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Pharmacognostical and standardization parameters of *Artemisia roxburghiana* Wall. ex Besser (Aerial parts)

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Abstract

The present study was undertaken with an objective to investigate the pharmacognosy of the aerial parts *Artemisia roxburghiana*, an ethno-medicinally important plant belonging to sunflower family (*Asteraceae*). Pharmacognostic evaluations were conducted in terms of macroscopic, microscopic, physicochemical and phytochemical analysis according to the WHO and other recommended procedures. Physicochemical investigation of plant point up that the total ash, acid insoluble ash, water soluble ash, sulphated ash values and loss on drying re 6.26%, 4.0%, 5.46%, 5.66% and 0.30%, respectively. In the present study it is noted that the extractive value of water is similar to the organic solvent investigated. Through the preliminary phytochemical assessment of the crude extracts indicated presence of metabolites like glycosides, flavonoids, phenols, saponins, steroid, which are responsible for pharmacological properties.

Pharmacognostical characters, when stem cut the section of petiole following feature are observe like vascular bundles, xylem, phloem, cambium cell, upper epidermis, lower epidermis & polygonal cells. Pluricellular covering trichomes and clavate trichomes are important diagnostic feature found in *A. roxburghiana*.

Keywords: *Artemisia roxburghiana*, Kunja, standardization, physicochemical, phytochemical

1. Introduction

Aromatic and medicinal plants are important sources of secondary metabolites, which have a wide range of applications in control of plant and human diseases, cosmetics, as well as in the pharmaceutical industry [1].

Artemisia, a large genus of the Anthemideae, and primarily produces many of sesquiterpene lactones, along with coumarins and acetylenes which have been evaluated for potent antibacterial, anti-inflammatory, antifeedant, cytostatic and antimalarial properties [2].

Artemisia has different vernacular name in India, Hindi: Nagadona, Jangali Ajwain, Sanskrit: Nagadaman, Garhwali: Kunja. [3]. *Artemisia* is a wind pollinated cosmopolitan genus, mainly distributed in temperate areas of mid to high latitudes of the northern hemisphere, colonizing in arid and semiarid environmental landscape, with only a few representatives in the southern hemisphere. Central Asia is its center of diversification, while the Mediterranean region and North West America are two secondary speciation areas [4, 5]. The genus is distributed worldwide, mainly across the temperate zones of the Northern Hemisphere, some species are found in the Southern Hemisphere. [6]. In India this plant is endemic in the sub alpine regions of Himachal Pradesh and Uttarakhand. *Artemisia roxburghiana* Besser var. *purpurascens* (Jacq.) Hook, is one of the important species of this genus that has been effectively used as an anti-pyretic tonic and for the treatment of skin allergies [7].



Fig 1a: Inflorescence of *Artemisia roxburghiana*



Fig 1b: Leaves of *Artemisia roxburghiana*

The plant grows as a perennial herb in Himalayas at heights of 1,000–4,300 m, with several or single, upright or ascending, 20 – 50 (-70) cm, sulcate, whitish hairy, often purplish tinged stems from horizontally creeping, c. 1 cm thick rootstock. Leaves with short winged petiole to almost sessile, oblong-ovate to almost broadly elliptic rotund, 5 – 18 x 3 – 10 cm, rachis without lobules, very sparsely hairy and green above, densely greyish-white arachnoid beneath, pinnatisect into obliquely patent, oblong primary lobes with lanceolate, 4 – 5 x c. 2 cm, acute-acuminate secondary segments; middle stem leaves auricled at the bases; uppermost in the floral region linear-lanceolate, entire or uni pinnatisect. Capitula heterogamous, sessile or very short peduncled, narrowly ± campanulate, 3 – 4 x 2 – 3 mm, erect to ± patent, solitary or 2 – 3 together in dense glomerulus, in narrowly spicate-racemose, up to 15 x 2 – 3 cm panicle with short, erecto-patent, 2.5 – 4.5 cm long branches. Involucre 4-seriate, phyllaries arachnoid hairy outside, purplish tinged; outermost ovate, 1.75 – 2 x 1 – 1.25 mm, narrowly membranous margined, acute; inner ones oblong-elliptic, c. 3 x 1 mm, obtuse, broadly scarious hyaline margined. Receptacle glabrous, ± hemispherical. Florets 20 – 38, usually purple-brown tinged, all fertile; marginal florets 5 – 8, with filiform, c. 1.25 mm long, 2-dentate corolla and long exerted style branches; disc-florets 15 – 30, with narrowly campanulate, c. 1.75 mm long, 5-toothed glandulosa corolla. Cypselas brown, c. 1.25 mm long.^[8]

The chemistry of this species is less explored compared to other species^[9, 10], leaving ample scope for further research on the phytochemistry and pharmacology of this plant. Till date, there is no report on the pharmacognostical evaluation of aerial parts of *A. roxburghiana* from Garhwal Himalaya. Therefore, the aim of the present study is to explore various pharmacognostical parameters of the herb, for its proper validation so that it cannot be easily depreciated.

2. Materials and Methods

2.1 Collection and Authentication of Plant Material

The plant *Artemisia roxburghiana* was collected from district of Rudraprayag { Kedarnath (Gaurikund) } (Latitude: 30° 39' 0.00" N, Longitude: 79° 01' 0.00" E and Altitude is about 3,583 m (or 11,755 ft) above sea level 18.00 m), Uttarakhand, India.. Identity of the plant was confirmed through Forest Research Institute, (Systematic Botany Discipline Botany Division) Dehradun, India. Voucher specimen of the plant (Voucher Number. Dis \550 \2016 \Syst. Bot. \Rev. Gen. \4-5) was deposited at the same Institute. The aerial parts of *Artemisia roxburghiana* was washed under running tap water, rinsed with distilled water, dried in shade at room temperature for two weeks. Subsequently dried plant parts, were kept in airtight containers, to free from moisture and humidity until further study.

2.1.1 Organoleptic (Sensory) Parameters

Different organoleptic (sensory) parameters of leaf and leaf powder such as colour, odour, taste and texture of dried leaf powder were evaluated by the sense organs and recorded.

2.1.2 Anatomical Studies- Transverse Sections

For anatomical studies, the required samples of stem and leaf were cut and removed from the plant and instantaneously fixed in FAA (formalin- 5 ml + acetic acid- 5 ml + 70% Ethyl alcohol- 90 ml). Further the specimens were left in the preservative for two days; and were washed in distilled water and processed. Standard microtome techniques were followed for anatomical investigation^[11]. Transverse sections were prepared and stained with reagent^[12]. Photomicrographs were taken using digital camera.

2.1.3 Anatomical Studies- Powder Microscopy

Pre-treatment: The cleaned sample is pulverized. The powder should pass through a No. 4 sieve (average internal diameter of aperture: 250 ± 9.9 µm) to obtain fine granules.

Mounting: Place the powdered material on the slides. Add 1-3 drops of testing agents; if necessary stir with a fine pointed needle to distribute testing agent evenly. Add cover slip. Remove any excess liquid that may exude from under the cover slip by blotting around its edges gently with filter paper^[13].

2.1.4 Preliminary Phytochemical Analysis

Powdered *A. roxburghiana* samples were successively extracted with petroleum ether, chloroform, ethyl acetate and ethanol. The extracts were filtered and concentrated using vacuum distillation. For the identification of various phytochemical constituents, the different extracts were subjected to qualitative tests as per the standard procedure^[14-17].

2.1.5 Physico-Chemical Evaluation

Physicochemical parameters such as foreign matter, moisture content, pH, ash constants and soluble extractive values were performed according to the official method prescribed and the WHO guidelines on quality control methods for medical plants material^[18].

2.1.6 Statistical analysis

All experiments were carried out in triplicate. Results are reported as mean ± standard error of the mean.

3. Results

3.1 Macromorphological Evaluation of *A. roxburghiana*

Organoleptic and macroscopic characteristics of *A. roxburghiana* are tabulated in Table 1 Fresh plant has bitter taste with distinctive odour. Dried drug powder is buff brownish grey in color with characteristic odour.

3.2 Anatomical Studies

In *Artemisia*, cells had irregular sinuous anticlinal walls. Different combinations of stomata occur on the same surface of the leaf. The basic stomatal type is adaxial anomocytic type. In addition to, anisocytic, diacytic, tri, tertacytic stomata are recorded. Pharmacognostical characters, when stem cut the section of petiole following feature were observed like vascular bundles, xylem, phloem, cambium cell, upper epidermis, lower epidermis & polygonal cells. Pluricellular covering trichomes and clavate trichomes are important diagnostic feature found in *A. roxburghiana* leaves.

Powder microscopy of *A. roxburghiana* showed the presence of fragments of thin walled cell, starch grains, lignified

sclerenchymatous cells, calcium oxalate crystals, aleurone grains and oil globules.

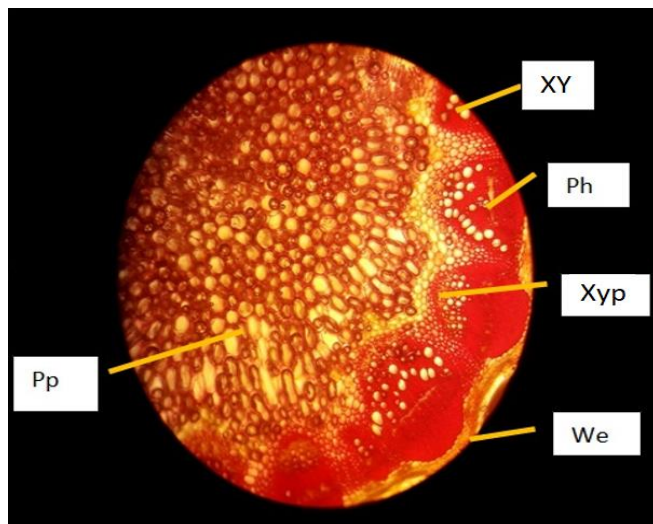


Fig 2A: Transverse section of stem of *Artemisia roxburghiana* (Section representing xylem, phloem, xylem parenchyma, pith parenchyma and wavy epidermis)

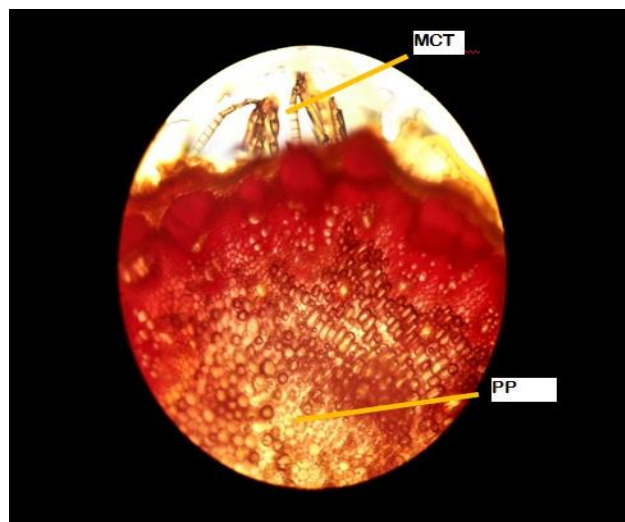


Fig 2B: Transverse section of stem of *Artemisia roxburghiana* (Section representing multicellular covering trichomes and pith parenchyma)

Legend = Xy- xylem, Ph-phloem, Xyp-xylem parenchyma, Pp-pith parenchyma, We-wavy epidermis, Mct- multicellular trichomes, Pp-pith parenchyma

3.3 Preliminary phytochemical analysis

The results of the study showed that depending on the polarity of the solvents, there was wide variation in the presence of the type of secondary metabolites observed (Table 2). It has been observed that in comparison to other solvent extracts, hydro

alcoholic extracts of aerial parts of *A. roxburghiana* contained a greater variety of secondary metabolites like, Flavonoids, Phenol, and Saponins in higher quantity.

3.4 Physicochemical Evaluation

Air-dried powdered material was used for quantitative determination of different physicochemical evaluation. The results pertaining to these investigations are summarized in Table 3. Aerial parts of *A. roxburghiana* were freshly collected hence there was no adherent inorganic matter. Thus the percentage of foreign matter was found to be nil. The physical constant investigation of the drugs is an important parameter in detecting adulteration or improper handling of drugs.

Table 1. Macroscopic/organoleptic characteristics of *Artemisia roxburghiana* (Aerial parts)

S. No.	Macroscopic characteristics	Inference
1.	Colour	Upper surface dark greenish and lower surface grayish green
2.	Odour	Aromatic
3.	Taste	Bitter
4.	Shape	Pinnate
5.	Size	1-3cm
6.	Habit	Shrub
7.	Margin	Ovate

Table 2. Phytochemical screening of various extracts of *Artemisia roxburghiana* (Aerial parts).

S. No.	Phytochemical tests	Petretether extract	Chloroform extract	Ethyl-acetate extract	Hydro alcoholic extract
1.	Carbohydrate	-	-	-	-
2.	Fats & oils	-	-	-	+
3.	Protein & amino acid	-	-	-	+
4.	Glycosides	-	+	-	+
5.	Phytosterol	-	-	--	-
6.	Alkaloids	-	-	-	-
7.	Flavonoids	+	+	-	+
8.	Phenols	+	-	+	+
9.	Saponin	+	-	+	+

Table 1. Physiochemical parameter of *Artemisia roxburghiana* (Aerial parts)

S. No.	Parameter	% value(w/w)
1	Loss on drying	0.30%
2	Total ash	6.26%
3	Acid insoluble	4.0%
4	Water soluble	5.46%
5	Sulphated ash	5.66%
6	Alcohol soluble (Cold maceration)	7%
7	Alcohol soluble (Hot extraction)	2.25%
8	Water soluble (Cold maceration)	7.25%
9	Water soluble (Hot extraction)	2.00%
10	Petroleum ether	5.5
11	Chloroform	3.4
12	Ethyl acetate	2.8
13	Hydro alcoholic	8.6

4. Conclusions

According to the World Health Organization, the macroscopic and microscopic description of a medicinal plant is the first step towards establishing the identity and the degree of purity of such materials and should be carried out before any tests are undertaken. Pharmacological studies are more reliable, accurate and inexpensive means to evaluate the plant drugs [19]. So in the current study essential diagnostic characters determining validity and purity of the medicinally important aerial parts of the plant were observed and recorded. Therefore, the result produced from this study would be handy in identification and standardization of the plant material towards quality assurance and also for preparation of a monograph on *A. roxburghiana*

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