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.

| 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 |

Different sections/peels were stained with safranin and observed under microscope and photographed.

**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 (x 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
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 3 Physicochemical analysis of different parts of *Aralia cachemirica*

| Physicochemical parameters (%) | 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 |
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 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 | -          |

### 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<sup>a</sup> |
| 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<sup>a</sup> |
| 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<sup>a</sup> |
| 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<sup>a</sup> |
| 19    | Powder drug + methanol           | Deep yellow green | Light orange | Grayish green |
| 20    | Powder drug + 5% potassium dichromate | Reddish brown | Light orange | Orange<sup>a</sup> |
| 21    | Powder drug + alcoholic potassium hydroxide | Strong olive green | Blackish orange | Grayish black |

<sup>a</sup>Diagnostic color
and cell inclusions, viz., pollen grains, starch grains, and calcium oxide 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].

**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

**Conclusion**

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

*aDiagnostic color*
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