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PHYTOCHEMICAL, SPECTRAL ANALYSIS AND METAL CONTENT OF AILANTHUS EXCELSA LEAVES

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ABSTRACT

The present study was carried out in *Ailanthus excelsa* leaves to analyze the phytoconstituent, to characterize the bioactive compounds using spectral analysis and metal content. Extraction was carried out by soxhlet apparatus method with various solvents such as aqueous, acetone, ethanol, chloroform, and petroleum ether. The phytochemical analysis revealed the presence of carbohydrate, alkaloids, flavonoids, phytosterols, tanin, phenol, protein and aminoacids. The FTIR analysis confirms the presence of methylene (-CH2-), carboxylic acid (C=O), alkenes (C=C), alcohols (-OH). Four metal i.e. copper, nickel, iron and cobalt determined by inductive coupling plasma spectroscopy which is superior to double atomic absorption spectroscopy. The results of the present study generated the FTIR spectrum profile for the medicinally important *Ailanthus excelsa* can be used to determine its therapeutic values for the development of new drugs.

KEYWORDS: Ailanthus excelsa, Phytochemical, Spectral analysis.

1. INTRODUCTION

Medicinal plant research includes much more than the discovery of new drugs. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for the new drug.^[1] This field has been expanding and includes diverse subjects as negotiation of power based on medicinal plant knowledge. [2] The different phytoconstituents present in plants include glycosides, bitter drugs, flavonoids, alkaloids, saponins, coumarins, phenol carboxylic acids and terpenes. These phytoconstituents confer specific characteristics and properties to plants. Therefore, the analysis of these constituents would help in determining various biological activities of plants. The spectroscopic technique has become a powerful and analytical tool for and qualitative quantitative analysis pharmaceutical and biological materials. Simple, costeffective and rapid tests for detecting phyto-components are necessary. Spectroscopic (UV-Vis, FTIR) methods together or separate can be used in this sense as well as conventional methods.[3] The Fourier Transform Infrared spectroscopy (FTIR) allows the analysis of a relevant amount of compositional and structural information in plants. Moreover, FTIR spectroscopy is an established time-saving method to characterize and identified groups.^[4] functional Ultraviolet-visible spectrophotometry (UV-Vis) related to the spectroscopy of photons in the UV-visible region. UV-visible

spectroscopy uses light in the visible ranges or its adjacent ranges. The colour of the chemicals involved directly affects the absorption in the visible ranges. Molecules undergo electronic transitions in these ranges of the electromagnetic Spectrum. [5] 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. [6] 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.^[7] The main objective of the present study is to identify the phyto-constituents, metal content of Ailanthus excelsa Roxb, leaves extract and UV-VIS spectrum and FTIR profile.

Taxonomical classification of Ailanthus excels

Kingdom : Plantae

Division : Magnoliophyta
Class : Magnoliotae
Order : Sapindales
Family : Simaroubaceae
Genus : Ailanthus
Species : Excelsa

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A traditional use of Ailanthus excels

Bark of Ailanthus excelsa is bitter, astringent, anthelmintic, febrifuge, appetizer, bitter tonic, taste bud stimulant. It is useful in diarrhea, amoebic dysentery, dyspepsia, abdominal spasm anorectal disease, hemorrhoids, fistula, fissures, ulcerative colitis and worm infection. It is also used as blood purifier in skin diseases, typhoid fevers, blood coagulation disorders, gouty arthritis, boils, carbuncle, scabies and allied skin chronic bronchitis, bronchial disease, asthama, pulmonary kochs, bronchiectasis, polyuria, diabetes mellitus, obesity, uterine disorders like dysmenorrhea and leucorrhoea. Leaf juice is given along with milk for post labor pains. In Chinese system of medicine bark of Ailanthus excelsa is used to treat diarrhea and dysentery. especially when there is a blood in stool. The root bark is used to cure epilepsy and heart troubles. In Africa the plant is used to treat cramps, gonorrhea epilepsy, tape warm infestation and high blood pressure.

Traditionally the mattress made from leaves of *Alianthus excelsa* is used as bed for children suffering from fever. The bark and leaves are of great repute as a tonic especially in debility after child birth in Bombay. They are used in dyspepsia, bronchitis and asthma. The juice of the leaves is usually given in khir, or the juice of the fresh bark is given with coconut juice and treacle or honey to stop pains in Konkan. It is also used to cure wounds and skin eruptions. The plant is used as natural antifertility agent in Tamil Nadu. The fresh juice of stem bark mixed with either honey is given to pregnant woman during evening for three consecutive days to induce permanent sterility. The pest of stem barks along with goat milk and neem oil is used for curing the nose rope wound in ox.

The bark is used as bitter, refrigerant, astringent, appetizer, anthelmintic, febrifuge, in dysentery, ear ache, skin disease, troubles of the rectum and fever. It is also used in gout and rheumatism.

In Ayurveda it is used to remove the bad taste of mouth. Fruits are used in diarrhea, polyurea, piles and fever. Leaves along with twigs are found to be suitable fodder for cattle, sheep and goats. The tree yields an inferior quality of bassora or hog gum. The plant is used as one of the host for silk worms. In France the tree is cultivated for its leaves, on which the caterpillar of the silk spinning Ailanthus moth (Bombyx Cynthia) is fed yielding a silk of more durable and cheaper than mulberry silk. The wood is used in the manufacture of paper and preparation of pencils. [8]

2. MATERIAL AND METHOD

The fresh leaves of *Ailanthus excelsa* are collected from Kada region, District Beed. The fresh leaves were dried under shade, powdered and pass through 40 mesh sieve and stored in closed bottle for further use. The powder was extracted with different solvent such as water,

acetone, ethanol, chloroform, and petroleum ether by Soxhlet apparatus.

2.1. PHYTOCHEMICAL ANALYSIS

Phytochemical examinations were carried out for all the extract as per the standard methods. [9]

- **2.1.1. 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 α -naphtha solution in a test tube. Formation of violet ring at the junction indicates the presence of carbohydrates.
- b) Benedict's test: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.
- c) Fehling's test: Filtrates were 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.
- **2.1.2. Detection of alkaloids:** Extracts were dissolved individually in dilute hydrochloric acid then filtered and alkaloids were detected using following test.
- a) Mayer's Test: Filtrates were 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.
- **2.1.3. Detection of glycosides**: Extract was hydrolyzed with dil. HCl, and then subjected to test for glycosides.
- a) Modified Borntrager's Test: Extracts were treated with ferric chloride solution and immersed in boiling water for about 5 minutes. 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 anthrnol glycosides.
- b) Legal's Test: Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

2.1.4. Detection of saponins

a) Froth Test: Extracts were diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for

15 minutes. Formation of 1cm 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 minutes it indicates the presence of saponins.

2.1.5. Detection of phytosterols

- a) Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow indicates the presence of phytosterol.
- b) Libermann Burchard's Test: Extracts were treated with chloroform and filtered. The filtrates were 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.

2.1.6. Detection of Phenols

a) Ferric chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish colour indicates the presence of phenols.

2.1.7. 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.

2.1.8. Detection of Flavonoids

a) Alkaline Reagent Test: Extracts were 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: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

2.1.9. Detection of protein and amino acids

- *a) Xanthoproteic Test:* The extracts were 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.

3. RESULT AND DISCUSSION

For present investigation an *Alianthus excelsa* leaves extracted with different solvent such as water, acetone, ethanol, chloroform, and petroleum ether. In aqueous extract there is presence of carbohydrate, alkaloid, glycosides, phytosterol, protein and amino acid, while in ethanol extract phyto-constituents like alkaloid, carbohydrate, glycoside, phytosterol, phenol, tannin, protein and amino acids are present. In chloroform extract tested positive for glycoside, while acetone extract shows only presence of phytosterol, phenol and tannin. While in petroleum ether extracts shows presence of glycoside and phytosterols [Table: 1].

Sr.	Chemical	Aqueous	Ethanol	Chloroform	Acetone	Petroleum
No.	constituents	extract	extract	extract	extract	ether extract
1	Carbohydrate	+++	+++			
2	Alkaloid	+++	+++			
3	Glycosides	+++	+++	+++		+++
4	Saponin	+++	+++			
5	Phytosterol		+++		+++	+++
6	Phenol		+++		+++	
7	Tanin		+++		+++	
8	Flavanoids	+++	+++	+++		+++
9	Protein and amino acid	+++	+++			
+++ - Present, Absent						

UV-visible spectral analysis of *Ailanthus excelsa in* aqueous extract shows presence of eight peaks and its λ max observed at 200 nm. Ethanolic extract of it shows $\lambda_{\rm max}$ at 669 nm and presence of fourteen peaks. Chloroform extract shows number of peaks four and $\lambda_{\rm max}$ observed at 248 nm. The number of peaks shown by acetone extract is ten, $\lambda_{\rm max}$ observed at 328 nm. Petroleum ether extract shows presence of ten peaks and its $\lambda_{\rm max}$ observed at 459 nm [Table.2].

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Sr. No.	Aqueous extract		Ethanol extract		Chloroform extract		Acetone extract		Petroleum ether extract	
	λ (nm)	O.D	λ (nm)	O.D	λ (nm)	O.D	λ (nm)	O.D	λ (nm)	O.D
1	200	6.30	669	4.91	248	4.65	328	5.34	459	4.56
2	213	4.44	661	4.79	239	4.50	665	4.87	219	4.51
3	221	4.80	234	4.59	663	4.24	331	4.74	460	4.05
4	271	4.38	250	4.55	441	2.70	658	4.43	467	4.05
5	378	4.09	279	4.39	-	-	335	4.36	449	4.05
6	-	-	666	4.27	-	-	376	4.13	680	4.14
7	-	-	377	4.08	-	-	379	4.12	378	4.07
8	_	-	379	4.10	=	-	463	2.75	212	3.71

Table 2: Absorption peaks in UV-Visible range of Ailanthus excelsa for different extracts.

Aqueous extract of *Ailanthus excelsa* shows absorption band at 3291 cm $^{-1}$ due to N-H stretch. The band founds at 1573 cm $^{-1}$ and 1395 cm $^{-1}$ due to presence of benzene ring and -NO₂ group. The band at 1049 cm $^{-1}$ indicates C-O stretch.

Ethanolic extract of *Ailanthus excelsa* shows absorption band at 2916 cm⁻¹ due to C-H stretching. The band founds at 1375 cm⁻¹ and 1066 cm⁻¹ due to -NO₂ group and C-O stretching. The band at 848 cm⁻¹ due to para substituted.

The chloroform extract shows absorption band at 2917 cm⁻¹ and 2849 cm⁻¹ due to C-H stretch. The band at 1710 cm⁻¹ and 1450 cm⁻¹ confirms C=O group and C=C of benzene ring. Whereas 1376 cm⁻¹ due to -NO₂ group. The bands founds at 1164 cm⁻¹, 834 cm⁻¹ due to para disubstituted and C-Cl stretching.

Acetone extract shows absorption band at 3379 cm⁻¹ due to O-H stretching. The band founds at 2917 cm⁻¹ and 2849 cm⁻¹ due to C-H stretching. The band founds at 1710 cm⁻¹ and 1449 cm⁻¹ confirms presence of benzene ring. The bands founds at 1376 cm⁻¹ due to -NO₂ group and band 719 cm⁻¹ due to C-Cl stretching.

Petroleum ether extract shows absorption band at 2917 cm⁻¹ and 2849 cm⁻¹ is due to C-H stretching. The band at 1731 cm⁻¹ and 1449 cm⁻¹ is due to presence of benzene ring. The band founds at 1082 cm⁻¹ and 719 cm⁻¹ due to C-O and C-Cl.

Ailanthus excelsa was tested for quantitative determination of nickel, copper, cobalt and iron. It was observed that the presence of nickel is 6.7 ppm, copper is 34.9 ppm, cobalt is 2.2 ppm and iron is 3634.5 ppm. The trend can be given as Fe > Cu > Ni > Co. This sample shows high concentration of iron in it.

Table 3: FTIR Peak value of Ailanthus excelsa for different extract.

Sr. No.	Extracts	I R Observed peaks (cm ⁻¹)
1	Water	3291, 1573, 1395, 1049.
2	Ethanol	2916, 1375, 1066, 1050, 1004, 989, 942, 909, 867, 848.
3	Chloroform	2917, 2849, 1710, 1450, 1376, 1164, 1063, 980, 834, 719.
4	Acetone	3379, 2917, 2849, 1710, 1449, 1376, 1035, 1007, 719.
5	Petroleum ether	2917, 2849, 1731, 1449, 1376, 1164, 1082, 982, 831, 719.

Table 4: Metal content of Ailanthus excels.

Sr. No.	Name of the metal	Content in (ppm)
1	Iron	3634.5
2	Copper	34.9
3	Cobalt	2.2
4	Nickel	6.7

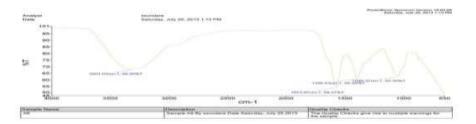


Fig. 1: FTIR of Ailanthus excelsa in aqueous extract.

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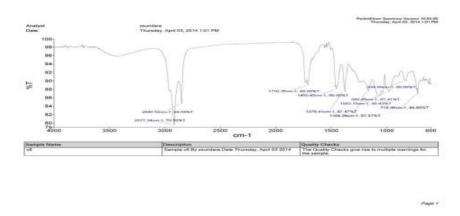


Fig. 2: FTIR of Ailanthus excelsa in ethanol extract.

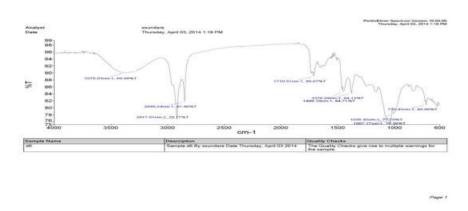


Fig. 3: FTIR of Ailanthus excelsa in acetone extract.

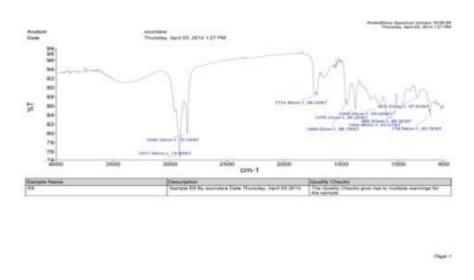


Fig. 4: FTIR of Ailanthus excelsa in petroleum ether extract.

4. CONCLUSION

The study of phytochemical composition, spectral analysis, and metal content of Ailanthus excelsa leaves

offers valuable insights into its potential applications in various fields. Through phytochemical analysis, the presence of bioactive compounds such as alkaloids,

flavonoids, phenolics, and terpenoids can be identified, indicating potential medicinal properties. Spectral analysis, including UV, Visible and FTIR provides detailed structural information about these compounds, aiding in their characterization and understanding their functional roles. Moreover, the determination of metal content helps assess the environmental quality and potential health risks associated with the consumption or utilization of Ailanthus excelsa leaves. Overall, this comprehensive analysis enhances our understanding of the chemical composition and properties of Ailanthus excelsa leaves, paving the way for further research and potential applications in medicine, agriculture, and environmental science.

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