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Pharmacognostic Standardization and Phytochemical Evaluation of *Ailanthus altissima* (Mill.) Swingle leaves

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ABSTRACT

The present study was focussed to carry out the detailed pharmacognostic studies in terms of organoleptic characters, physicochemical parameters, macroscopical, microscopical characters and phytochemical screening for major groups of compounds and other WHO recommended parameters for standardization of the leaves *Ailanthus altissima* (Mill.) of family Simaroubaceae. Phytochemical screening of methanolic and aqueous extracts of leaves of *Ailanthus altissima* demonstrated the presence of Carbohydrates, flavonoids, tannins, cardiac glycosides, phenols, saponins, diterpenes, fats and oils. However alkaloids and anthraquinone glycosides were found absent in all the extracts. Information obtained from phytochemical and pharmacognostical analysis can be used as markers in the identification and standardization of this plant for monograph development and as herbal remedy.

Keywords: Tree of heaven, Phytochemical, Pharmacognostic analysis, Bioactive compounds, *Ailanthus altissima*.

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INTRODUCTION

Description

Ailanthus altissima commonly known as smoke tree or tree of heaven belongs to family simaroubaceae. The plant is locally known as Handoon or Alamthar in Kashmir. *A. altissima* is a medium-sized tree that reaches heights between 17 and 27 metres with a diameter at breast height of about 1 metre [1]. The bark is smooth and light grey. The twigs are stout, smooth to lightly pubescent, and reddish or chestnut in colour. They have lenticels as well as heart-shaped leaf scars with many bundle scars around the edges. The buds are finely pubescent, dome shaped, and partially hidden behind the petiole [2]. The branches are light to dark gray in colour, smooth, lustrous, and containing raised lenticels that become fissures with age. The ends of the branches become pendulous. All parts of the plant have a distinguishing strong odour that is often likened to peanuts, cashews or rotting cashews [2,3,4]. The leaves are large, odd- or even-pinnately compound, and arranged alternately on the stem. They range in size from 30 to 90 cm in length and contain 10-41 leaflets organised in pairs, with the largest leaves found on vigorous young sprout. When they emerge in the spring, the leaves are bronze then quickly turn from medium to dark green as they grow [5]. The rachis is light to reddish-green with a swollen base. The leaflets are ovate-lanceolate with entire margins, somewhat asymmetric and occasionally not directly opposite to each other. Each leaflet is 5 to 18 cm long and 2.5 to 5 cm wide. They have a long tapering end while the bases have two to four teeth, each containing one or more glands at the tip. The leaflets' upper sides are dark green in colour with light green veins, while the undersides are a more whitish green. The petioles are 5 to 12 mm long. The flowers are small and appear in large panicles up to 50 cm in length at the end of new shoots. The individual flowers are yellowish green to reddish in colour, each with five petals and sepals [1,2]. They appear from mid-April in the south of its range to July in the north. *A. altissima* is dioecious, with male and female flowers being borne on different individuals. Male trees produce three to four times as many flowers as the females, making the male flowers more conspicuous. Furthermore, the male plants emit a foul-smelling odour while flowering to attract pollinating insects. Female flowers contain ten (or rarely five through abortion) sterile stamens (stamenoides) with heart-shaped anthers. The fruits grow in clusters; a fruit cluster may contain hundreds of seeds. The seeds borne on the female trees are 5 mm in diameter and each is encapsulated,

appearing July through August, but can persist on the tree until the next spring [6].

Distribution

A. altissima is native to subtropical/warm temperate climates but is able to invade climates ranging from cool temperate to tropical. In South Africa it invades forest margins, roadsides and riverbanks in cool, moist regions unlike other members of the genus *Ailanthus*, it is found in temperate climates rather than the tropics. The tree was first brought from China to Europe in the 1740 and to the United States in 1784. It has become an invasive species due to it's due to its ability both to colonise disturbed areas quickly and to suppress competition with allelopathic chemicals. It is considered a noxious weed in Australia, United States, New Zealand and many countries of easten and southern Europe [7].

Traditional uses

Ailanthus altissma has been traditionally used in Chinese folk medicine as an astringent, antispasmodic, antihelminthic, parasiticide and narcotic [8,9]. Fresh stem bark was used to treat diarrhoea and dysentery; root bark for heat ailments, epilepsy, and asthma; fruits as an emmenagogue and to treat ophthalmic diseases; and leaves as astringent used in lotions for seborrhoea and scabies [8]. Recent pharmacological studies indicate a broad array of possible uses of quassinoids, for example to treat malaria. Epstein–Barr-virus infection and HIV infection. In addition, Ailanthus is used for homoeopathic remedies [10,11,12,13].

MATERIALS AND METHODS

Plant Material Collection

In the present study, the fresh leaves of the plant *Ailanthus altissima* were collected from Botanical Garden, University of Kashmir, Srinagar, Kashmir, at an altitude of 1585m in the month of August. The plant was identified and authenticated by Prof. Akhtar H. Malik, KASH Centre for Biodiversity and Taxonomy University of Kashmir under specimen voucher number 2322-KASH. A sample specimen of collected material was deposited in herbarium for future reference.

Macroscopic and microscopic analysis

Macroscopic and microscopic analysis of the leaves of *Ailanthus altisimma* was studied according to the method mentioned in the Trease and Evans pharmacognosy [14,15].

Preparation of extracts

After collection and authentication, leaves of the plant material were shade dried and powdered. The powdered sample was then passed through sieve no. 40 and subjected to extraction. A weighed quantity of the powdered drug was extracted in a soxhlet with methanol. Aqueous extract was prepared by decoction method. The extracts were evaporated to dryness under reduced pressure and controlled temperature 40-50ºC [12]. The extracts were then kept in desiccators to remove remaining moisture, and finally stored in air tight containers at 4ºC for further use.

Physicochemical analysis

The leaves were shade dried and powdered for the determination of physicochemical parameters like ash value, extractive values, Flourescence analysis study of powdered drug material reagents was carried out to observe the color reactions. The drug was treated with various chemical reagents and exposed to visible and ultra violet light to study their fluorescence behaviour[16,17].

The methanolic and aqueous extracts so obtained were subjected to preliminary phytochemical screening. Phytochemical studies were performed to identify the presence of various constituents according to the standard methods [18,19,20].

RESULTS AND DISCUSSION

Macroscopic characters

The leaves are large, odd or even pinnately compound, and arranged alternately on the stem. They range in size from 30 to 90 cm in length. The leaflets are ovate-lanceolate with entire margins, somewhat asymmetric and occasionally not directly opposite to each other.

Microscopic characters

The microscopic characters of the leaves reveals the presence of epidermal cells, collenchyma, spongy mesophyll, palisade cells, covering trichomes, glandular trichomes, xylem and phloem vessels (Figure 1). The powder microscopy of leaves reveals the presence of epidermal cells, paracytic stomata, palisade cells, covering trichomes and crystals of calcium oxalate (Figure 2).

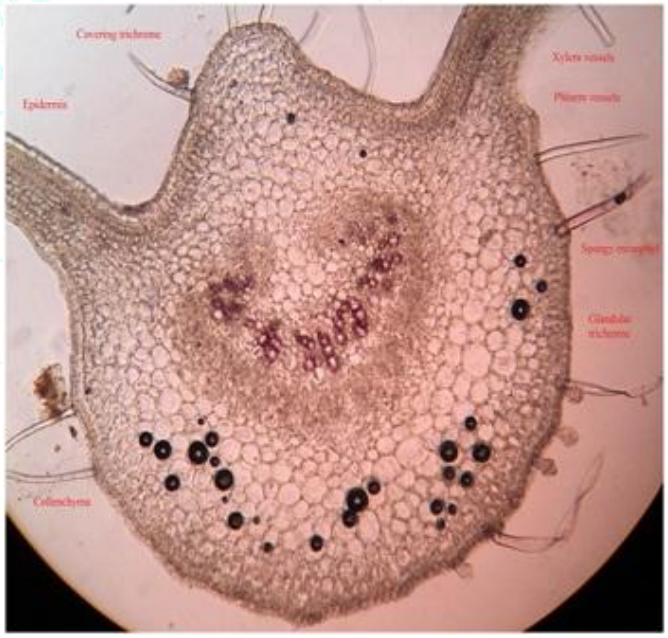
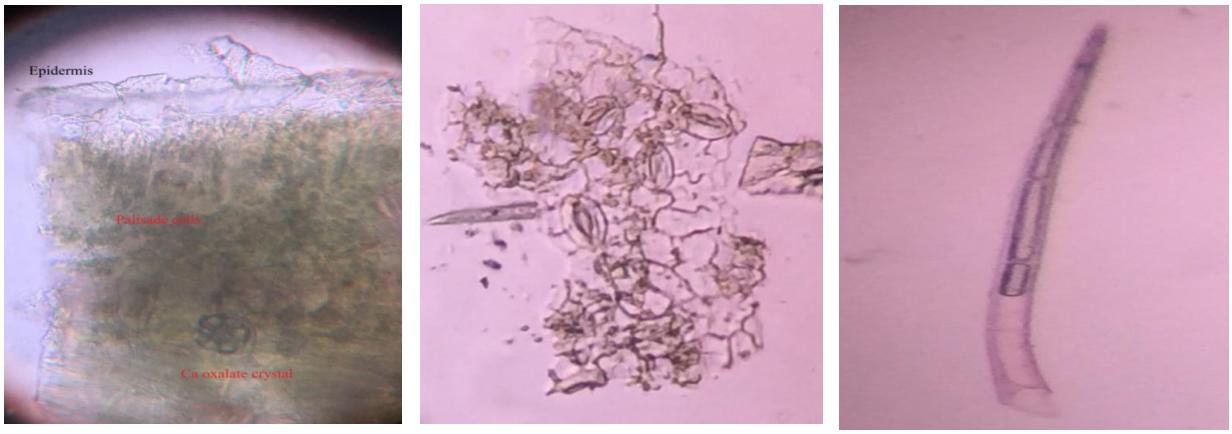


Figure 1: Transverse section of leaf of *Ailanthus altissima* showing epidermis, collenchyma, spongy mesophyll, Xylem vessels, phloem vessels, covering trichrome, glandular trichrome and ground tissue



(a) (b) (C)

Figure 2: Microscopic structures of leaf of *Ailanthus altissima (*a): Epidermis with palisade cells and calcium oxalate crystals; (b): Paracytic stomata; (c): Covering trichomes

Physicochemical analysis

Various physicochemical parameters like ash value, extractive values, Flourescence analysis were determined and are shown in Table- 1, 2 and 3 respectively.

Table 1: Extractive values of metanolic and aqueoua extracts *of Ailanthus altissima*

Extractive value	Solvent	Leaves
Cold extractive value	Petroleum ether	0.3325%
	Chloroform	0.8625%
	Methanolic	3.1325%
	Ethylacetate	1.72%
	Aqueous	3.865%
Hot extractive value	Petroleum ether	18.764%
	Chloroform	20.325%
	Methanolic	21.376%
	Ethylacetate	19.487%
	Aqueous	23.235%
Successive extractive value	Petroleum ether	0.448%
	Chloroform	0.986%
	Methanolic	3.436%
	Ethylacetate	1.698%
	Aqueous	3.423%

Table 2: Ash values and other physicochemical constants of leaves of *Ailanthus altissima*

Physico-chemical Parameters	Values
pH 1% solution pH 10% solution	4.7 4.1
Loss on drying at 105°C	6.651%
Swelling index	2
Total Ash value (% *w/w*)	4.37%
Foaming index	

Table 3: Flourescence analysis of leaves of *Ailanthus altissima*

Treatment	Day light	Ultraviolet (254nm) Short wavelength	Ultraviolet (266nm) Long wavelength
Powder as such	Green	Green	Light green
Powder with distilled water	Green	Green	Light green
Powder with Iodine	Blackish green	Light green	Fluorescent green
Powder with $FeCl_3$	Dark green	Blackish green	Green
Powder with Picric acid	Green	Greenish black	Fluorescent green
Powder with NaoH	Green	Green	Light green
Powder with $CHCl_3$	Fast green	Fast green	Green
Powder with Ethylacetate	Green	Light green	Greyish green
Powder with Concentrated HNO_3	Light green	Orange	Black

Powder with concentrated Hcl	Green	Greyish green	Brown
Powder with $K_2Cr_2O_7$	Orange	Black	Fluorescent green
Powder with Pet. ether	Green	Green	Light green
Powder with Methanol	Green	Dark green	Green
Powder with Concentrated H_2SO_4	Black	Black	Dark green
Powder with ammonia	Green	Light green	Light green
Powder with concentrated Hcl and distilled water	Green	Light green	Green
Powder with concentrated H_2SO_4 and distilled water	Black	Black	Brown
Powder with concentrated HNO_3 and distilled water	Light green	Brown	Black
Powder with GAA	Brown	Olive green	Light green
Powder with alcoholic KOH	Green	Light green	Light green

Phytochemical Screening

In the preliminary phytochemical screening of methanolic and aqueous extracts of *Ailanthus altissima* secondary metabolites like carbohydrates, tannins, flavonoids, phenols, phytosterols, proteins, saponins, diterpenes, cardiac glycosides, anthraquinones and alkaloids were tested. Phytochemical screening of methanolic and aqueous extracts of leaves of *Ailanthus altissima* demonstrated the presence of Carbohydrates, flavonoids, tannins, cardiac glycosides, phenols, saponins, diterpenes, fats and oils. However proteins, alkaloids and anthraquinone glycosides were found absent in all the extracts and phytosterols were also found absent only in aqueous extract. (Table-4).

Table 4: Preliminary phytochemical analysis of metanolic and aqueoua extracts *of Ailanthus altissima*

Tests	**Inference**	**Extracts**	
		Methanolic	**Aqueous**
Carbohydrates			
Molisch's test	Violet ring	+	+
Fehling's test	Brick red ppt.	+	+
Benedict's test	Orange red ppt.	+	+
Tannins			
5% $FeCl_3$ test	Yellow color	+	+
Lead acetate test	White ppt.	+	+
Gelatin test	White ppt.	+	+
Flavonoids			
Shinoda test	Pink color	+	+
Alkali reagent test	Intense yellow color Which becomes colorless on addition of dil. acid	+	+
Lead acetate test	Yellow color ppt.	+	+
Phenols			
1% $FeCl_3$	Bluish color	+	+
Phytosterols			
Salkowski test	Golden yellow ring at junction	+	_
Libermann's test	Brown ring at junction	+	_
Proteins			
Xanthoproteic test	Yellow color	_	_
Biuretic test	Blue color	_	_
Saponins			
Foam test	Foaming	+	+
Ftoth test	Frothing	+	+
Diterpenes			
Copper acetate test	Emerald green color	+	+
Cardiac glycosides			
Keller killiani test	Brown ring at junction	+	+
Legal test	Pink color	+	+
Alkaloids			
Mayer's test	Cream ppt.	_	_
Hager's test	Yellow ppt.	_	_
Dragendroff's test	Orange ppt.	_	_
Wagner's test	Reddish brown ppt.	_	_

CONCLUSION

From the present study, it was concluded that *Ailanthus altissima* showed the presence of a number of active secondary metabolites such as carbohydrates, tannins, flavonoids, phenols, phytosterols, saponins, diterpenes, cardiac glycosides. From the results, it was noted that the extracts of *Ailanthus altissima* were found to be a rich source of variety of active secondary metabolites. The leaf constants also play the major role for the standardization of plant and one of the backbone of microscopical evaluation. Information obtained from above study can be used as markers in the identification and standardization of this plant or herbal drugs which provide the healthy and maximum potent drug in the market.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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