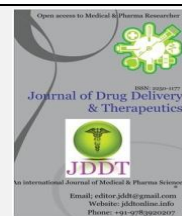




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Research Article

## Morphoanatomical and physicochemical studies on *Ailanthus excelsa* roxb. stem bark: a tree of heaven

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### ABSTRACT

*Ailanthus excelsa* Roxb. (Simaroubaceae) is a traditional medicinal plant used widely in India and China in various health conditions. The morphology and microscopical evaluation are most preferred quality control parameter, in order to establish its quality and purity, we report some important pharmacognostic profile of *A. excelsa* stem-bark for the purpose of its identification and differentiation from related species. The study of the fresh, powdered and anatomical sections of the stem bark were carried out to determine the morphological, microscopical, some physicochemical and phytochemical parameters. Presence of lignified multicellular trichome, stone cells, scleroids, lignified pericyclic fibre, phloem fibre, prismatic calcium oxalate, starch grains and uni to multiserrate non-lignified medullary rays observed as distinguishing microscopical characteristics in transverse section and powder studies. The result of preliminary phytochemical screening indicated presence of alkaloids, glycoside, steroids, carbohydrates, proteins, phenolic compounds, tannins, flavonoids and saponins. In addition, quantitative phytochemical analysis revealed significant amount of total phenolic and flavonoid content. The present study will be useful for its identification prior to carrying out further research work.

**Keywords:** *Ailanthus excelsa*; stem-bark; pharmacognostic; quantitative phytochemical analysis.

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### INTRODUCTION

*Ailanthus excelsa* Roxb. is a deciduous tree belonging to the family Simaroubaceae and is widely distributed in Asia. Its native origin is China and it is known as 'Tree of Heaven' and used in the Indian system of medicine for variety of purposes.<sup>1</sup> It is used in wounds, skin eruption, febrifuge, bronchitis, asthma and in conditions of diarrhea and dysentery.<sup>2,3</sup> Tribals in Nilgris region traditionally used it in antifertility, fever and been scientifically evaluated.<sup>4,5</sup> Previous phytochemical studies with *A. excelsa* have demonstrated the presence of quassinoids,<sup>6,7</sup> alkaloids, terpenoids.<sup>8,9</sup> *A. excelsa* extracts and some isolated compounds have demonstrated pharmacological properties such as significant antileukemic,<sup>10</sup> antifungal, antibacterial,<sup>11,12</sup> and antimalarial.<sup>13</sup> Phytochemically, it found to contains  $\beta$ - Sitosterol, Quassinoids, Ailanthic Acid, vitexin; ailanthione, glalucarubinone, malanthine, excelsin, glaucarubol, 2-6 Dimethoxy-Benzoquinone and Melanthin.<sup>14</sup> Though the plant species could be easily distinguished on the basis of the flowers, it becomes very difficult when the crude drug is in the form of dried and cut pieces. Therefore, present investigation was planned to have a detailed study

on its pharmacognostic, physicochemical parameters and preliminary phytochemical investigation with regards to its identification, characterization and standardization of plant *A. excelsa*.

### MATERIAL AND METHODS

The *A. excelsa* stem bark was collected from Shirpur and nearby places, Dhule district of Maharashtra, and positively authenticated at Taxonomy department, SSVPS College, Dhule, Maharashtra. The voucher specimen and raw drug were deposited at Pharmacognosy department herbarium library and raw drug depository, respectively. The bark was washed, cleaned, shade dried, powdered, and passed through a 40-mesh sieve. It was stored in a tightly closed container.

### Macroscopy

The bark was separated from other parts, washed, cleaned and dried for further use. The stem bark of *A. excelsa* was subjected to macroscopic studies which comprised of organoleptic characteristics viz. color, odour, appearance, taste, shape, touch, texture, fracture, etc (Figure 1). of the drug. These parameters are considered as quite useful in

quality control of the crude drug and were evaluated as per standard WHO guidelines.<sup>15</sup>

### Microscopy

Fresh barks of *A. excelsa* were utilized for the microscopical studies. The free hand thin transverse sections were taken and examined microscopically for distinguishing cell arrangement (Figure 2). Furthermore, small quantity of the powdered leaves was cleared, mounted, and observed for diagnostic powder characteristics histochemical reactions were applied with staining reagents on transverse sections and on bark powder by reported methods<sup>16</sup> (Figure 3). Photomicrographs of the microscopical sections and powder were taken with the help of MOTIC photomicroscope provided with Motic Images plus 2.0 software.

### Physicochemical and phytochemical analysis

Physicochemical parameters such as percentage of ash, extractive values, foreign matter content, loss on drying and proximate chemical analysis as per Kalaskar and Surana<sup>16</sup> (Table 1 and 2). In addition, the total phenolic and flavonoid content of the extracts was determined by the already method.<sup>17,18</sup> The mean of three readings was used and the total phenol content and total flavonoid content was expressed in milligram of gallic acid equivalents/g extract and quercetin equivalents/g extract, respectively (Figure 4; Table 3).

## RESULTS

### Macroscopic features

The bark has a thickness of about 2 to 4 mm and yellowish outside and creamish yellow inside. The bark has soft, brittle, and rough surface, with horizontal and vertical wrinkles, curved, the young bark was smooth to touch, fracture was found to be splintery and laminated and exfoliating. The

stem bark showed typical scars remain after leaf detachment (Figure1).

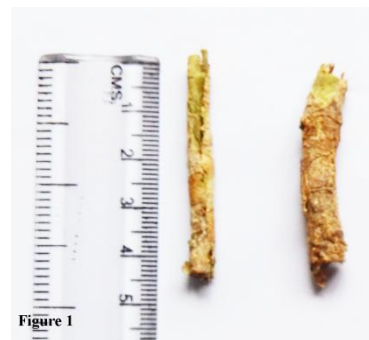


Figure 1: Dried stem bark of Ailanthus

### Microscopical characteristics

Bark differentiated into outer bark as periderm and inner bark or secondary phloem. Periderm well differentiated into phellem, phellogen and phelloderm. Phellem zone was about 7-8 layers of tangential compressed cells, rectangular with thicker cell wall. The phellem cell was dead and suberized and even in transsection, phellem showed the presence of multicellular uniseriate lignified covering trichome. Phellogen layer was found to present below phellem. The phellogen is 3-5 layered, dead, cellulosic cell walled, and rectangular cells. Two -three layers of lignified stone cells present below the phellogen, they are smaller in size compared to other stone cells present in phelloderm. The phelloderm layer composed of multiple layers of polygonal parenchymatous cells. Large scleroids in the group of 8-20, scattered in the phelloderm layer. The 3-4 layers of lignified pericyclic fibers present at the end of phelloderm, and above the secondary phloem i.e. in between outer bark and inner bark.



Figure 2: Transverse section of *A. excelsa* stem bark a) phellem b) phellogen c) lignified stone cells layer d) phelloderm f) scleroids h) pericyclic fibre i) phloem fibres j) medullary rays g) lignified trichomes.

The phloem was differentiated into inner intact non-collapsed zone and outer collapsed phloem zone. The outer collapsed phloem region showed the wider medullary rays with bunches of lignified phloem fibres, while, in non-collapsed phloem region the phloem fibres are few and scattered in small clusters. The phloem regions has groups of sieve tubes associated with companion cells and phloem parenchyma. The medullary rays are uniseriate to multiseriate. Prismatic calcium oxalate is abundant at

cortical parenchyma, phloem parenchyma and medullary rays.

### Powder drug analysis

The microscopical powder analysis of stem bark showed presence of multicellular lignified covering trichomes, lignified phloem fibers, sclereids in the form of stone cells, prisms of calcium oxalate and abundant starch grain in parenchymatous cells observed (Figure 3).

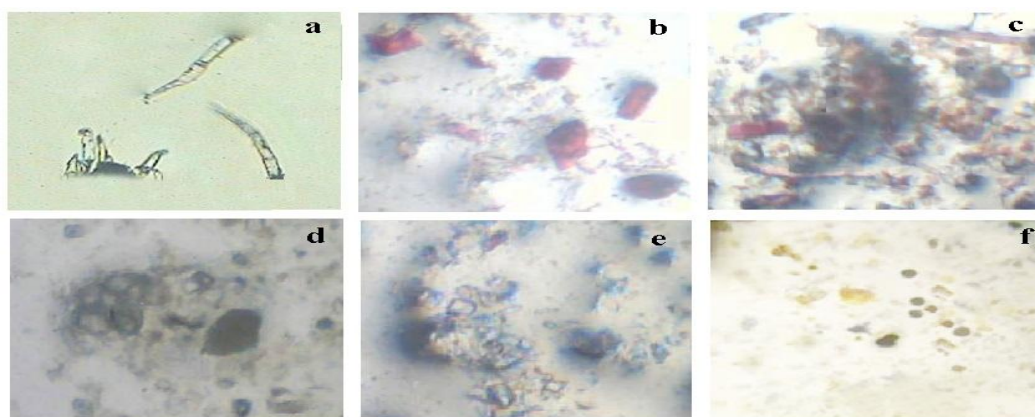


Figure 3

**Figure 3: Powder microscopy of *A. excelsa* stem bark a) multicellular uniseriate covering trichome b) scleroids and stone cells c) lignified fibres d) phellem (cork) e) calcium oxalate prisms f) starch grains**

### Physicochemical analysis:

The physicochemical standards are important to check the quality, purity and adulteration of given crude drug. The foreign matter, LOD, ash, and extractive values were determined and summarized in table 1.

**Table 1: Physicochemical analysis of *A. excelsa* stem bark.**

Types of ash value/extractive values	% w/w
<b>Ash values</b>	
Total ash	12.47 ± 0.64
Acid insoluble ash	1.19 ± 0.56
Water soluble ash	3.14 ± 0.51
Sulfated ash	5.14 ± 0.82
<b>Extractive values</b>	
Petroleum ether (40-60°)	2.12 ± 0.12
Alcohol	6.57 ± 0.94
Water	3.35 ± 0.26
<b>Foreign organic matter</b>	1.59 ± 0.33
<b>Loss on drying</b>	1.35 ± 0.48

Mean; n=3 ± SD

### Phytochemical analysis

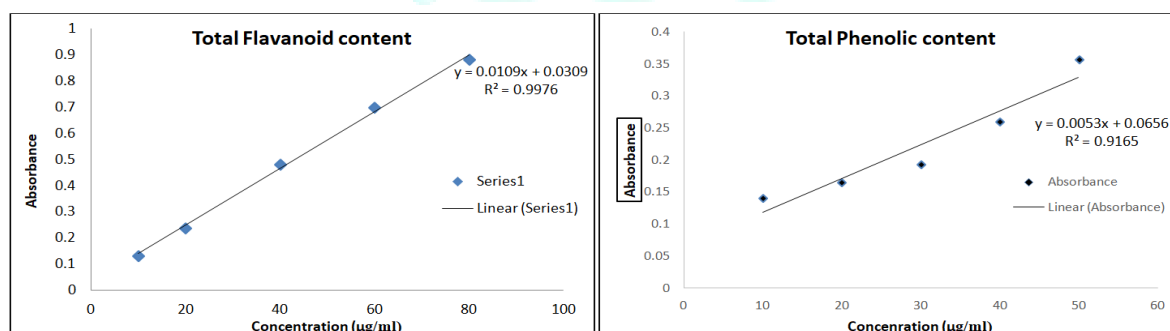
The preliminary phytochemical analysis of methanol bark extracts showed the presence of range of secondary metabolite includes carbohydrates, proteins, alkaloid, tannins, flavonoids, steroids, and saponins (Table 2).

**Table 2: Preliminary phytochemical screening of methanol extracts of *Ailanthus excelsa* stem bark**

Test	Observation
Carbohydrate	+
Protein	+
Alkaloid (General test)	+
Glycoside (General test)	-
Steroid	+
Triterpenoids	-
Coumarin	+
Flavonoids	+
Tannins and phenols	+

'+' present; '-' absent

The quantitative phytochemical analysis of bark extract showed significant amount of phenolic and flavonoid content (Table 3).



**Figure 4: Calibration curves of gallic acid for total phenolic content and quercetin for total flavonoid content**

**Table 3: Total phenolic and flavonoid content of *A. excelsa* stem bark**

Quantitative estimation	Methanol extract
Total Phenolic content	13.52 ± 0.21
Total Flavonoid content	11.46 ± 0.03

Values are mean; n=3 ± SD

### DISCUSSION

The bark is one of the important medicinal plant part, used traditionally for therapeutic purposes. The bark is very complex in structure and has the potential of containing many primary and secondary metabolites. The complex structure of the bark can be utilized for correct identification to maintain the quality and purity of the drug. According to the WHO reports, the macroscopic and microscopic



description of a medicinal plant is the first step towards establishing the identity and degree of purity and should be carried out before any other tests are undertaken. Thus, the present work was taken up with an objective to lay down detail pharmacognostical and phytochemical standards that will contribute significantly to quality control of medicinally useful *A. excelsa*.

Microscopy of *A. excelsa* showed the distinct differentiation in macroscopy and microscopy. The bark has yellowish color with very few distinct transverse and vertical ridges. Microscopically the phellogen cells are tangentially elongated, thick, and suberized. In addition, the phellogen was bordered by sclereids as stone cell layer. The large lignified scleroids groups scattered in phellogen. The broadly dilated multiserrate medullary rays towards the cortex region associated with pericyclic fiber. The phloem fibers are distinct, lignified present in bundles. The medullary rays and parenchyma showed the presence of starch and rhomboidal calcium oxalate crystals. The present macroscopic and microscopic observations of stem bark of *A. excelsa* provide useful information for quality control parameters for the crude drug.

Evaluation of physicochemical parameters is an important part in the preparation of herbal monograph. The extractive values were used to find out sum of chemical constituents present, quantitatively. The ash value is product obtained after incineration, i.e. it measures amounts of inorganic mineral present in and along with the plant part. The total ash contains the sum of all indigenous minerals such as oxalate, malate, carbonate etc. The acid insoluble ash used to determine silica or sand present in the crude drugs, it is indicative of the contamination with earthy materials. The water-soluble ash was used for the measurement of inorganic elements.<sup>19</sup> In present study, the ash value was found to be more than 5% w/w; it may be due to presence of calcium oxalate. *A. excelsa* showed lowest acid-insoluble ash value.

Loss on Drying (LOD) determines the moisture content along with volatile components present in a drug. The high amount moisture present in the crude drug may leads to hydrolysis of medicinally important secondary metabolites and thus decreases its quality and efficacy.<sup>19</sup> The final dryness of the drug and rate of moisture removal are equally important and it was observed that the moisture content in stem bark of *A. excelsa* was found to be 1.35%.

The plants are considered as biosynthetic laboratory, it involves in synthesis of variety of secondary metabolites responsible for therapeutic effect. The results of preliminary phytochemical screening of stem bark of *A. excelsa* showed the presence of carbohydrates, protein, phenolic compounds, flavonoids, alkaloids, saponins, sterols and tannins, in the methanol extract.

Furthermore, the quantitative analysis of alcoholic extract of *A. excelsa* stem bark were determined. The phenolic and flavonoid compounds act as antioxidants. These compounds are also reported to have anticancer, antimicrobial, anti-inflammatory and antiallergic activities etc.<sup>20</sup>

## CONCLUSION

The present study provides in-depth macroscopical and microscopical features, and preliminary identification and quantification of biologically active phytoconstituents which also provide pharmacopoeia standards for easy

identification of the *A. excelsa* stem bark. Hence, differentiating it from closely related species.

## ACKNOWLEDGMENT

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## CONFLICT OF INTEREST

Authors declare, there is no conflict of interest

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