Phytochemical Screening And Antimicrobial Activity Of Stem Of *AILANTHUS EXCELSA*

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Abstract
Ayurveda, one of the most traditional systems of medicine is based on drugs obtained from plants and animals. The research was based on phytochemical screening to identify various phytoconstituents present in plants and also evaluating the antimicrobial effect of the plants especially stem. The plant was collected from region of Bareilly and was authenticated. The dried powdered drug material was extracted by 9 different solvents by cold maceration for 12 hrs at room temperature. Mayer's, Hager's tests, Fehling's Wager's and Molisch test, Foam test and lead acetate test were used for detection of alkaloids, glycosides, saponins, terpenoids etc. Then the stem was screened for antimicrobial activity by using cup plate method. In conclusion, this research suggests, that novel molecules should be identified and isolated responsible for specific pharmacological activity.

Keywords: *Ailanthus excelsa*, antibacterial, phytochemical screening, antibacterial activity.

INTRODUCTION

In India, the traditional medicine system also known as Ayurveda is based on ancient writing that relies on natural approach to physical and mental health. Standardization of herbals means confirmation of its identity, quality and purity. Ayurveda, the science of life, prevention and longevity is believed to be the oldest and most holistic or comprehensive medical system available. Ayurveda is one of the most ancient systems of life, health and cure. Ayurveda is a highly evolved and codified system of life and health science based on its own unique and original concepts and fundamental principles1. Standardization of herbals means confirmation of its identity, quality and purity. Ayurveda, the science of life, prevention and longevity is believed to be the oldest and most holistic or comprehensive medical system available. Ayurveda is one of the most ancient systems of life, the traditional medicine system also known as Ayurveda is based on ancient, health and cure.

These are tradional medicine which are safe, easily available and have less side effects[1]. Ayurveda is also known as Ancient Indian Medical System. WHO has set certain standards to check quality and purity of drugs. The process is known as Standarization of herbal drugs. This process is evaluated with the help of physical, organoleptic, microscopic and chemical process. According to ayurveda, the human body is composed of tissues (*dhatus*), waste (*malas*), and biomaterials (*doshas*).The seven *dhatus* present in body are plasma (*rasa*), blood (*rakta*), muscles (*māmsa*), fat (*meda*), bone (*asthi*), marrow (*majja*), and semen (*shukra*). Like the medicine of classical antiquity, Ayurveda has historically divided bodily substances into five elements like earth, water, fire, air and ether. These are organized in ten pairs: heavy/light, cold/hot, unctuous/dry, dull/sharp, stable/mobile, soft/hard, non-slimy/slimy, smooth/coarse, minute/gross, and viscous/liquid[2].

According to WHO, it is estimated that around 80 % of the people in developing countries still rely on traditional medicines for their primary health care as they have less side effects. The drugs obtained from natural sources are simple, easy to prepare, cheap and have less side effects[3]. In recent times there is a need to screen traditional medicine as everyone wants scientific support before using traditional medicines.

Ayurveda is one of the most promising branch of medical science. It is cheap, highly effective and easily available. Ayurveda along with allopathic medicine serve as a boon to medical science[4]. Quality of herbal medicine should be checked in order to ensure maximum effectiveness. This effectiveness helps in maximum sales of herbal drugs[5].

Ailanthus excelsa is a common plant that is widely used all over India. It is also known as tree of heaven.[6] It is most commonly found in India and Sri Lanka. It is a tree belonging to family Simaroubaceae. *Ailanthus excelsa* Roxb. (Simaroubaceae) is commonly known as Mahanimba due to its resemblance with the neem tree (*Azadirachita indica*) and Maharukha due to its large size. The word Ailanthus is derived from ailanto which means tree of heaven and is the name for one of the species in the Moluccas, while in Latin excelsa means tall[7]. The plant is known by different names like tree of heaven in English.

Botanical Distribution

It is a large tree, and is 18 - 25 m tall; trunk straight, 60 to 80 cm in diameter; dark light 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 lanced shaped from very unequal base.[8] Flower cluster lobed at leaf base, shorter than leaves, much branched; flowers many, mostly male and females on different trees. The generic name Ailanthus came from ailaanthos[9] (tree of heaven) It thrives best on porous loamy soil. The tree can be raised from both seeds and stumps[10,11,12]. The leaves are rated as highly palatable and nutritious fodder for sheep and goats and an average tree yields about 500-700 kg of green leaves twice a year.

MATERIALS AND METHOD

The plants were collected from Bareilly region, Uttar Pradesh and authentication was done. The collected plant material was dried in shade and ground in the grounder. The dried powdered drug material was extracted by 9 different solvents by cold maceration for 12 hrs at room temperature. The extracts were filtered and concentrated at 40°C. The residues were stored in a freezer until further tests.

Preparation of extracts

Plant material of *Ailanthus excelsa*[13] *was* collected from Bareilly region of Uttar Pradesh, India, in the month of October. The collected plant material was dried in shade and grounded in the grounder. The dried powdered drug materials were extracted by 9 different solvents by cold maceration for 12 hrs at room temperature (pharmacognostical). The extracts were filtered and concentrated. The residues were stored in a freezer until further tests.

Extractive Values: For calculation of extractive value 5 gm air dried powder of stem was taken, coarsely powdered and macerated with 100 mL of solvents in a closed flask for 24 hr, shaken frequently during first 6 hr and allowed to stand for another 18 hr, filter, evaporate and finally weighed to calculate extractive value.

PHYTOCHEMICAL SCREENING

Phytochemical Qualitative Analysis

The plant extracts were screened for different for identification of different phytoconstituents:

1. Detection of Alkaloids: Extracts were prepared by dissolving specific quantity of drug in definite amount of solvent. Then the extracts were tested for presence of alkaloids:

Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow colored precipitate indicates the presence of alkaloids.[14]

Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Hagers Test : Filtrates were treated with Hagers Reagent . Formation of yellow ppt indicates the presence of alkaloids.

2. Detection of Glycosides:

Fehling's test: Fehling's solution A and B was diluted with distilled water and boiled for 1min. To this clear blue solution, 8 drops of plant extract was added. After that it was mixed with 1ml of Fehling's solution and boiled in a water bath for 5 min. The formation of brick red precipitation indicates the presence of glycosides[15].

3. Detection of Saponins:

Foam test: About 2g of the plant extract was mixed with 10ml of distilled water and shaken vigorously for a stable persistent froth. Appearance of froth indicates the presence of saponins.[16]

4. Detection of Tannins:

Ferric chloride test: 0.5g of the dried powdered sample was boiled in 20ml of water in a test tube and then filtered. A few drops of 0.1% $FeCl_3$ was added and observed for brownish green-black or a blue-black coloration.

Lead acetate test: 2ml of plant extract was combined with 2ml of distilled water. 0.01g lead acetate was added to this combined solution and shaken well. Development of white turbidity and precipitate indicates the presence of tannins.[17]

5. Detection of Flavonoids:

Sodium Hydroxide test: A small amount of extract was treated with aqueous NaOH and HCl, and observed for the formation of yellow orange color.

Sulfuric acid test: A fraction of the extract was treated with Conc.H_2SO_4 and observed for the formation of orange color.[18,19]

6. Detection of Terpenoids:

2.0 ml of chloroform was added with the 5 ml plant extract and evaporated on the water bath and then boiled with 3 ml of sulphuric acid. A grey color appear which indicates presence of terpenoids.

7. Detection of Steroids:
2 ml of chloroform and concentrated H_2SO_4 were added with the 5 ml aqueous plant crude extract. Red color appeared that indicated the presence of steroids.

8. **Test for Reducing Sugars and Carbohydrates**
1) Molish test (General test)
To 2-3 mL extract of individual solvents add few drops of α-naphthol solution in alcohol, shake and then add concentrate H_2SO_4 from sides of test tube. Violet ring at the junction of two liquids is formed.

2) Fehling's test : It is used for detection of reducing sugars. Dissolve 34.66 gm of copper sulphate in distilled water; make volume up to 500 mL (solution A). Dissolve 17.3 gm of potassium sodium tartaret and 50 gm sodium hydroxide in distilled water and make volume up to 50 mL. (Solution B). Mix two solutions in equal volume prior to use.
Mix 1 mL Fehling's A and Fehling's B solution boil for one minute. Add equal volume of test solution. Heat in boiling water bath for 5-10 min. First a yellow then brick red ppt observed.

Antimicrobial Activity:
Bacteria were incubated at 37º C in incubator for 24 hr. They were further stored at 4º C in the refrigerator. Here, qualitative antimicrobial screening was carried out using the cylinder plate or cup-plate method.[20,21]

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 50º C temperature, 20 mL media was poured into the petriplates containing bacterial suspension or fungal 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, four bores were made[22]. Out of which, one is meant for addition of standard, one for control and remaining two 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 37º C for about 21 hr for bacterial growth. The diameter of zone of inhibition was accurately measured for bacterial growth in each treated plate. The zone of inhibition of bacterial growth and fungal growth by the test solution was compared with the zone of inhibition by the standard at tested concentrations.

RESULT AND DISCUSSIONS
The extraction was performed in various solvents and extractive value was obtained. The result shows that maximum extraction occurs in chloroform extract and minimum in petroleum ether stem extract.

Table 1. Extractive values of the extract

S. No	Solvent	Extractive value (stem)
1.	n-hexane	1.3%
2.	Dimethylsulphoxide	4.2 %
3.	Petroleum ether	1.1 %
4.	Chloroform	4.9 %
5.	Water	4.8%
6.	Toluene	2.4 %
7.	Acetone	1.5 %
8.	Methanol	3.5%
9.	Ethyl acetate	1.9 %

Table No. 1 : Percentage extractive value of *Ailanthus excelsa* stem

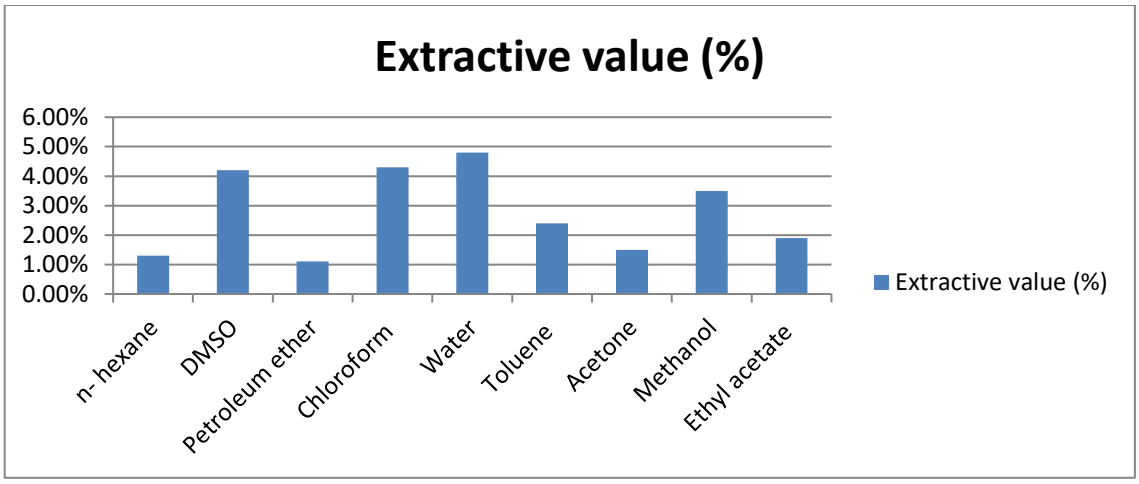

Figure No 1: Extractive value of *Ailanthus excelsa* stem

PHYTOCHEMICAL SCREENING

All the extracts were screened for identification of phytoconstituents like alkaloids, glycosides, carbohydrates, tannins , terpenoids, saponin etc. The test was performed and the samples were tested.

Table 2. Preliminary Phytochemical Screening (Stem)

S.No	Chemical Test	Acetone extract	Methanol extract	Ethyl acetate extract	Aqueous extract	Chloroform extract
1.	Carbohydrate	-	-	-	-	-
2.	Alkaloid	-	+	+	+	+
3.	Glycosides	+	+	+	+	+
4.	Saponins	-	+	+	+	-
5.	Tannins	+	+	+	+	-
6.	Amino acid	+	+	-	+	-
7.	Terpenoid	+	+	+	+	-
8.	Steroid	+	+	+	-	-
9.	Flavanoids	-	+	+	-	+

Table 3. Antibacterial and antifungal activity of *Ailanthus excelsa* stem in different solvent

S. No.	Name of organism	Aqueous extract (mm)	Methanol extract (mm)	Chloroform extract (mm)	Ethyl acetate extract (mm)	n- Hexane extract (mm)
1	E.Coli	7.6	7.0	4.3	5.2	--
2	S.aurus	4.3	---	---	---	--
3	A.niger	5.6	3.4	4.4	2.6	--
4	A.flavus	5.4	2.4	4.2	3.8	0.8

CONCLUSION

Ailanthus excelsa is a tree of heaven and has highest significance for its valuable secondary metabolites.. Extractive value is essential to determine the quality and purity of crude drug. Phytochemical screening was performed to identify various phytoconstituents like alkaloids, glycosides, tannins , terpenoids and saponin etc. This phytochemical screening gives an insight about the uses and constituent present in plant. Maximum extractive value was obtained in chloroform extract and minimum in petroleum ether extract . Phytochemical screening was done in various solvents and Maximum diversity of chemical constituents was found in methanol, ethyl acetate and chloroform extracts in stem. The extracts were screened for antimicrobial activity. Methanol, chloroform and ethyl acetate extract were found to be effective as antibacterial and

antifungal agent and n hexane extract shows least activity. The plant products plays an important role in the treatment of diseases without any side effects, there is a need to search new drugs from natural sources. Now there is a need to look back towards traditional medicine which can serve a novel therapeutic agent as they are effective, cost efficient and has less side effects. Standarization of herbal drug is need of society and attempts should be taken to do it in order to have number of traditional medicines.

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CONFLICT OF INTEREST
Authors have declared for none conflict of interest.

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