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Pharmacognostic standardization of *Aralia cachemirica*: a comparative study

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Abstract

Background: *Aralia cachemirica* Decne. is an endemic and an important medicinal plant species of Kashmir Himalaya. Despite having immense medicinal importance, little information is available on the standardization parameters of the species. For this reason, present work was carried out for providing comprehensive report on the quality control and standardization parameters of *A. cachemirica*. In this connection, different parts (leaves, stem, and root) of the plant were examined. Methods like microscopy and macroscopy, physicochemical parameters, extractive values, and fluorescence analysis were used to establish pharmacognostical standards.

Results: The macroscopic, microscopy, and physicochemical parameters of different parts of *A. cachemirica* revealed various diagnostic characteristics in the species.

Conclusion: This is the first study providing complete pharmacognostic profile of *A. cachemirica* and hence will be useful for correct identification and authentication of the species for future studies.

Keywords: *Aralia cachemirica*, Fluorescence, Pharmacognosy, Physicochemical, Extractive yield

Background

Plants happen to be serving human beings as a natural source of cure for various ailments and diseases since ages. The world has seen huge increase in plant research in recent times, and numerous evidences show vast potential of medicinal plants used in various traditional systems [1]. In Ayurveda, about 2000 plant species are labeled as source of medicinal value, while in Chinese Pharmacopoeia 5700 traditional medicines are listed [2], most of which are still used in conventional medical practice [3]. These are now getting more attention than ever because they have potential of multitude benefits to society or indeed to all mankind, especially in medicine and pharmacological studies [4]. Therefore, there is a need to evaluate phytoconstituents obtained from traditional medicines, based on various phytochemical screening and pharmacological and analytical methods [5]. The World Health Organization (WHO) supports, suggests, and encourages traditional/herbal remedies in national health care programs as these drugs are easily available at low cost, safe, and people have reliance in them [6]. Proper identification, quality assurance, and establishing pharmacognostic standards are very important parameters for evaluation of medicinal plants. Macroscopic and microscopic characters, physicochemical studies, and fluorescence analysis of these are prime steps for their evaluation. According to the WHO, the macroscopical and microscopical account of a medicinal plant is the first step towards ascertaining the identity and the degree of purity of such material [6].

Aralia cachemirica Decne. is commonly known as "Kashmir spikenard" and locally known as "khoree". It is a shrubby herb, 1 to 3-m tall, growing at various altitudes, and belongs to the family Araliaceae. It is found distributed in temperate Himalayas from Kashmir to Sikkim at 2100 to 4000-m altitude [7, 8]. The following phytoconstituents have already been isolated from the plant: octadec-6-enoic acid, 8-primara-14, 15-diene-19-oic acid, aralosides A&B [9, 10]. Nonane, a hexacosane

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derivative, petroselinic acid, stigmasterol, and β-sitosterol [10, 11] are other phytoconstituents isolated from the plant. Anti-inflammatory activity also has been reported in this plant [10]. Bhat et al. [10] reported hypoglycemic activity from the roots of *Aralia cachemirica*. Furthermore, isolation of continentalic acid from *A. cachemirica* and its immunomodulatory activity has already been reported [12].

Despite having great medicinal importance, little information is available on the standardization parameters of *A. cachemirica*. Hence, present work can only be an attempt for providing comprehensive report on the quality control and standardization parameters of *A. cachemirica*. In this connection, different parts (leaves, stem, and root) of the plant were examined. Methods like microscopy and macroscopy, physicochemical parameters, extractive values, and fluorescence analysis were used to establish pharmacognostical standards. These parameters in turn can facilitate the quality of the drug and be helpful for the assemblage of appropriate monograph for its proper identification.

Methods

Preparation for pharmacognostic studies

Healthy and disease-free plants of *A. cachemirica* were collected from Ferozpur Nallah area of Jammu and Kashmir. The collected specimens were identified and deposited in Kashmir University Herbarium (KASH) under voucher number 2689-KASH. The plant collections were made quite judiciously throughout the course of the present study. The plant materials were fragmented into different parts (leaves, stem, and root) and dried under shade at room temperature for 15–20 days. After shade drying, the plant materials were pulverized to coarse powder using grinder and stored under proper conditions for future use. The pharmacognostic studies were carried out on different parts (leaves, stem, and root) separately.

Organoleptic evaluation

It refers to the evaluation of plant material by color, odor, taste, shape, texture etc. Different dried parts of *A. cachemirica* were considered for macroscopical evaluation [13].

Macroscopic evaluation

Fresh and healthy plants of *A. cachemirica* were assessed for their external characteristics.

Microscopic evaluation

Anatomy

Transverse sections of fresh materials of different parts of *A. cachemirica* were cut with the help of sharp blades. Peels were obtained from fresh leaves by forceps. Different sections/peels were stained with safranin and observed under microscope and photographed.

Table 1 Macroscopical attributes of *Aralia cachemirica*

Habit	Herbaceous perennial
Root	Very thick, branched
Stem	Upright stems, 1 to 3-m tall
Leaves	Large bright green tri-pinnate leaves, imparipinnate. Petiole long, leaflets ovate, apex acuminate, glabrous
Inflorescence	Inflorescence of umbels in axillary or terminal panicles
Calyx	Toothed, persistent
Corolla	Petals ovate
Androecium	Stamens 5, filaments longer than the petals, broader at the base and alternating with the petals
Gynoecium	Styles 5, united at the base, persistent. Ovary 5—locular
Flowering	July–August (–September)
Fruit	A 5-angled drupe, purplish black

Powder microscopy

For the analysis of plant powder, pinch of fine powder is taken in a test tube and boiled in chloral hydrate solution for few minutes. A few drops of powder were smeared on a slide mounted with phloroglucinol followed by few drops of concentrated HCl [13]. The prepared slides were then observed under a microscope and photographed.

Physicochemical parameters

Various physicochemical parameters (foreign matter, moisture content, ash value, fat content, pH, swelling index, foaming index, fluorescent analysis, extractive value) were analyzed [13–17].

Results

Macroscopic and organoleptic description

The macroscopic and organoleptic description of various parts of *A. cachemirica* is presented in Tables 1 and 2 and Fig. 1a–e.

Table 2 Organoleptic evaluation of different parts of *Aralia cachemirica*

Plant parts	Organoleptic characters			
	Color	**Odor**	**Taste**	**Texture**
Leaves	Dark green	Characteristic	Astringent	Soft
Stem	Purplish green	Aromatic	Bitter	Rough
Root	Dark brown	Aromatic	Bitter	Hard

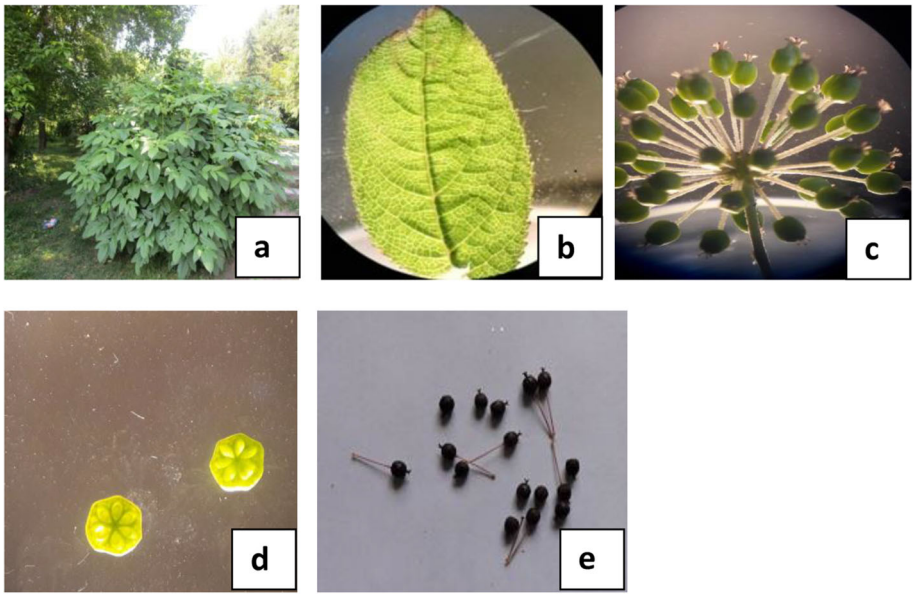

Fig. 1 Morphological attributes of *A. cachemirica*. **a** Habit (perennial herb). **b** Leaf ovate. **c** Inflorescence umbel. **d** Ovary 6–locular. **e** Purplish black fruit

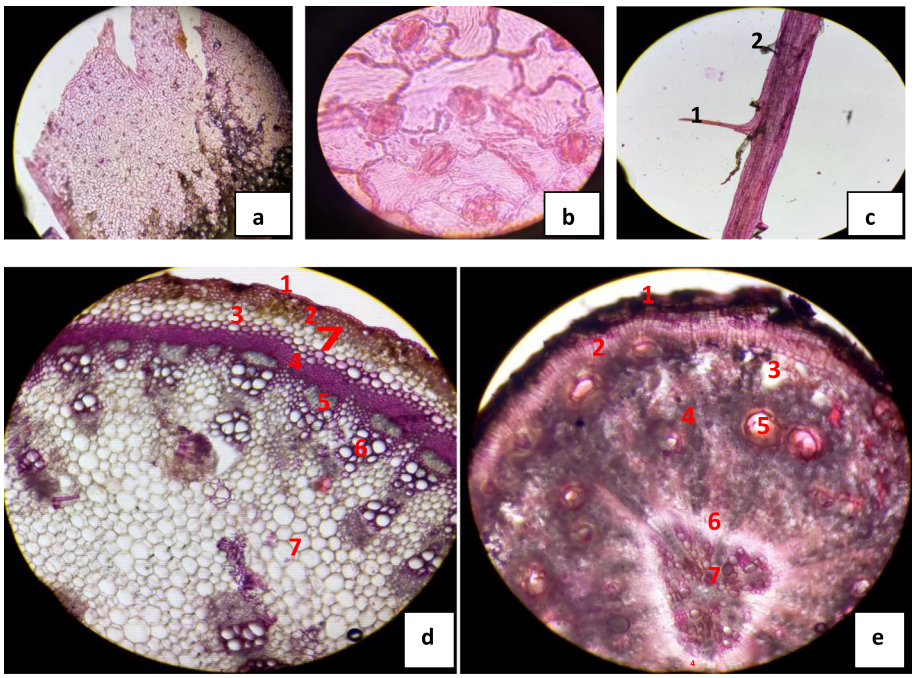

Fig. 2 Anatomical features of *A. cachemirica* leaf. **a** A patch of stomata (× 10). **b** Anomocytic stomata with wavy epidermal cells (× 100). **c** 1 Unicellular trichome (× 40), 2 glandular trichome. Anatomical features of *A. cachemirica* stem. **d** Transverse section of stem (× 10): 1 epidermis, 2 hypodermis, 3 cortex, 4 sclerenchymatous sheath, 5 phloem, 6 xylem, 7 pith. Anatomical features of *A. cachemirica* root. **e** Transverse section of root (× 10): 1 phellem, 2 phelloderm, 3 intercellular spaces, 4 cortex, 5 mucilage canal, 6 phloem, 7 xylem

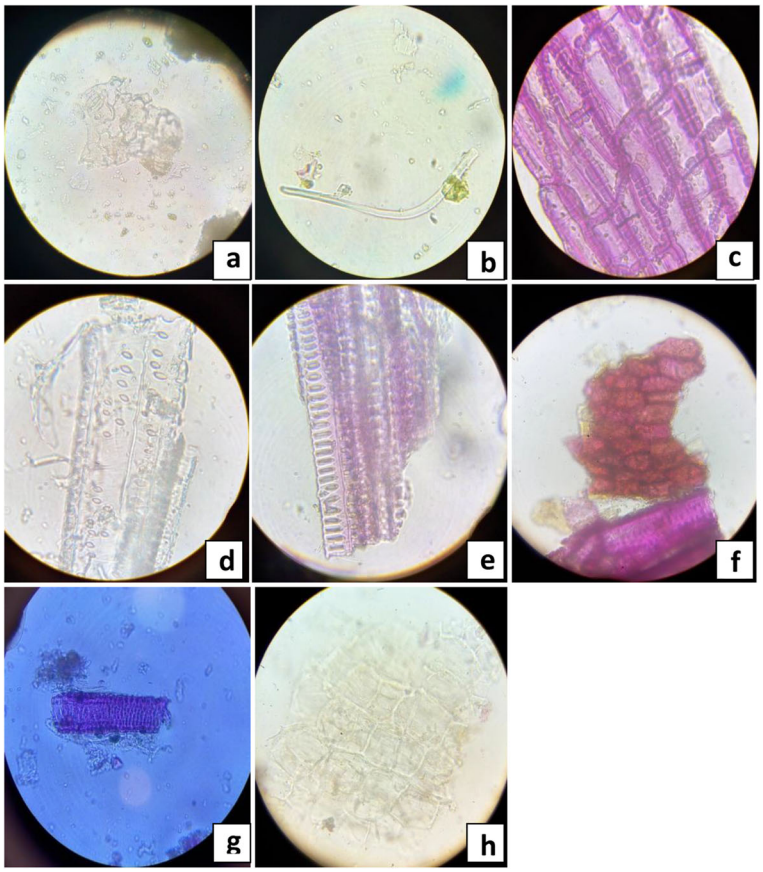

Fig. 3 Powder microscopy of *A. cachemirica* leaf. **a** Stomata with wavy epidermal cells (× 40). **b** Unicellular trichome (× 40). Powder microscopy of *A. cachemirica* stem: **c** reticulate parenchyma (× 40), **d** bordered pitted xylem vessel (× 40), **e** scalariform vessel (× 40). Powder microscopy of *A. cachemirica* root: **f** lignified cork cells (× 40), **g** pitted vessel (× 40), **h** parenchyma cells (× 40)

Table 3 Physicochemical analysis of different parts of *Aralia cachemirica*

Physicochemical parameters (%age)	Plant parts		
	Leaves	Stem	Root
Total ash	17.745	6.54	8.14
Acid insoluble ash	10.076	0.99	1.99
Water soluble ash	8.221	1.80	6.33
Foreign matter	0	0	0.75
Loss on drying	6.61	9.67	9.45
Swelling index	10	100	60
Foaming index	< 100	< 100	< 100
1% pH	6.19	5.86	6.10
10% pH	5.87	5.14	5.68
Total fat content	8.21	2.57	12.58

Microscopy

Anatomy

The anatomical studies of different parts of *A. cachemirica* revealed presence of various diagnostic features as depicted in Fig. 2a–e.

Powder microscopy

The result of powder microscopy of different parts of *A. cachemirica* revealed many important features which are illustrated in the Fig. 3a–h.

Physicochemical parameters

The results attained from various physicochemical parameters in different parts of *A. cachemirica* are presented in Table 3. The detailed results of cold extraction, hot extraction, and successive extraction values are presented in Table 4. The fluorescence characteristics of powdered leaves, stem, and root of *A. cachemirica* were observed in visible, short, and long UV light. The observations are presented in Tables 5, 6, and 7 showing the variation in color.

Table 4 Extractive values of *Aralia cachemirica* various parts using different solvents

Plant parts	Solvent	Cold	Hot	Successive
Leaf	**Hexane**	1.33	8.21	4.105
	Chloroform	0.32	6.71	2.135
	Ethyl acetate	2.92	6.73	1.575
	Methanol	5.74	16.13	8.415
	Aqueous	21.17	19.84	12.685
	Hydroalcohol	13.31	34.934	-
Stem	**Hexane**	0.79	2.57	1.285
	Chloroform	0.77	2.30	0.805
	Ethyl acetate	2.75	3.35	1.010
	Methanol	1.07	18.03	10.925
	Aqueous	16.10	18.39	4.155
	Hydroalcohol	14.16	21.227	-
Root	**Hexane**	7.04	12.58	6.29
	Chloroform	9.09	18.23	1.51
	Ethyl acetate	9.64	14.75	0.905
	Methanol	6.53	22.04	6.475
	Aqueous	10.41	11.77	9.59
	Hydroalcohol	8.965	18.68	-

Discussion

Proper identification, quality assurance, and establishing pharmacognostic standards are very significant factors for evaluation of medicinal plants. According to the World Health Organization (WHO), the macroscopical and microscopical account of a medicinal plant is the first step towards ascertaining the identity and the degree of purity of such material and should be accomplished before any tests are undertaken [6, 13].

Microscopic assessment of the plant material is crucial for the detection of source materials. The anatomical attributes are employed as a criterion for unraveling the species, genera, and even families. Also, anatomy gives the idea of diagnostic features of a plant material such as cork cells, cortex, secondary phloem, and fibers which forms the vital factors for the quality control and standardization of herbal drugs [18]. Investigations of the powdered plant material offer the comprehensive structural information of the raw drugs by discovering the identified histological characters in the drugs. The powdered examination of herbal material is based on the cytomorphological parameters, for instance collenchyma, parenchyma, trichomes, vessels, and secretory cells,

Table 5 Fluorescence analysis of *Aralia cachemirica* leaf

S.No.	Reagents	Visible light	UV 254 nm	UV 366 nm
1	**Powder drug**	Dull green	Dark black	Light black
2	**Powder drug + distilled water**	Olive green	Reddish green	Gray green
3	**Powder drug + 10% aq. sodium hydroxide**	Yellowish green	Light red green	Grayish
4	**Powder drug + ammonia**	Light green	Orange	Green[a]
5	**Powder drug + conc. sulfuric acid**	Reddish black	Dark orange	Grayish green
6	**Powder drug + sulfuric acid + water**	Light green	Light orange	Brownish green
7	**Powder drug + conc. hydrochloric acid**	Blackish green	Orange gray	Light black
8	**Powder drug + hydrochloric acid + water**	Dark olive	Brownish orange	Grayish black
9	**Powder drug + conc. nitric acid**	Orange	Light orange	Light green[a]
10	**Powder drug + nitric acid + water**	Light orange	Dark orange	Dark gray
11	**Powder drug + iodine**	Brownish green	Moderate orange	Grayish black
12	**Powder drug + 5% ferric chloride**	Black green	Blackish orange	Dark grayish
13	**Powder drug + picric acid**	Yellow green	Grayish orange	Black gray
14	**Powder drug + picric acid + water**	Light yellow green	Light orange	Blackish gray
15	**Powder drug + glacial acetic acid**	Light brown green	Orange blackish	Bluish green[a]
16	**Powder drug + petroleum ether**	Moderate olive green	Grayish orange	Grayish white
17	**Powder drug + chloroform**	Light olive	Light orange	Reddish black
18	**Powder drug + ethyl acetate**	Mild yellow green	Grayish black	Dark orange[a]
19	**Powder drug + methanol**	Deep yellow green	Light orange	Grayish green
20	**Powder drug + 5% potassium dichromate**	Reddish brown	Light orange	Orange[a]
21	**Powder drug + alcoholic potassium hydroxide**	Strong olive green	Blackish orange	Grayish black

[a]Diagnostic color

Table 6 Fluorescence analysis of *Aralia cachemirica* stem

S.No.	Reagents	Visible light	UV 254 nm	UV 366 nm
1.	**Powder drug**	Light brown	Grayish orange	Light olive green
2.	**Powder drug + distilled water**	Brown	Moderate orange	Dark olive green
3.	**Powder drug + 10% aq. sodium hydroxide**	Dark brown	Light orange	Dark olive green
4.	**Powder drug + ammonia**	Moderate brown	Blackish orange	Green[a]
5.	**Powder drug + conc. sulfuric acid**	Brownish black	Blackish orange	Black
6.	**Powder drug + sulfuric acid + water**	Black	Moderate orange	Light black
7.	**Powder drug + conc. hydrochloric acid**	Chocolate brown	Blackish orange	Grayish black
8.	**Powder drug + hydrochloric acid + water**	Light brown	Light orange	Blackish green[a]
9.	**Powder drug + conc. nitric acid**	Moderate orange	Dark orange	Grayish black
10.	**Powder drug + nitric acid + water**	Light orange	Orange	Greenish black
11.	**Powder drug + iodine**	Brown	Dark orange	Greenish black
12.	**Powder drug + 5% ferric chloride**	Grayish black	Blackish orange	Grayish black
13.	**Powder drug + picric acid**	Yellow	Light orange	Greenish black
14.	**Powder drug + picric acid + water**	Yellowish brown	Light orange	Dark olive green
15.	**Powder drug + glacial acetic acid**	Dark brown	Moderate orange	Light gray
16.	**Powder drug + petroleum ether**	Light brown	Grayish orange	Light gray
17.	**Powder drug + chloroform**	Dark brown	Blackish orange	Grayish white
18.	**Powder drug + ethyl acetate**	Moderate brown	Blackish orange	Light pink[a]
19.	**Powder drug + methanol**	Brown	Light orange	Greenish gray
20.	**Powder drug + 5% potassium dichromate**	Yellowish dark brown	Moderate orange	Black
21.	**Powder drug + alcoholic potassium hydroxide**	Moderate brown	Blackish orange	Greenish gray

[a]Diagnostic color

and cell inclusions, viz., pollen grains, starch grains, and calcium oxalate crystals [19, 20].

Physicochemical parameters are also vital for the standardization and quality control of herbal drugs which included foreign matter analysis, loss on drying, ash content, pH, swelling index, and foaming index. Herbal materials should be devoid of any kind of contamination, so foreign matter analysis of powdered drugs can be considered as an important parameter in order to check the purity of herbal drugs [21]. Loss on drying is commonly used test procedure for determination of moisture content in a powdered sample. Moisture content of drugs should be at minimal level to discourage the growth of bacteria, yeast, or fungi during storage [22]. Ash values are used to determine quality and purity of crude drug. It indicates presence of various impurities like carbonate, oxalate, and silicate. The water soluble ash is used to estimate the amount of inorganic compound present in drugs. The acid insoluble ash consist mainly silica and indicate contamination with earthy material [22]. The pH values provide information about acidic or basic nature of the chemical constituents present in the crude drug. Foaming index is seen to be less than 100 in all the parts of the select species which reveals absence or very little amount of saponins. Swelling index indicates the presence of gums and mucilage, hemicellulose, or pectin in the natural drug [21]. Estimation of extractive values determines the amount of the active constituents in a given amount of plant material when extracted with a particular solvent. The extractions of any crude drug with a particular solvent yield a solution containing different phytoconstituents. The compositions of these chemical constituents depend upon the nature of the drug and the solvent used. It also provides an indication whether the crude drug is exhausted or not [22, 23]. Fluorescence analysis is also an important pharmacognostic parameter. Some constituents show fluorescence in the visible range in daylight. The ultraviolet light produces fluorescence in many natural products which do not visibly fluoresce in daylight. If substance themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by applying different reagents. Hence, crude drugs are often assessed qualitatively in this way, and it is an important parameter for pharmacognostic evaluation of crude drugs [24, 25].

Conclusion

The study may possibly provide a foundation for further undertakings towards generating understanding about

Table 7 Fluorescence analysis of *Aralia cachemirica* root

S.No.	Reagents	Visible light	UV 254 nm	UV 366 nm
1	**Powder drug**	Light brown	Dark orange	Bluish gray
2	**Powder drug + distilled water**	Moderate brown	Dark orange	Olive green
3	**Powder drug + 10% aq. sodium hydroxide**	Chocolate brown	Light orange	Moderate green
4	**Powder drug + ammonia**	Dark brown	Light orange	Green[a]
5	**Powder drug + conc. sulfuric acid**	Reddish black	Blackish orange	Black
6	**Powder drug + sulfuric acid + water**	Black	Moderate orange	Olive green
7	**Powder drug + conc. hydrochloric acid**	Chocolate brown	Dark orange	Dark olive green
8	**Powder drug + hydrochloric acid + water**	Moderate brown	Light orange	Moderate olive green
9	**Powder drug + conc. nitric acid**	Moderate orange	Moderate orange	Grayish black
10	**Powder drug + nitric acid + water**	Light orange	Moderate orange	Grayish black
11	**Powder drug + iodine**	Grayish black	Light black	Greenish black
12	**Powder drug + 5% ferric chloride**	Light black	Blackish orange	Black
13	**Powder drug + picric acid**	Dark yellow	Light orange	Greenish black
14	**Powder drug + picric acid + water**	Blackish yellow	Moderate orange	Greenish black
15	**Powder drug + glacial acetic acid**	Moderate brown	Light orange	Bluish gray
16	**Powder drug + petroleum ether**	Moderate brown	Orange	Bluish green
17	**Powder drug + chloroform**	Chocolate brown	Blackish orange	Light olive green
18	**Powder drug + ethyl acetate**	Moderate brown	Blackish orange	Light green
19	**Powder drug + methanol**	Light brown	Light orange	Bluish green[a]
20	**Powder drug + 5% potassium dichromate**	Yellowish brown	Light orange	Black
21	**Powder drug + alcoholic potassium hydroxide**	Dark brown	Grayish black	Green[a]

[a]Diagnostic color

medicinal plants of Kashmir Himalaya. The pharmacognostic studies are the first step towards ascertaining the identity and the degree of purity of herbal materials. The pharmacognostic analysis is not reported previously in this plant species thus making this first report which provides inclusive pharmacognostic profile of *A. cachemirica* and thereby will be helpful for correct identification and authentication of the species for future studies.

Abbreviations

WHO: World Health Organization; KASH: Kashmir University Herbarium

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Plant authentication

Healthy and disease-free plants of *A. cachemirica* were collected from Ferozpur Nallah area of Jammu and Kashmir. The collected specimens were identified by Dr. Anzar A. Khuroo (senior assistant professor, Centre for Biodiversity & Taxonomy, Department of Botany, University of Kashmir) and deposited in Kashmir University Herbarium (KASH) under voucher number 2689-KASH.

Authors' contributions

NM and SN carried the experimental work; WYR helped in the compilation of data; IAN and ZAB helped in the result analysis and supervision of the work. All authors have read and approved the final manuscript.

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Availability of data and materials

Data and material are available upon request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

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Competing interests

No conflict.

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