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Screening of phytoconstituents and antibacterial effects of leaf & stem of Ailanthus excelsa against E. Coli, S. Aureus, A. Niger & A. Flavus

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***Abstract*---**The research was aimed as screening of phytoconstituents and antibacterial effects of Leaf & Stem of *Ailanthus excelsa* against E. *Coli, S. Aureus, A. Niger & A. Flavus*. The plants were collected from Bareilly region, Uttar Pradesh and authentication was done by CIMAP, Lucknow. 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. Mayer's, Hager's tests, Fehling's Wager's and Molisch test were used for detection of alkaloids, glycosides, saponins, terpenoids etc. After the extraction was complete, it was tested against various species such as *E. coli, S. aureus, S. niger and A. Flavus* for its antimicrobial potential. The results indicate that methanolic extract showed highest level of phytoconstituents such as alkaloids, glucosides, saponins, terpenoids, flavonoids, and carbohydrates. Extract showed significant antibacterial potential against all the species tested with. In conclusion, this research suggests, that novel molecules should be identified and isolated responsible for specific pharmacological activity.

***Keywords*---**Ailanthus Excelsa, antibacterial, A. niger, E. coli, phytoconstituents.

Introduction

In India, the traditional medicine system also known as Ayurveda is based on ancient writings 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 principles. 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 (Bhandari & Gupta, 1972; Bhatia & Sahai. 1985). The traditional drugs are simple, easy to prepare, cheap and has less side effects. In recent there is a need to screen traditional medicine as everyone wants scientific support before using traditional medicines.

Ayurveda is continuing as a main approach in the treatment due to its easy availability coupled with safe, effective and sustainable claims. Indian people have a tremendous passion for medicinal plants and use them for a wide range of health-related applications, from common cold to memory improvements and enhancement of general immunity.

Ailanthus excelsa is a common plant that is widely used all over India. It is also known as tree of heaven (Hukkeri et al. 2002; Khan & Shamsuddin, 1978). 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. The plant is known by different names like tree of heaven in English.

Botanical Distribution

It is a large deciduous tree, 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 (Kumar et al. 2010a; Chevallier et al. 1996). 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 (Sahai & Bhatia, 1985; Kumar et al. 2010). It thrives best on porous loamy soil. The tree can be raised from both seeds and stumps. 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 (Kumar et al. 2011; Chopra et al. 1958; Database, 2000).

Materials And Methods

The plants were collected from Bareilly region, Uttar Pradesh and authentication was done by CIMAP, Lucknow. 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^{o}C. The residues were stored in a freezer until further tests.

Preparation of Extracts

Plant material of *Ailanthus excelsa was* collected from Bareilly region of Uttar Pradesh, India, in the month of October. The herbarium will be authenticated by Central Institute of Medicinal and Aromatic Plants. 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 and leaves was taken, coarsely powdered and macerated with 100 mL of respective solvents in a closed flask for 24 hr, shaken frequently during 6 hr and allowed to stand for 18 hr, filter, evaporate and finally weighed to calculate extractive value.

Phytochemical Screening

Phytochemical Qualitative Analysis

The plant extracts were screened for different phytoconstituents to check their presence.

Detection of Alkaloids

Extracts were dissolved individually in dilute HCl and filtered.

Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow-colored precipitate indicates the presence of alkaloids (Kundu & Laskar, 2008; IP, 1996).
Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.
Hager's Test: Filtrates were treated with Hagers Reagent . Formation of yellow ppt indicates the presence of alkaloids.

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.

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 (Khandelwal, 2002; Nag & Matai, 1994).

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 (Bhandari & Gupta, 1972; Bhatt & Dhyani, 2012).

Detection of Flavonoids

NaOH test: A small amount of extract was treated with aqueous NaOH and HCl, and observed for the formation of yellow orange color.
H_2SO_4 test: A fraction of the extract was treated with Conc.H_2SO_4 and observed for the formation of orange color.

Detection of Terpenoids

2.0 ml of chloroform was added with the 5 ml aqueous plant extract and evaporated on the water path and then boiled with 3 ml of H_2SO_4 concentrated. A grey color formed which showed the entity of terpenoids.

Detection of Steroids

2 ml of chloroform and concentrated H_2SO_4 were added with the 5 ml aqueous plant crude extract. In the lower chloroform layer red color appeared that indicated the presence of steroids.

Test for Reducing Sugars and Carbohydrates

Molish test (General test)

To 2-3 mL extract of individual solvents add few drops of α-naphthol solution in alcohol, shake and add concentrate H_2SO_4 from sides of test tube. Violet ring at the junction of two liquids.

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 tartrate and 50 gm sodium hydroxide in distilled water and make volume up to 50ml. (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 37° C in incubator for 24 hr. They were further stored at 4° C in the refrigerator. Here, qualitative antimicrobial screening was carried out using the cylinder plate or cup-plate method (Shete et al. 2013; Trease & Evans, 1996). 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 petri-plates. As soon as nutrient agar attained 50° C temperature, 20 mL media was poured into the petri-plates 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. 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 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. 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 (Table 1).

Table No. 1: Percentage extractive value of Ailanthus excelsa leaves

S. No.	Solvent	Extractive value (leaves)
1.	n-hexane	1.2%
2.	Dimethylsulphoxide	5.4 %
3.	Petroleum ether	1.5 %
4.	Chloroform	5.1 %
5.	Water	6.2%

6.	Toluene	3.2 %
7.	Acetone	4 %
8.	Methanol	3.7 %
9.	Ethyl acetate	2.6 %

The result shows that maximum extraction occurs in water extract and minimum in n-hexane leaves extract. It exhibited as a highest efficient source of alkaloids, glycosides, flavonoids and terpenoids etc. (Table 2).

Table No. 2: Percentage extractive value of Ailanthus excelsa stem

S. N.	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 %

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. Highest extraction value as noted in solvent of Dimethyl-sulphoxide, chloroform and water as 5.4, 5.1 and 6.4 respectively which indicates about its efficient solubility efficacy. 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 3).

Table No. 3: Phytochemical Screening of leaves of *Ailanthus excelsa*

S. N.	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	-	+	-	-	+

Antibacterial and antifungal activity of *Ailanthus excelsa* leaves

The antifungal activity of the A. excelsa leaves might be due to its deteriorating potential on cell membrane and cell wall. It decomposes the proteins and nucleotides that is needed for the synthesis of nucleic acids such as DNA and DNA. Better activity was found in methanolic and ethyl acetate extract because of higher efficient extraction (better solubility) (Table 4).

Table No. 4: Antibacterial and Antifungal activity of *Ailanthus excelsa* leaves

S. No.	Name of organism	Aqueous extract (mm)	Methanol extract (mm)	Chloroform extract (mm)	Acetone extract (mm)	Ethyl acetate extract (mm)	N hexane extract (mm)
1	*E. Coli*	--	7.2	5.6	6.2	7.4	--
2	*S. aureus*	2.3	6.0	-	5.7	5.5	--
3	*A. niger*	-	5.2	4.6	-	5.6	1.1
4	*A. flavus*	3.0	6.9	4.2	6.0	6.2	1.2

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 water and chloroform extract and minimum in n-hexane and 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 leaf. The extracts were screened for antimicrobial activity. Methanol, chloroform and ethyl acetate extract were found to be effective as antibacterial and antifungal agent. The plant products play an important role in the treatment of diseases

without any side effects, there is a need to search new drugs from natural sources. India is a home to a variety of traditional medicine system that relay to a very large extent on native plant species for new drug materials therefore now there is the need to look back towards traditional medicine which can serve a novel therapeutic agent. Pharmacognostical evaluations also give valuable information which is essential to standardize the drug.

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Conflict of Interest
None

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