Indian Journal of Natural Products and Resources
Vol. 14(2), June 2023, pp. 285-291
DOI: 10.56042/ijnpr.v14i2.4213

Pharmacognostical, physicochemical, and phytochemical investigation of *Allium wallichii* Kunth (An unexplored and underutilised traditional aromatic plant from Himalayan regions)

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Received 11 November 2022; revised received 15 May 2023; accepted 19 May 2023

Allium wallichii (*A. wallichii*) Kunth is considered one of the underutilised, threatened, and unexplored medicinal plant species having several beneficial health-promoting actions. Moreover, being so popularised in both ethnobotanical and traditional systems, still, the plant is less studied in terms of proper pharmacognostic standardisation. The main objective of the current work is to establish standard pharmacognostic, physiochemical and phytochemical analytical parameters for routine quality control of *A. wallichii* Kunth. Intact leaves, powdered plant materials, and various extracts viz petroleum ether, chloroform, ethyl acetate, methanol, ethanol, hydro-alcohol and water (aqueous) were investigated following the prescribed guidelines of World Health Organization (WHO) standardisation of herbs. Morphological, microscopic, physiochemical (including all extractive, loss on drying and ash values) and preliminary phytochemical analysis of *A. wallichii* Kunth is presented and discussed to establish the standard quality control parameters. Additionally, high performance thin layer chromatographic (HPTLC) fingerprinting for *A. wallichii* Kunth extract and fractions were also incorporated. The outcome of the current research will add value for routine quality control to secure the safety and efficacy of *A. wallichii* Kunth (both crude and processed drug) in terms of future commercialisation.

Keywords: *Allium wallichii* Kunth, Ethnobotany, Medicinal plants, Pharmacognostic, Physiochemical and Phytochemical

IPC Code; Int. cl. (2021.01) – A61K 36/00

Introduction

The demand for herbs-based quality products is continuously growing rapidly worldwide for many healthcare requirements. Several academic institutions, pharmaceutical companies, and research labs throughout the globe are regularly conducting extensive research to establish standardisation parameters for plant-based materials[1-2]. Additionally, current regulatory requirement is very astringent on the quality of herbal drugs, including efficacy, safety, and acceptability of the finished product[3-4]. Therefore, standardisation of herbal drugs to achieve consistent quality before proceeding with any commercialisation is highly required[5-7].

Allium wallichii Kunth (Family: Amaryllidaceae) is a higher altitude perennial monocot herb having several medicinal and healthcare properties mainly founds in Asia continent[8-13]. Evidence from rich traditional and ethnobotanical literature suggests its utilisation for culinary, nutraceutical and medicinal purposes by several local communities[13]. The plant is also considered an underutilised and unexplored (or least studied) herb regarding standardisation methods, phytochemicals investigation, and pharmacological activities[8,13].

An earlier study identified diosgenin, 1,2 bis (methylthio) ethene, tigogenin, 2,4 dimethyl thiophene, dimethyl disulfide and trisulfide as some major phytochemicals among flavonoids, glycosides, steroids, reducing sugars, and terpenoids[13]. Additionally, *A. wallichii* has also shown the presence of carbohydrates, proteins, fibres, fats, minerals, and vitamins[14]. Pharmacologically plants have been majorly validated for antimicrobial, antioxidant, anti-inflammatory, anti-diabetic, and anticancer activities via laboratories and animal experimental models[13-15].

Since ancient times plant had rich traditional and medicinal applications, but still, the plant is highly

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neglected for quality control method development, and only a few studies, including pharmacognostic and phylogenetic, have been carried out so far in past[8,11,16-17]. Therefore, the current work was carried out to investigate *A. wallichii* thoroughly regarding herbal monograph development for setting standard pharmacognostic parameters by strictly adhering to the guideline of regulatory requirements.

Materials and Methods

Plant materials

Plant samples of *A. wallichii* were collected from Darkot (Elevation: 2200 m), Munsiyari, Pithoragarh (Uttarakhand, India) from August to September 2021 and authenticated by Dr. M. C. Bharti (Botany Department, Hemwati Nandan Bahuguna Central Garhwal University, Srinagar, Uttarakhand, India). Voucher specimens of the plant (No. Herbarium/ bot./1070) have been deposited at the Department of Botany, Hemwati Nandan Bahuguna Central Garhwal University, Srinagar, Uttarakhand, India.

Reagents and chemicals

Solvents, reagents, and chemicals were of analytical grade and purchased from Merck (Darmstadt, Germany). Further, Precoated silica gel $60F_{254}$ ready-to-use TLC plates were purchased from Merck (Darmstadt, Germany).

Macroscopic studies

The macroscopic features, including colour, shape, odour, taste and size of the leaves obtained from the fresh plant of *A. wallichii,* were determined using the standard reported methods earlier[18-19].

Microscopical studies

Surface preparations of the fresh leaves and powdered samples of *A. wallichii* by anatomical sections were developed, and microscopic studies were carried out according to the standard methods reported earlier[18-19].

***Physiochemical studies* (*Proximate analysis*)**

A. wallichii leaves were dried in the shade and powdered for analysis of moisture content ash values, and successive solvent extractive values[18-19]. For elemental analysis, 1 g of powdered leaves material was taken in concentrated 1 mL nitric acid and boiled at 100°C. Then after completion of digestion for 1 h, the solution was fileted, and the volume was adjusted to 100 mL with distilled water. This solution was further used for the determination of elements such as copper, iron, sodium, potassium, magnesium, manganese, and zinc by atomic absorption spectroscopy[20-22].

Quantitative microscopy

Quantitative microscopy was performed on the anatomical section, and the fresh epidermal layers of the plant leaves were used to determine stomata type, stomata number, and stomatal index through standard methods[18-19].

Preparation of extracts and phytochemical investigations

For screening of phytochemicals, the powdered leaves of *A. wallichii* Kunth were subjected to maceration in hydro-alcohol (30:70 v/v) for 72 h. After complete maceration, the filtrate was concentrated under reduced pressure, and the % yield, consistency and colour were determined[18,19,23,24]. Further, 1 g of extract was diluted with 10 mL of methanol to get the stock solution for qualitative chemical tests, including alkaloid, saponin, glycosides, steroids, terpenoids, and tannins as per standard reported method[18-19, 23-24].

Column chromatographic studies of leaves extract

Exactly 2 g of hydro-alcohol extract was kept in a column (length 50 cm, diameter 1.8 cm, borosil glass chrome sintered column), already packed with 60 g of silica gel of 60-120 mesh in 100 mL petroleum ether. The experiment was followed by the subsequent addition of 400 mL of petroleum ether, 500 mL of chloroform, and 500 mL of methanol to collect a total of 1-70 fractions of 15 mL each.

HPTLC fingerprinting of leaf extract

HPTLC fingerprinting was performed with standard methods using a Camag HPTLC system (LINOMAT 5 applicator) with nitrogen flow, TLC Visualizer 2, a TLC scanner 4, and vision CATS software[25-28]. In brief, filtered hydro-alcohol extract and fractions (10 mg/mL) of *A. wallichii* were applied with an 8 mm band on a 10 × 10 silica gel $60F_{254}$ TLC plate (Merck, Darmstadt, Germany). The samples-loaded plate was kept in a TLC twin trough developing chamber using methanol: ethyl acetate: water: formic acid (1.4: 7.1: 1: 0.1 v/v). The developed plates were air-dried and kept in a photo documentation collection chamber for capturing images or photographs at 254 and 366 nm.

Results and Discussion

The study of pharmacognosy generally implies setting several standard methods for identifying and characterising crude drugs obtained from natural

Fig. 1 — Morphological character of *Allium wallichii* Kunth

Table 1 — Macroscopic features of *A. wallichii* Kunth leaves

Macroscopic features	Observation
Colour	Green
Shape	Linear to oblong-lanceolate or lanceolate, fibrous to sub-reticulate
Odour	Alliaceous (resembling garlic or onion)
Taste	Slightly aromatic and pungent
Size	30-40 cm

sources, mainly from plant origin. These methods broadly involve proper authentication of plant samples through morphology, anatomy, physiochemical, phytochemical and pharmacological analysis[29]. Indeed, the high demand for herbal materials in the current market leads to adulteration or substitution with similar products, either intensely or accidentally. Therefore, the primary concern involving crude drugs is the proper identification of genuine drugs pertaining to commercialisation. Interestingly, standardisation with reference to standard pharmacognostic parameters may support correct authentications of crude drug[30-31]. Additionally, exact identification and quality assurance play an important role in the reproducibility of the quality of the medicinal plants or herbal drugs for the efficacy of the drug[18]. In this connection, the current work has been designed to investigate pharmacognostic standardisation parameters with reference to *A. wallichii.* The results of the studies are presented herein with the following heads.

Macroscopic studies

The result of macroscopic studies is given in Fig. 1 and Table 1. The study demonstrated a similar pattern

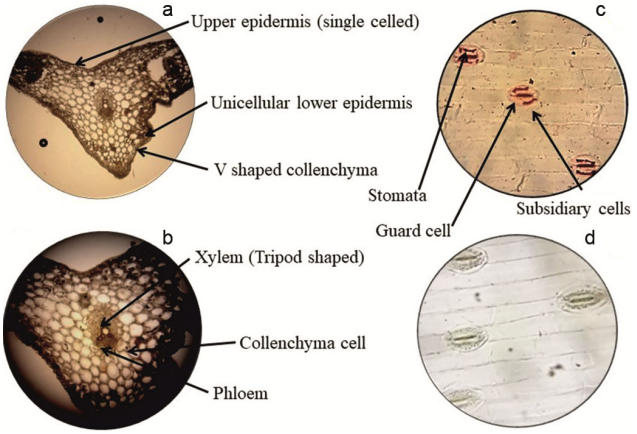

Fig. 2 — (a-b) Transverse section (T. S.) through midrib of *Allium wallichii* Kunth is shown; and c-d) shows the presence of stomata on the upper and lower epidermis, respectively.

as discussed in an earlier study, including plant height (40-65 cm), bulb (yellowish brown, cylindric and solitary/clustered), fibrous/sub-reticulate tunic, fleshy-non-fleshy root, 2-4 number flat linear green colour leaves, subequal pedicel, brilliant magenta-deep purple-pale red flower, ovate-lanceolate subequal dark purple tepal, shorter anther filaments and having garlic like aroma[32].

Microscopical studies

The result of microscopic studies of the anatomical section of leaves was presented in Fig. 2. In this study transverse section of the leaf reveals a triangular wedge-shaped midrib region with extended lamina in opposite directions. The midrib region of leaves was found to have two vascular strands; however, each lateral side of the lamina may have 6-7 vascular

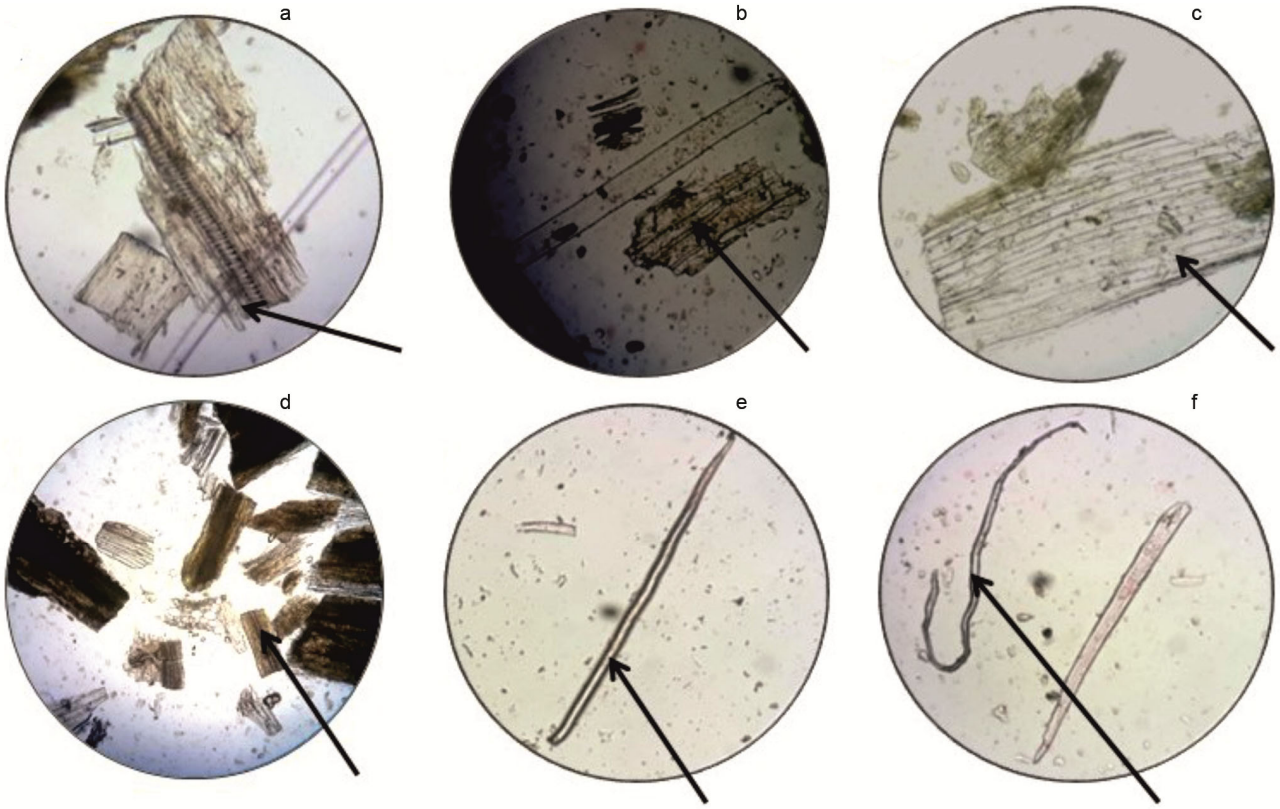

Fig. 3 — Powder microscopic feature of *Allium wallichii* Kunth, a) vascular tissues or vessels; b) vein islets; c) epidermis; d) fragments of reticulate vessels; and e-f) xylem fibers.

strands. Further, each vascular strand was found to have a collateral arrangement of both phloem and xylem. Subsequently, tracheary elements in a group or little scattered with intervening parenchyma cells were seen in the xylem region. The mesophyll of leaves was arranged homogenous rarely with spongy parenchyma along with scattered occasional lactiferous canals. The epidermis layer is very distinct, with large tangential elongated cells and covered by a very prominent thick cuticle, which often extends to some distance in the radial walls. The outcome of the study follows similar results as earlier studies conducted in a similar direction[8]. Moreover, an earlier study on epidermal cells presented information on stomata cell length (5-7 µm), stomata cavity length (3-6 µm), length of long cells (160-300 µm), and shape, i.e. rectangular[33]. Further, powder microscopy reveals the characteristics of the anatomical section of the leaf and is presented in Fig. 3.

Physiochemical studies (Proximate analysis)

Moisture contents and ash values determinations of *A. wallichii* leaves are given in Table 2. Similarly, different extractive values as per requirement were presented in Table 3. Higher extractive values were found in methanol, ethanol, hydro-alcohol, and water may be due to the presence of more polar compounds. Further, the result of elemental analysis demonstrates the presence of Copper (0.11 mg), Magnesium (0.47 mg), Iron (1.54 mg), Calcium (8.04 mg), Lead (2.01 mg) and Zinc (0.25 mg) per 100 g of samples, respectively. However, higher lead may signify soil toxicity to the particular geographical area of plant habitat[34-35].

Table 2 — Moisture contents and ash values analysis on *A. wallichii* Kunth leaves

Parameters	Values* (% w/w)
Moisture content	5.69±0.25
Total ash	12.4±0.255
Acid insoluble ash	7.45±0.324
Sulfated ash	6.88±0.26
Water soluble ash	5.81±0.26

*n=3 (Average + Standard Error Mean)

Quantitative microscopy

The quantitative microscopy, including stomatal number (upper epidermis 4 and lower epidermis 6) and stomatal index (upper epidermis 25 and lower epidermis 28.57) for the anatomical section of epidermal layers following our study published earlier[17].

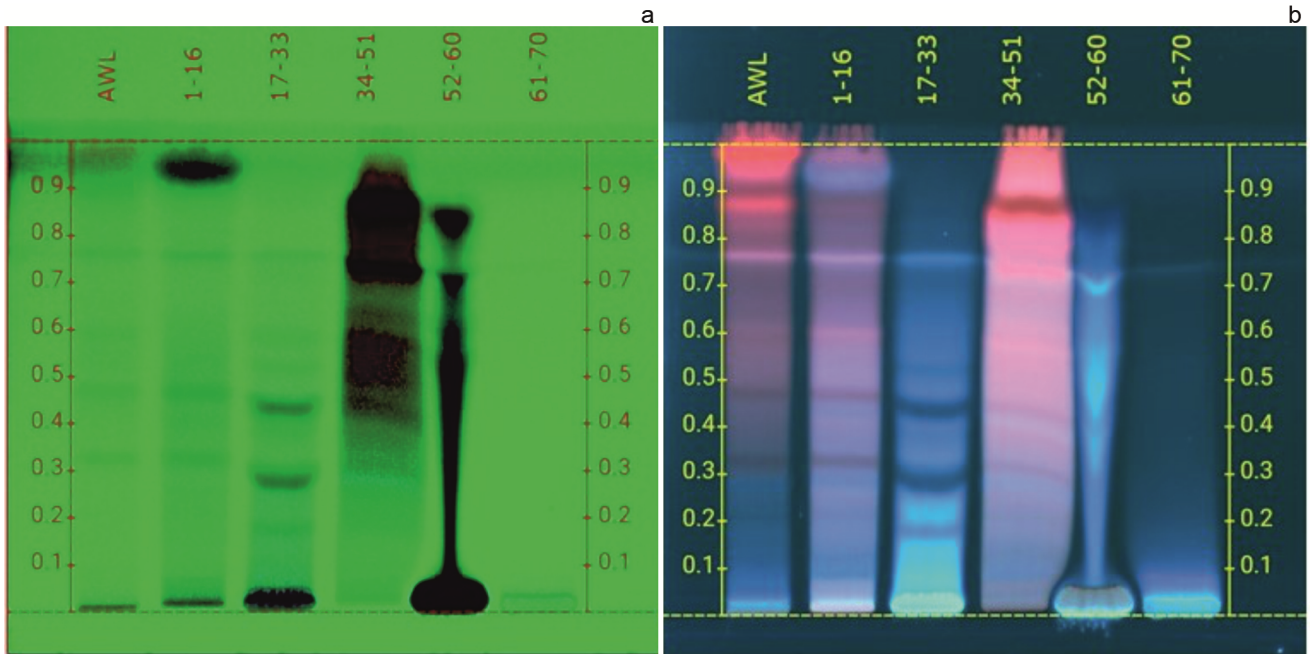

Fig. 4 — HPTLC chromatograms of *Allium wallichii* Kunth hydroalcohol extract and different fractions obtained after column chromatography using methanol: ethyl acetate: water and formic acid (1.4: 7.1: 1: 0.1 v/v). Where Plate a was detected in UV-254 nm and plate b was detected in UV-366 nm.

Table 3 — Extractive values parameters analysis of *A. wallichii* Kunth leaves

Menstruum	Consistency	Colour	Odour	Taste	Extractive values* (% w/w)
Petroleum ether	Semisolid	Green	Characteristics	Waxy slightly pungent	2.0±0.11
Chloroform	Semisolid	Dark green	Characteristics	Waxy slightly pungent	1.8±0.17
Ethyl acetate	Semisolid	Dark green	Characteristics	Waxy slightly pungent	2.4±0.20
Methanol	Semisolid	Dark green	Characteristics	Waxy slightly pungent	10.73±0.35
Ethanol	Semisolid	Light brown	Characteristics	Bitter pungent	21.13±0.40
Hydro-alcohol	Semisolid	Brown	Characteristics	Bitter pungent	21.86±0.17
Aqueous	Solid	Dark brown	Characteristics	salty pungent	25.13±0.26

*n=3 (Average + Standard Error Mean)

Preparation of extracts and phytochemical investigations

The extract was prepared by the maceration method, and the resultant extract was tested through various chemical tests. The hydro-alcohol extract contained different classes of secondary metabolites, as shown in Table 4.

Column chromatographic studies of leaf extract

Column chromatography resulted in 70 separated fractions (15 mL each), which were further chromatographed by TLC to identify a similar pattern of phytoconstituents. After that fractions possessing similar phytochemicals bands were mixed to get a total of 5 fractions, i.e. mixing of fractions 1 to 16, mixing of fractions 17 to 33, mixing of fractions 34-51, mixing of fractions 52-60, and mixing of fractions 61-70, respectively. All five fractions again confirm the presence of various phytoconstituents through HPTLC.

Table 4 — Chemical test on *A. wallichii* Kunth leaves

Chemical Test	Observation
Alkaloids	Not found
Tannins	Not found
Flavonoids	Present
Reducing Sugars	Present
Coumarins	Not found
Glycosides	Present
Quinone	Not found
Steroids	Present
Terpenoids	Present
Saponins	Not found

HPTLC fingerprinting of leaf extract

The chromatographic pattern through high performance thin layer chromatography of hydro-alcohol extract and all five fractions were presented in Fig. 4. As evidenced from the Fig. 4, both

hydroalcoholic extracts of *A. wallichii* and its fraction contains several phytoconstituents, mainly phenolics and can be a source for delivering requisite action like a drug.

Conclusion

Herbs are gaining significant attention worldwide due to the long historical traditional background of safety and efficacy. In this context, many herbs, including *A. wallichii*, still need to be explored. Therefore, the current studies were performed to establish standard parameters as a distinct feature for identification and setting authenticity of *A. wallichii* in the near future for trade and industry standards. These standards value may further support the evaluation of the purity of drugs to act as a reliable standard for selecting and identifying raw materials of optimum quality for industry production and setting standards for herbal monographs.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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