ecological role and potential pharmacological applications[13].

Stomatal number

The stomatal number in *Androsace globifera* leaves was determined using a chloral hydrate solution to clear the leaf's middle portion for observation. A Camera Lucida setup was employed with a drawing board, and a stage micrometer was

used to draw a 1 mm square to scale. The prepared slide was placed on the stage, and the epidermal cells and stomata were traced. The number of stomata within the 1 mm² area of the leaf surface was counted, including cells where at least half of their area fell within the square[14]. This process enabled the calculation of the average number of stomata per square millimeter of the leaf surface, providing quantitative data on the stomatal distribution of *Androsace globifera*.

Stomatal index

The determination of the stomatal index in *Androsace globifera* leaves involved placing a prepared slide on the microscope stage and counting the number of stomata and epidermal cells present per square millimeter of leaf surface[15]. Using a Camera Lucida setup and a drawing board, a 1 mm² square was drawn to scale with the aid of a stage micrometer (E.q.1)

$$SI = \frac{S}{E} X100 \text{--- (1)}$$

Where: S: the number of stomata per mm², E: the number of epidermal cells per mm².

Vein islet number

To determine the vein islet number in *Androsace globifera* leaves, a microscope slide containing a leaf portion was placed on the stage. Using a Camera Lucida setup and a drawing board, a 1 mm² square was drawn to scale with the assistance of a stage micrometer. Within this square, the number of vein islets present was carefully counted and recorded. Vein islets are the small vascular openings or gaps between veins that facilitate nutrient and water transport within the leaf tissue[16]. This method enabled precise calculation of the vein islet density per square millimeter of the leaf portion, providing valuable anatomical data for understanding the leaf's vascular architecture and physiological functions in *Androsace globifera*.

Vein termination number

To determine vein islet number and average vein termination number in *Androsace globifera* leaf portions, a comprehensive approach using a microscope and Camera Lucida was employed. A microscope slide with the leaf sample was placed on the stage, and using a drawing board, multiple 1 mm² squares were drawn to scale with the assistance of a stage micrometer. The number of vein islets and vein terminations within each square was meticulously counted. For vein islets, the count from four adjoining squares was averaged to determine the density per square millimeter of the leaf portion. Similarly, the average number of vein terminations per square millimeter was calculated based on their occurrences within the drawn squares[17].

Palisade ratio

To determine the palisade ratio in *Androsace globifera* leaf portions, a methodical approach using a microscope and Camera Lucida was employed. A microscope slide containing the leaf sample was placed on the stage, and using a drawing board, multiple 1 mm² squares were meticulously drawn to scale with the aid of a stage micrometer. Four cells of the leaf epidermis were traced within these squares, and the number of palisade cells located directly beneath each epidermal cell was counted. The palisade ratio was calculated by dividing the total number of palisade cells counted by the number of epidermal cells traced. This process was repeated across several squares to ensure the accuracy and representativeness of the data[18].

Physicochemical Evaluation

Physicochemical investigations of *Androsace globifera* powder (from leaves and roots) were conducted to evaluate its purity and quality. Parameters such as moisture content, ash values (total, acid-insoluble, water-soluble), and extractive values were assessed following WHO guidelines. These analyses provide essential data for standardizing *Androsace globifera* as a medicinal plant, ensuring consistency and quality in its therapeutic applications[19].

Assessing of Loss on drying of leaves and roots of *Androsace globifera*

Approximately 4 g of dried leaves and roots (powdered drug) of *Androsace globifera* were accurately weighed into wide-mouthed flat weighing bottles. These bottles were placed in an oven set at 105 ± 2°C for 2 h to ensure uniform drying. After heating, the bottles were carefully removed, their mouths protected, and they were transferred to a desiccator to cool to room temperature. Subsequently, the bottles were re-weighed after cooling, and this process of reheating and weighing was repeated until two successive weightings did not differ by more than 5 mg. The percent loss on drying was then calculated as the weight loss (in mg) per gram of air-dried material, providing a measure of the moisture content in the *Androsace globifera* sample. This procedure ensures an accurate determination of moisture content, critical for assessing the stability and quality of the dried plant material[20].

Assessing of total ash

Approximately 4 g of dried powder from *Androsace globifera*'s leaves were placed in a silica crucible and subjected to high-temperature heating in a furnace ranging between 400-550°C. The heating process was continued until the material turned white, indicating complete combustion and removal of carbonaceous matter, thus achieving carbon-free ash. After cooling the ash in a desiccator for approximately 30 min to ensure it reached room temperature, it was promptly weighed.

This method was repeated similarly for the dried roots (powder drug) of *Androsace globifera* to determine their total ash content. The weight of the total ash obtained from both the leaves and roots was expressed as milligrams per gram of the original dried material. This standardized procedure ensures accurate determination of total ash content, providing insights into the inorganic mineral composition of *Androsace globifera*, which is essential for evaluating its medicinal and pharmacological properties[21].

Assessing of acid-insoluble ash

Approximately 25 mL of dilute hydrochloric acid was added to the crucible containing the total ash from *Androsace globifera*, both from leaves and roots separately. The mixture was boiled for 5 min, and then the crucible was covered with a watch glass. After cooling, the watch glass was rinsed with hot water, and the liquid was carefully transferred back into the crucible. Any insoluble matter was collected on a filter paper, transferred back to the original crucible, and dried on a hot plate until a constant weight was achieved.

The remaining residue in the crucible, after cooling in a desiccator for 30 min, was weighed. The weight of the acid-insoluble ash was then calculated as milligrams per gram of air-dried material for both the leaves and roots of *Androsace globifera*. This meticulous procedure ensures the accurate determination of acid-insoluble ash content, providing crucial information about the plant's mineral composition, which is essential for assessing its quality and medicinal potential[22].

Assessing of water-soluble ash

Approximately 25 mL of distilled water was added to the crucible containing the total ash from *Androsace globifera*'s leaves and roots separately. The mixture was boiled for 5 min to dissolve the soluble ash components. Insoluble matter was collected on filter paper, washed with hot water, and then transferred into a crucible. The contents were then burned in the crucible at 450°C for 15 min to ensure complete combustion of organic matter. After cooling, the residue remaining in the crucible was weighed, and this weight was subtracted from the weight of the total ash to determine the weight of the water-soluble ash. The water-soluble ash content was calculated as milligrams per gram of air-dried material for both the leaves and roots of *Androsace globifera*. This methodical procedure ensures an accurate determination of water-soluble ash content, providing important information about the plant's soluble mineral components, which are significant for evaluating its quality and medicinal properties[23].

Assessing of extractive value of leaves and roots of *Androsace globifera*

Approximately 4 g of powdered air-dried material from *Androsace globifera* leaves and roots were placed in separate conical flasks. To each flask, 100 mL of distilled water and various organic solvents were added, and the flasks were reweighed to include the weight of the solvent. The contents were thoroughly shaken and allowed to stand for 1 h, followed by refluxing for an additional hour. After cooling, each flask was reweighed to determine any weight loss due to evaporation. The flasks were readjusted to their original weight using distilled water or the respective organic solvent. Then, 25 mL of the filtrate from each flask was transferred to a separate flask and evaporated in a water bath. The residue was dried at 105°C for 90 min, cooled in a desiccator for 20 min, and then promptly weighed. The extractive value was calculated as milligrams of extractable material per gram of air-dried material for both the leaves and roots of *Androsace globifera*. This meticulous procedure ensures an accurate determination of extractive values, providing crucial data on the plant's soluble constituents in water and various organic solvents, which are important for assessing its medicinal potential and quality[24].

3. RESULTS

Macroscopic Evaluation

The morphological characteristics of *Androsace globifera* were thoroughly examined. The leaves were noted to be elliptical with a speculated shape, varying in size from small (3-4 cm), medium (7-9 cm), to large (10-12 cm) in length. They exhibit an astringent taste, and aromatic and pungent odor, and are brittle when dry. The stem is straight, varying in size, and colored brownish-green with a flat shape adorned with small hairs. The flowers are pinkish in color, leaf-shaped, and arranged in clusters of 12-15 on a single stem. Each flower has five petals with a yellow center, and the sepals are longer than the petals, visible from above. The style is 1.5 mm long, and the capsule is 3 mm wide with a smooth, brittle fracture when dried. These detailed observations provide a comprehensive understanding of the macroscopic characteristics of *Androsace globifera*, essential for botanical identification and medicinal plant classification.

Organoleptic evaluation

The organoleptic study of *Androsace globifera*'s powdered drug highlights distinct characteristics essential for plant identification and standardization. The powder derived from leaves exhibits a greenish color, with an astringent and nauseating odor, accompanied by a bitter taste. In contrast, the powder from the roots displays a brown color, an acrid taste, and an aromatic odor. Additionally, *Androsace globifera* produces umbels of five-stellate flowers typically from May to June, with fruit formation occurring between June and July. These unique traits are instrumental in accurately identifying and standardizing the plant for medicinal purposes, ensuring consistency and quality in its applications.(Show in Table.1)

Table 1: Organoleptic evaluation of *Androsace globifera's* leaves & flowers and roots

Parameters	Leaves	Flower	Roots
Color	Dark green upper surface whitish green lower surface	Pink numerous (12-15)	Brown
Shape	Simple spatulate leaves but are whorled	Leafy, rounded sepals, notched petals, stigma capitated, capsule globose included in the calyx, umbels of five-stellate flowers.	Surface not smooth
Size	Large leaves; 10-12 cm Medium leaves; 7-9 cm Small leaves ; 3-4 cm	Sepals are longer than petals & are visible from the top, The style is 1 mm long, capsule is 3 mm in size	Large maximum up to 20 cm long
Taste	Astringent, nauseating	Slight	Aromatic
Odor	Aromatic	Pungent	acrid
Fracture	Brittle when dry	Smooth, brittle when dry	Smooth

***Androsace globifera's* leaf constants**

The leaf constants for *Androsace globifera* were determined as follows: the stomatal number is 5, the stomatal index ranges from 2.5 to 7.0, the vein islet number ranges from 11 to 17, the veins termination number ranges from 9 to 12, and the palisade ratio is 2:6. These parameters provide quantitative data on the leaf's anatomical features, crucial for understanding its physiological functions and ecological adaptations.(Show in Table.2)

Table 2: Determination of *Androsace globifera's* leaves constants.

Parameters	Values
Stomatal number	5.0
Stomatal index	2.5-7.0
Vein islet number	11-17
Vein termination number	9-12
Palisade ratio	2:6

Physicochemical analysis. Ash values

The physicochemical analysis of *Androsace globifera*'s leaves and roots reveals significant data on their ash content and loss on drying. For the roots, the total ash content is 25%, with 10% being water-soluble ash and 12.5% acid-insoluble ash. In comparison, the leaves have a total ash content of 22.5%, with 9% acid-insoluble ash and 8.9% water-soluble ash. The loss on drying is determined to be 3% for the leaves and 2.5% for the roots. These findings provide essential information for evaluating the plant's quality and suitability for medicinal applications, ensuring consistency and adherence to standardization protocols.(Show in Table.3)

Table 3: Ash values of *Androsace globifera's* leaves & roots (Total, water-soluble and acid insoluble Ash)

Part of plant	Total Ash (%)	Water soluble (%)	Acid insoluble (%)	Loss on drying (%)
Roots	25.0	10.0	12.5	3.0
Leaves	22.5	8.9	9.0	2.5

Extractive value

The extractive values for *Androsace globifera*'s leaves and roots were determined through solvent extraction. For the leaves, the extractive values were found to be 1% for water, 2.25% for ethanol, 8.5% for chloroform, 7% for ethyl acetate, and 13% for methanol. In comparison, the roots exhibited extractive values of 0.8% for water, 3.98% for ethanol, 8% for chloroform, 9% for ethyl acetate, and 11% for methanol. These values represent the percentage of soluble constituents extracted from the plant material, highlighting the different solvent affinities for extracting bioactive compounds from *Androsace globifera* leaves and roots, essential for assessing their medicinal potential and standardization.(Show in Table.4)

Table 4: Extractive value determination of *Androsace globifera's* leaves and roots in different organic solvents (ethanol, methanol, ethyl-acetate, chloroform) and water.\

Solvents	Extractive value (%)	
Part of plant	Leaves	Roots
Water	1.00	0.80

Ethanol	2.25	3.98
Methanol	13.00	11.00
Ethyl-acetate	7.00	9.00
Chloroform	8.50	8.00

Fluorescent study

Fluorescent studies were conducted on the powdered drug of *Androsace globifera*'s leaves and roots. For the leaves, the examination included observations under ordinary light as well as under UV light sources, both short-range and long-range. Various organic and inorganic reagents and chemicals were employed to assess fluorescence characteristics. These studies provide insights into the fluorescent properties of *Androsace globifera*'s leaf powder, which are important for identifying specific compounds and understanding their potential applications in medicinal and pharmaceutical fields.(Show in Table.5)

Table 5: Fluorescent studies of *A. globifera's* leaves and roots (powdered drug)

Treatment	Ordinary light	Leaves powdered drug		Roots Powdered drug	
		UV-short range	UV-long range	UV-short range	UV-long range
Acetic acid	Medium green	Brown	Grey black	Brownish black	Grey-brown
Ammonia sol.	Light green	Yellow-green	Grey green	Brownish black	Dark grey
Benzene	Dark green	Brown	Brown	Light brown	Black
CCl_4	Dark green	Dark brown	Greenish grey	Black	Blackish brown
Con H_2SO_4	Light green	Dark brown	Light green	Greyish black	Grey-brown
Con HCl	Light green	Light green	Dark green	Black	Brown
Diethyl ether	Medium green	Brown	Grey	Grey black	Black
Ethanol	Dark green	Brown	Grey	Black	Dark grey
Fecl3	Dark green	Dark brown	Light Brown	Black	Grey
Iodine sol.	Yellowish green	Grey	Greenish Grey	Black	Brown
NaOH sol.	Light green	Light grey	Grey	Brownish black	Brown
Normal	Green	Light brown	Green	Brown	Dark brown
Toluene	Dark green	Brown	Light green	Black	Dark grey

Histochemical Analysis

Histochemical investigations were conducted on the powdered drug of *Androsace globifera*'s leaves and roots using various reagents. The study revealed the presence of several biochemical constituents in the leaves, including lignified cell walls, aleurone grains, calcium oxalate, fatty acids, resins, inulin, mucilage, and tannins. In contrast, the roots of *Androsace globifera* showed the presence of cellulose cell walls, lignified cell walls, cuticular cell walls, calcium oxalate, tannins, and hydroxyl anthraquinones. These findings provide detailed information about the chemical composition and potential pharmacological properties of *Androsace globifera*, essential for understanding its medicinal uses and applications. (Show in Table.6)

Table 6: Histochemical analysis of *A globifera's* leaves and roots (powder drug).

Type of cell	Test applied	Observation	Sample	
			Leaves	Root
Cellulose cell walls.	Iodinated Zinc Chloride	Blue to blue violet color	-	+
Lignified cell walls	Phloroglucinol Hcl test.	Pink to cherry red color	+	+
Cuticular cell walls.	Sudan red test	Orange red or red	-	+
Aleurone grains.	Iodine ethanol test	Yellowish brown	+	-
Calcium carbonate	Acetic acid/HCl	Dissolve with effervescence	-	+
Calcium oxalate	Acetic acid /HCl /sulfuric acid	Insoluble in acetic acid/dissolved in HCl and sulfuric acid	+	+
Fats, fatty oils, volatile oils and resins	Sudan red test/ethanol	Fatty subs: orange-red to red, Don't dissolve in ethanol Volatile oils and resins dissolve in ethanol	+	-
Hydroxyl-anthra- quinones.	Potassium hydroxide	Stain red	-	+
Inulin	Naphthol / sulfuric acid	Brownish red and dissolve	+	+
Mucilage	Chinese ink	Transparent spherical dilated fragments	+	-

Tannin	Ferric chloride test	Greenish black.	+	+
+: Present, -: Absent				

4. DISCUSSION

Natural products have historically been pivotal in drug research, contributing significantly to the development of numerous medicines, many of which are either natural products themselves or derivatives thereof. Even today, natural products continue to yield innovative clinical candidates and drugs. Current research focuses on isolating and chemically characterizing bioactive compounds from medicinal plants to address various prevalent diseases. Organoleptic studies of *Androsace globifera* reveal characteristics such as an astringent taste, aromatic odor, and brittle fracture, with a straight stem and brownish-green coloration. Its pinkish flowers, numbering ranging from 12-15, feature five petals and seven sepals, with a style length of 1.5 mm and capsules measuring 3 mm. The powdered leaves and roots exhibit greenish and brown colors respectively, with an astringent and aromatic odor and bitter and acrid taste. Microscopic and physicochemical studies highlight vascular bundles, and upper and lower epidermal cells, and indicate moisture contents of 2.5% in roots and 3% in leaves. Ash content analysis shows total ash percentages of 25% in roots and 22.5% in leaves, with acid-insoluble ash levels of 12.5% in roots and 9% in leaves, and water-soluble ash contents of 10% in roots and 8.9% in leaves. Additionally, extractive values of roots and leaves in various solvents include water (0.8% and 1%), ethanol (4% and 2.25%), chloroform (8% and 8.5%), ethyl acetate (9% and 7%), and methanol (11% and 13%). Leaf constants encompass stomatal number (5), stomatal index (ranging from 2.5 to 7), vein islet numbers (ranging from 11 to 17), vein termination numbers (ranging from 9 to 12), and a palisade ratio of 2:6. These comprehensive findings provide crucial insights into the botanical and chemical characteristics of *Androsace globifera*, supporting its potential medicinal applications.[25,26,27,28,29,30]

Standardization is a critical step following the collection and drying of plants, ensuring adherence to protocols established by the WHO. It plays a vital role in the certification and documentation of medicinal plants, encompassing the development of herb/drug monographs that detail macroscopic and microscopic characteristics, as well as phytochemical methods. A key method of standardization involves determining leaf constants, which are specific to each plant species and aid in accurate identification and detection of adulteration. For *Androsace globifera*, the leaf constants were established as follows: stomatal number of 5, stomatal index ranging from 2.5 to 7, vein islet number between 11 and 17, vein termination number ranging from 9 to 12, and a palisade ratio of 2:6. These constants provide standardized metrics essential for verifying the identity and quality of *Androsace globifera* in medicinal applications.[31,32,33,34,35,36]

Ash values are crucial indicators in the standardization of herbal drugs, providing insights into the mineral content and purity of plant materials. After incineration of *Androsace globifera*'s roots and leaves, their ash content was determined, representing the residue remaining after combustion. This residue reveals the presence of inorganic compounds naturally occurring in the plant or potentially introduced as adulterants. Three types of ash values were measured: total ash, water-soluble ash, and acid-insoluble ash. Total ash differentiates between non-physiological ash, which includes extraneous materials adhering to the plant surface, and physiological ash derived from plant tissues. Water-soluble ash indicates the soluble portion of the total ash, highlighting the presence of water-soluble minerals in the plant. Acid-insoluble ash quantifies silica content, particularly sand, within the plant fragments. These values are critical criteria for evaluating the quality of *Androsace globifera* as a medicinal plant. For *Androsace globifera*, the roots exhibited a total ash of 25%, with 10% water-soluble ash and 12.5% acid-insoluble ash. Leaves, on the other hand, showed total ash of 22.5%, with 8.9% water-soluble ash and 9% acid-insoluble ash. Additionally, the loss on drying (LOD) test is another standardization parameter, assessing the amount of moisture and volatile substances in a sample under specific drying conditions. *Androsace globifera* leaves had an LOD of 3%, indicating higher moisture content compared to the roots, which had an LOD of 2.5%. Higher moisture content in leaves can promote bacterial and fungal growth and may degrade formulations through oxidation and hydrolysis, highlighting the importance of maintaining optimal moisture levels in herbal drug preparations.

Extractive value is a crucial parameter in the standardization of herbal drugs, indicating the solvents best suited for extracting bioactive compounds from plant material. For *Androsace globifera*, the extractive values were determined for various solvents. The water extractive value for leaves was 1%, while ethanol yielded 2.25%, methanol 13%, chloroform 8.5%, and ethyl acetate 7%. Similarly, for roots, water extractive value was 0.8%, ethanol 3.98%, methanol 11%, chloroform 8%, and ethyl acetate 9%. Notably, methanol showed the highest extractive value among all solvents tested, indicating its effectiveness in extracting bioactive constituents from both leaves and roots of *Androsace globifera*. This underscores methanol as the most suitable solvent for extracting medicinal components from this plant species.

Fluorescent studies were conducted on both roots and leaves of *Androsace globifera* using a variety of chemical solutions including concentrated hydrochloric acid, concentrated sulfuric acid, ferric chloride, iodine solution, carbon tetrachloride, diethyl ether, ethanol, sodium hydroxide solution, acetic acid, toluene, benzene, and ammonia solution. Different colors were observed under ordinary light as well as under UV light with both short and long ranges. For the leaves' powdered drug, UV short range exhibited a brown fluorescence, while UV long range showed a green fluorescence. Similarly, the roots' powdered drug showed a brown fluorescence under UV short range and a dark brown fluorescence under UV long range. These fluorescent characteristics provide valuable information about the chemical composition and potential applications of *Androsace globifera* in medicinal and other fields.

Histochemical evaluations are crucial for the standardization of medicinal plants, providing insights into their chemical composition and aiding in species identification. Various tests such as Iodinated Zinc Chloride, Phloroglucinol HCl test, Sudan red test, Iodine ethanol test, Acetic acid/HCl, Acetic acid/HCl/sulfuric acid, Sudan red test/ethanol, Potassium hydroxide, Naphthol/sulfuric acid, Chinese ink, and Ferric chloride test were applied to *Androsace globifera*'s leaves and roots. The studies revealed the presence of lignified cell walls, aleurone grains, calcium oxalate, fatty acids, resins, inulin, mucilage, and tannins in the leaves. In the roots, cellulose cell walls, lignified cell walls, cuticular cell walls, calcium oxalate, tannins, and hydroxyl anthraquinones were identified. These findings contribute to understanding the medicinal properties and potential applications of *Androsace globifera* in pharmacology and other fields.

All standardization parameters, including leaf constants, ash values, loss on drying, extractive value, and histochemical evaluation, have been established for *Androsace globifera* based on the first-time measurements for this plant species. These protocols were conducted per the guidelines outlined in monographs by the World Health Organization. The development of these monographs ensures that *Androsace globifera*'s standardization values are recognized as benchmarks for this herb, providing comprehensive data essential for its characterization and utilization in various applications.

5. CONCLUSION

Androsace globifera is valued for its phytochemical content, particularly saponin, and its traditional uses in treating liver and kidney diseases, amenorrhea, skin allergies, Leucorrhoea, and as an abortifacient. Comprehensive pharmacognostic evaluation included organoleptic, macroscopic, microscopic, and powder drug studies. Leaf constants such as stomatal number, stomatal index, vein islet number, vein termination number, and palisade ratio were determined. Standardization parameters such as ash values (total ash, water-soluble ash, and acid-insoluble ash) were assessed. Fluorescent studies using various solvents revealed distinct colors for roots and leaves. Histochemical analysis highlighted the presence of cellulose cell walls, lignified cell walls, cuticular cell walls, aleurone grains, calcium oxalate, fats, fatty oils, volatile oils and resins, hydroxyl-anthraquinones, inulin, mucilage, and tannins. This comprehensive research, essential for establishing a monograph, represents the first detailed study of *Androsace globifera*, contributing significantly to its standardization and evaluation as a crude drug.

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