



## PHYSICO-CHEMICAL, PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF *AILANTHUS EXCELSA* LEAVES

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### ABSTRACT

In the present investigation, *Ailanthus excelsa Roxb* plants were collected from Beed district, which is used widely as traditional medicine in treatment. The study was carried out to evaluate the physico-chemical, phytochemical and potential antimicrobial activity against five types of bacteria (*Staphylococcus aureus*, *Salomella typhimurium*, *Proteus vulglaris*, *Pseudomonas aeruginosa* and *B. megaterium*) and two fungi (*Aspargillus niger* and *Aspargillus flavus*) of five extracts. Cylinder plate or cup plate method was performed to assess the antimicrobial activity of five extracts. The obtained results showed a potential effect as maximum zone of inhibition was 1 mm in chloroform and acetone extract.

**Key words:** Ash value, Extractive value, Antibacterial activities, Aqueous extract, Ethanol extract, Chloroform extract, Acetone extract, Petroleum ether extract.

### INTRODUCTION

*Ailanthus excelsa Roxb.* belonging to family Simaroubaceae is commonly known as Maharukha. The traditional claims, phytochemical investigation, pharmacological evaluation and some ayurvedic formulations provide the backbone to make this tree, a plant of Heaven<sup>1</sup>. Traditionally or in Indian system of medicine, *Ailanthus excelsa Roxb.* is used in treatment of asthma, cough, colic pain, cancer, diabetes and also used as antispasmodic and bronchodilator<sup>2</sup>. *Ailanthus excelsa* is a large tree originally from China, which is known as the 'Tree of Heaven'. Different parts of this plant are used widely in traditional medicine for a variety of diseases. The bark is used as bitter, refrigerant, astringent, appetizer, antihelmintic, febrifuge, in dysentery, skin disease, troubles of the rectum, and fever due to tridosha and

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allays thirst. It is also used in gout, rheumatism, dyspepsia, bronchitis and asthma. *Ailanthus* is used to cure wounds and skin eruptions as mentioned in traditional medicine. Stem bark extracts showed potent antibacterial and antifungal activities<sup>3</sup>.

*Ailanthus excelsa* Roxb. is commonly known as Mahanimba. *Ailanthus* is a genus of trees belonging to the family of Simaroubaceae, The genus is native from east Asia south to northern Australasia. It's Hindi name is maharuk. It is a fast growing tree and is extensively cultivated in many parts of India in the vicinity of villages. It is cultivated as an avenue tree for its deep shade and can be used for anti-erosion purposes<sup>4</sup>. *Ailanthus excelsa* is a large deciduous tree, 18-25 m tall; trunk straight, 60-80 cm in diameter; bark, light grey and smooth, becomes grey-brown and rough on large trees, aromatic, slightly bitter. Leaves alternate, pinnately compound, large, 30-60 cm or more in length; leaflets 8-14 or more pairs, long stalked, ovate or broadly lance shaped from very unequal base, 6-10 cm long, 3-5 cm wide, often curved, long pointed, hairy gland; edges coarsely toothed and often lobed. *Ailanthus excelsa* is really a plant of heaven<sup>1</sup>. They are fast-growing trees growing to 25-45 m tall, with spreading branches and large (40-100 cm). The small yellow to greenish flowers are borne on branched panicles. They turn reddish later and eventually brown. They stay on the tree for a long time. The fruit is a samara drawn out into a long wing with the seed in the middle. The wood is fine grained and satiny. *Ailanthus excelsa* has the antibacterial activity against different types of bacterial strains<sup>5</sup>. The bark has been used in Asian and Australian medicine to counteract worms, excessive vaginal discharge, malaria and asthma<sup>6</sup>.

## EXPERIMENTAL

### Materials and methods

Plant material was first dried under shed and then powdered. The air dried powder was extracted in Soxhlet's assembly with acetone, petroleum ether, ethanol, distilled water and chloroform. The extract obtained in each solvent was concentrated by distilling off the solvent and evaporated to dryness. Extract obtained in each solvent was concentrated, solidified and weighed.

### Ash analysis

Ash value is helpful in determining the quality and purity of crude drug, especially in powder form<sup>7</sup>.

### Total ash

About 3 g of powdered leaves was accurately weighed and taken in a silica crucible, which was ignited and weighed. The powder was spread as a fine, even layer on the bottom

of crucible. The crucible was incinerated gradually by increasing temperature to make it dull red hot until free from carbon. The crucible was cooled and weighed. The procedure was repeated to get constant weight<sup>8</sup>.

### **Water soluble ash**

The ash obtained as described in the determination of total ash was boiled for 5 minutes with 25 mL of water. The insoluble matter was collected on ashless filter paper and washed with hot water. The insoluble ash was transferred in to silica crucible, ignited for 15 minutes and weighed. The procedure was repeated to get constant weight. The weight of insoluble matter was subtracted from the weight of the total ash. The difference of weight was considered as water soluble ash.

### **The acid insoluble ash**

The above obtained ash was boiled with 25 mL of 2N HCl for 5 min. The insoluble ash was collected on an ashless filter paper and was washed with hot water. The insoluble ash was transferred into a silica crucible, ignited and weighed. The procedure was repeated to get constant weight<sup>9</sup>.

### **Extractive value**

Extractive value of crude drug are useful for their evaluation, especially when the constituents of a drug cannot be readily estimated by any others means. Further, these values indicate the nature of constituents present in crude drug<sup>10</sup>.

### **Phytochemical analysis**

The solvent free extract obtained as above was then subjected to qualitative test for the identification of various plant constituents from the sample<sup>11</sup>.

### **Detection of carbohydrates**

Extracts were dissolved individually in 5 mL distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

**(a) Molisch's test:** Filtrate was treated with 2 drops of alcoholic  $\alpha$ -naphthol solution in a test tube. Formation of a violet ring at the junction indicates the presence of carbohydrates.

**(b) Benedict's test:** Filtrate was treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

**(c) Fehling's test:** Filtrate was hydrolyzed with dil. HCl, neutralized with alkali

and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

### **Detection of alkaloids**

Extracts were dissolved individually in dilute hydrochloric acid and filtered.

**(a) Mayer's test:** Filtrate was treated with Mayer's reagent (Potassium mercuric iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

**(b) Wagner's test:** Filtrate was treated with Wagner's reagent (Iodine in potassium iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

**(c) Dragendroff's test:** Filtrate was treated with Dragendroff's reagent (solution of potassium Bismuth iodide). Formation of red precipitate indicates the presence of alkaloids.

**(d) Hager's test:** Filtrate was treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

### **Detection of glycosides**

Extract was hydrolyzed with dil. HCl, and then subjected to test for glycosides.

**(a) Modified Borntrager's test:** Extract was treated with ferric chloride solution and immersed in boiling water for about 5 min. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthrnl glycosides.

**(b) Legal's test:** Extract was treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

### **Detection of saponins**

**(a) Froth test:** Extract was diluted with distilled water to 20 mL and this was shaken in a graduated cylinder for 15 min. Formation of 1 cm layer of foam indicates the presence of saponins.

**(b) Foam test:** 0.5 g of extract was shaken with 2 mL of water. If foam produced persists for ten min., it indicates the presence of saponins.

### **Detection of phytosterols**

**(a) Salkowski's test:** Extract was treated with chloroform and filtered. This filtrate was treated with few drops of Conc. sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of phytosterol.

**(b) Libermann Burchard's test:** Extract was treated with chloroform and filtered. This filtrate was treated with few drops of acetic anhydride, boiled and cooled, Conc. sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

### **Detection of phenols**

**(a) Ferric chloride test:** Extract was treated with 3-4 drops of ferric chloride solution. Formation of bluish colour indicates the presence of phenols.

### **Detection of tannins**

**(a) Gelatin test:** To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

### **Detection of flavonoids**

**(a) Alkaline reagent test:** Extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

**(b) Lead acetate test:** Extract was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

### **Detection of protein and amino acids**

**(a) Xanthoproteic test:** The extract was treated with few drops of Conc. nitric acid. Formation of yellow colour indicates the presence of protein.

**(b) Ninhydrin test:** To the extract, 0.25% Ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

### **Screening of antimicrobial activity**

#### **Bacterial and fungal strains**

The test organisms were purchased from NCIM, NCL Pune. Bacteria were incubated at 37°C in incubator for 24 hr. They were further stored at 4°C in the refrigerator to maintain

stock culture. Here, qualitative antimicrobial screening was carried out using the cylinder-plate or cup-plate method.

### **Cylinder-plate or cup-plate method**

All the sterilized materials were kept in the aseptic area in the Ultra-Violet laminar air flow. Bacterial suspensions (3 mL) were then poured in the petriplates. As soon as nutrient agar attained 50°C temperature, 20 mL media was poured into the petriplates containing bacterial suspension and plates were rotated to mix the suspension with media. When the agar got solidified, bores were made in the plate with sterile borer of 8 mm diameter. In each plate, six bores were made. Out of which, one is meant for addition of standard, two for negative control of blank solvents of standard and sample and remaining three bores for addition of same concentrations of sample. 0.1 mL of sample was added in each cylinder. The plates were kept to allow diffusion at room temperature for three hr and then incubated in the upright position in incubator at 37°C for about 21 hr for bacterial growth. The diameter of zone of inhibition was accurately measured for bacterial growth in each treated plate (Table 4). The zone of inhibition of bacterial growth by the test solution was compared with the zone of inhibition by the standard at tested concentrations<sup>12</sup>.

## **RESULTS AND DISCUSSION**

Preliminary physico-chemical, phytochemical and antimicrobial screening has been done of leaves extract and results are incorporated in Tables 1, 2, 3 and 4. Results in Tables 1 and 2 showed the ash value and extractive value in different solvents. Results of the phytochemical screening were presented in Table 3. It revealed the presence of alkaloids, glycosides, phytosterols, saponins, phenolic compounds, tannins and proteins.

In earlier study, different medicinal compounds such as alkaloids, glycoside, phenol, phytosterols, saponins and tannins were present<sup>13-15</sup>. Different types of secondary metabolites such as glycosides, phenol, saponins and tannins were presented while alkaloids, flavonoids and sterols were not present in *Ailanthus excelsa* Roxb. These compounds are known to have curative activity against several pathogens and therefore, can be suggested for the treatment of different diseases. Plant leaves are very useful for treatment of different types of disease such as diabetes. It is a good source of drug for human health. Results of antimicrobial activity are presented in Table 4, which shows maximum zone of inhibition 1 mm for ethanol and acetone extract while aqueous, chloroform and petroleum ether extracts do not show zone of inhibition.

**Table 1: Ash analysis of *Ailanthus excelsa* leaves**

S. No.	Type of ash	Percentage (w/w)
1	Total ash	15.5%
2	Acid insoluble ash	13.2%
3	Water soluble ash	1.0%

**Table 2: Percentage extractive values of *Ailanthus excelsa* leaves**

S. No.	Type of extractive value	Percentage (w/w)
1	Water	1.66%
2	Ethanol	0.9%
3	Chloroform	1.2%
4	Acetone	0.6%
5	Petroleum ether	0.7%

**Table 3**

S. No.	Chemical constituents	Aqueous extract	Ethanol extract	Chloroform extract	Acetone extract	Petroleum ether extract
1	<b>Test for carbohydrate</b>					
	a) Molisch test	+++	+++	---	---	---
	b) Benedicts test	+++	+++	---	---	---
	c) Fehling test	+++	+++	---	---	---
2	<b>Test for alkaloids</b>					
	a) Mayer's test	+++	+++	---	---	---
	b) Wagner's test	+++	+++	---	---	---
	c) Dragendorff's test	+++	+++	---	---	---
	d) Hagner's test	+++	---	---	---	---
3	<b>Glycosides</b>					
	a) Modified borntrager's test	+++	+++	+++	---	+++
	b) Legal's test	+++	+++	+++	---	+++

Cont...

S. No.	Chemical constituents	Aqueous extract	Ethanol extract	Chloroform extract	Acetone extract	Petroleum ether extract
4	<b>Saponins</b>					
	a) Froth test	---	+++	---	---	---
	b) Foam test	+++	+++	---	---	---
5	<b>Phytosterols</b>					
	a) Salkowski's test	---	+++	---	+++	+++
	b) Libermann Burchard's test	+++	+++	---	+++	+++
6	<b>Phenols</b>					
	a) Ferric chloride test	---	+++	---	+++	---
7	<b>Tanin</b>					
	a) Gelatin test	---	+++	---	+++	---
8	<b>Flavanoids</b>					
	a) Alkaline test	---	---	---	---	---
	b) Lead acetate test	---	---	---	---	---
9	<b>Proteins &amp; amino acid</b>					
	a) Xanthoproteic test	+++	+++	---	---	---
	b) Ninhydrin test	+++	+++	---	---	---

**Table 4: Antibacterial and antifungal activity of *Ailanthus excelsa* leaves in different solvent**

S. No.	Name of organism	Aqueous extract (mm)	Ethanol extract (mm)	Chloroform extract (mm)	Acetone extract (mm)	Petroleum ether extract (mm)
1	<i>Staphylococcus aureus</i>	-	-	-	1	-
2	<i>Salomonella typhimurium</i>	-	-	-	1	-
3	<i>Proteus vulgaris</i>	-	-	-	-	-
4	<i>Pseudomonas aeruginosa</i>	-	1	-	-	-
5	<i>B. megaterium</i>	-	1	-	1	-
6	<i>Aspargillus niger,</i>	-	-	-	-	-
7	<i>Aspargillus flavus</i>	-	-	-	-	-



Attempt was made to study antimicrobial activity of *Ailanthus excelsa* and to isolate the constituent of active extract responsible for activity. The present work has clearly proved that the acetone and ethanol extracts of plant have considerable antimicrobial activity, which may be due to presence of phytosterols compounds as confirmed by phytochemical analysis of this extract. Though there are number of antibacterial and antifungal drugs available in the market, they produce many side effects and hence, to improve the status of therapy for various ailments; the plant extract like *Ailanthus excelsa* will be much useful. From the result obtained, it is clear that if detail research is carried out on *Ailanthus excelsa*, some useful drug may develop for treatment of fungal infection.

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*Revised : 07.08.2014*

*Accepted : 08.08.2014*