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Research Article

Extracts of *Ailanthus excels* an Essential Medicine in Ayurveda: Pharmacological evaluation and preliminary screening of phytochemicals

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ABSTRACT

Ailanthus excels belongs to family *Simaroubaceae* known as tree of Heaven and Mahanimba commonly found in the India and China. The present study involves preliminary screening, qualitative analysis and pharmacological evaluation of extract. The preliminary photochemical screening performed using petroleum ether, chloroform and methanol extract. Analytical techniques like; TLC, FTIR and HPTLC also performed for qualitative and quantitative determination. Various chemical tests also performed for qualitative determination of alkaloids, flavanoids, phenols, terpenoids, steroids and saponins in plant extracts. Plant extract finally subjected to antibacterial and antifungal activities and findings of study suggested that plant extracts possesses potent antibacterial and antifungal activities.

Keywords: *Ailanthus excelsa*, antibacterial, antifungal, preliminary screening, TLC, HPTLC.

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INTRODUCTION

Ayurveda the traditional system of India practicing word widely and its acceptance increasing day by day in global health community. The Ayurveda system provokes health as well as wellness in living creatures. The medicinal knowledge transformed from good to sages and then to Ayurveda preacher to serve mankind. The Hindu god Dhanvantari name first time found in book *Sushruta Samhita* wrote by *Sushruta*, which taught us about magical medicine treatment. The therapies includes complex herbal, mineral and metal based *Bhasm*, churns and extract which treat kidney stone, sutures, diabetes and bone fractures. In modern Ayurveda system various Ayurveda medicines are patent but found to contain toxin which includes heavy metals. The Ayurveda system generation to generation moved with preachers from Indus Valley Civilization in *Vedic* period to non-Vedic systems includes Buddhism and Jainism in their classical Ayurveda texts.

Ayurveda preaches us a great thought to protect human life from illness and disease. The word ayurveda derived from Sanskrit i.e. ayur and veda meaning life and knowledge respectively. Thus ayurveda teaches or provides knowledge to increase life span without sickness. The information given in ayurveda literature was collected by our sages (*rishi, muni and sant*), through their deep meditation and spiritual power¹⁻⁵.

The *Ailanthus excels* also known as Mahanimba belongs to family *Simaroubaceae* and widespread in India and China. The *Ailanthus excels* tall and widely spread tree possesses active constituents for the management diarrhoea, malaria, hypoglycaemia and used as hepatoprotective. It utilized in treatment of dysentery asthma, antihelminthic, gout, rheumatism, dyspepsia bronchodilator, antispasmodic, astringent, appetizer colic pain, refrigerant, cough, cancer and diabetes. It is also used to cure wounds and skin eruptions and extract showed potent antibacterial and antifungal activities. The plant reported to contain phytochemicals as Quassinoids, excelsin, glaucarubin, ailanthone, glaucarubinone, Glaucarubilone and 13,18-dehydroexcelsin and glaucarubolailexcelone and ailexcelol, together with ocotillone, malabaricol, epoxyamarabicol, lupeol

Considering importance of bark, present investigation were planned to perform phyto-chemical and pharmacological evaluation of bark extracts of *Ailanthus excelsa*. The study was aimed to explore folklore use of plant bark⁵⁻⁷.

MATERIALS AND METHODS

Sample collection

The *Ailanthus excels* was collected from the Indore (*Malwa*) region India. The authentication of species was completed in the Ayurveda department Indore. The bark of plant was scraped with the help of knife, in the month of November

2016 and dried in shade. After drying the bark was grinded into coarse powder and stored in plastic vessel.

Extract preparation

The coarse powder of bark of *Ailanthus excels* was divided into three parts. Each part having 25 g of bark powder extracted with petroleum ether, chloroform and methanol. The first 25 g bark powder was wrapped into thimble and kept in upper chamber of Soxhlet apparatus and in lower portion solvent was present, heated to evaporation and solvent reaches to thimble (upper portion) and passes through the sample powder as a result extraction was starts. same procedure was adopted for chloroform and methanol extracts⁹⁻¹⁰. The petroleum ether, chloroform and methanolic extracts were collected, filtered with Whatmann No. 1 filter paper, evaporated till drying, stored in airtight container and analyzed further¹²⁻¹³.

Thin Layer Chromatography

The thin layer chromatography profiling of dried extracts of petroleum ether, chloroform and methanol was performed using silica gel plate. The three TLC plates were taken and 50 µl crude extracts applied on 1 centimeter above the TLC plate with the help of micro-pipette. After sample application the plates were dried and kept in the chamber equipped with solvent system; Ethyl Acetate: Chloroform: Water: methanol 5:3:1:1. The solvent mixture was allowed to travel $\frac{3}{4}$ th of plate height, after that plate was removed and dried. The dried plate kept in UV chamber and in the iodine chamber to detect spots. The spots were scraped and analyzed further for chemical evaluation¹⁴. The scraped bands of TLC plate were dissolved in 10mL respective solvent, filtered and then subjected to following chemicals tests⁴⁻⁸.

Chemical Test

Test of alkaloids:

a. Mayers test

The 0.5 mL filtrate of each extract taken in test tube and two drops of Mayer's reagent was added and observed for appearance of white or creamy color precipitation.

b. Wagners test

The 0.5 mL filtrate of each extract taken in test tube and two drops of Wagner's reagent were added and observed for appearance of reddish-brown color precipitation.

Test of flavonoids Ammonia Reduction Test

The 0.5 mL filtrate of each extract taken in test tube and few drop of dilute NaOH solution added, an intense yellow colour generated and becomes colourless on addition of a few drop of dilute acid showed test as positive.

Test of phenol by Ferric Chloride Test

The 0.5 mL filtrate of each extract taken in test tube, dissolved in 1 mL of distilled water then 5-6 drops of 10% ferric chloride solution was added. A dark green colour showed positive test for phenolic compounds.

Test of terpenoids by Salkowski's test

The 0.5 mL of extract dissolved in chloroform (1 mL) and 1.5 ml concentrated sulphuric acid was added to the solution. The reddish brown coloured showed positive test for terpenoids.

Test of steroids by Salkowski's test

The 0.5 mL of extract dissolved in 5 ml of chloroform and 5 mL of concentrated sulphuric acid was added from sides of

test tube. The top layer showed red appearance and sulphuric acid layer showed yellow colour with green fluorescence as positive test for steroids.

Test of Saponins

The 0.5 mL filtrate diluted with distilled water to 10 ml and shaken in a graduated cylinder for 15 minutes 1 cm layer of foam showed presence of saponin.

FTIR (Fourier transform infrared) Spectroscopy

The dried petroleum ether, chloroform and methanol extract of plant *Ailanthus excels* kept in oven for drying then triturated in mortar pestle along with dried KBr. The blank was taken as KBr to avoid the interference of it in sample. The triturated extract kept in sample cell of FTIR and instrument allowed to run spectra which generated peaks of functional group present in sample¹⁰.

High Performance Thin Layer Chromatography (HPTLC)

The silica gel GF HPTLC plate were used for analysis and activated in oven prior to spotting. The sample of dried petroleum ether, chloroform and methanol extract of plant was applied with an automatic applicator. The solvents used as mobile phase were composed of Ethyl Acetate: Chloroform: Water: methanol 5:3:1:1. The solvent mixture was filtered and kept in chamber for saturation. The silica gel GF HPTLC plate kept in HPTLC chamber and mobile phase was allowed to run for few hours. After development plate was removed from chamber dried to avoid contamination. The plate was kept in UV chamber for confirmation of spot. The TLC scanner used to detect spot at 200 and 800 nm¹⁵.

Pharmacological evaluation

The pharmacological evaluation of extracts of *Ailanthus excels* was performed as follows:

a. Anti-bacterial activity: Cup-plate method

The culture medium was made with nutrient agar in Ultra-Violet laminar air flow and sterilization was done at 60°C in an autoclave. The medium was poured on glass plate and mixed with bacterial suspension and after drying cups were prepared over it. The samples were then poured over bacterial cup and plate and activity was measured by visual inspection.¹⁶⁻¹⁸

b. Antifungal activity by inhibitory zone estimation:

Disk diffusion method was used to determine antifungal activity. Sample was prepared and disk was dipped in it with solid culture medium. All plates of different extract were kept for incubation at 37°C in UV chamber for 48 h. after incubation inhibition was measured by the round scale.¹⁶⁻¹⁸

RESULTS AND DISCUSSION

The plant sources were widely used for medicinal purpose since ancient times due to active chemical constituents present in it. The results of phytochemical screening are compiled in table-1 which confirms presence of secondary metabolite which contributed towards antimicrobial activity. The TLC was also performed using mixture of ethyl acetate: chloroform: water: methanol as mobile phase. The alkaloid was present in the petroleum ether extract and methanolic extract of plant respectively at R_f value 0.42 and 0.41. The alkaloid was confirmed in scrap part of TLC by Mayer's Test and Wagner's Test. The flavonoid was present in chloroform and methanol extract at R_f value 0.30 and 0.31 respectively confirmed by ammonia reduction test. The phenol was present, chloroform and methanol extract at R_f value 0.73 and 0.71 respectively confirmed by Ferric Chloride test.

The terpenoids was confirmed by Salkowski's Test at R_f value 0.51 in petroleum ether extract. The steroid was present in chloroform extract of plant at R_f value 0.61. The saponin was confirmed by Froth Test at R_f value 0.83 and

0.79 in petroleum ether and methanol extract respectively. The investigation confirmed presence of important phytochemical in plant extracts which are known for therapeutic value.

Table 1: Phytochemical screening, chemical test and TLC results (R_f Value) of extracts of *Ailanthus excelsa*

S. No.	Constituents	Test performed	Extracts of <i>Ailanthus excelsa</i>					
			Petroleum Ether Extract		Chloroform Extract		Methanolic extract	
			Results	R_f Value	Results	R_f Value	Results	R_f Value
1	Alkaloids	Mayer's Test	+ve	0.42	-ve	-	+ve	0.41
		Wagner's Test	+ve		-ve		+ve	
2	Flavanoids	Ammonia Reduction Test	-ve	-	+ve	0.30	+ve	0.31
3	Phenol	Ferric Chloride Test	-ve	-	+ve	0.73	+ve	0.71
4	Terpenoids	Salkowski's Test	+ve	0.51	-ve	-	-ve	-
5	Steroids	Salkowski's Test	-ve	-	+ve	0.61	-ve	-
6	Saponins	Froth Test	+ve	0.83	-ve	-	+ve	0.79

The FTIR technique was used to identify functional group present in the extract. The narrow NH group peak obtained at 3502 cm^{-1} , broad -OH peak at 3400 cm^{-1} , =C-H peak at 2200 cm^{-1} , -C-H peak at 2000 cm^{-1} , C-O peak at 1800 and -CHO peak at 1700 cm^{-1} . The results of IR study confirmed presence of characteristics peaks of Alkaloids, Flavanoids, Terpenoids, Steroids and Saponins.

The HPTLC also performed which confirmed the presence of Alkaloids, Flavanoids, Phenol, Terpenoids, Steroids and Saponin in the extract. The spots for Alkaloids, Flavanoids, Phenol, Terpenoids, Steroids and Saponins found in TLC respectively at R_f value 0.42, 0.30, 0.73, 0.51, 0.61 and 0.83.

The TLC and HPTLC results were calculated and found similarity between both.

The cup-plate technique applied to determined the antibacterial activity of extract (petroleum ether, chloroform and methanolic) of *Ailanthus excelsa*. The petroleum extract showed negative result against *Staphylococcus aureus* and positive result against *Salomonella typhimurium*. The chloroform extract showed positive result against *Staphylococcus aureus* and *Salomonella typhimurium* (Table 2). The methanolic extract showed positive result against *Staphylococcus aureus* and negative result against *Salomonella typhimurium*.

Table 2: Anti-bacterial activity of petroleum ether, chloroform and methanolic extract of *Ailanthus excelsa*.

S.No.	Bacteria	<i>Ailanthus excelsa</i> Extract		
		Petroleum Ether Extract	Chloroform Extract	Methanol extract
1	<i>Staphylococcus aureus</i>	-ve	+ve	+ve
2	<i>Salomonellatyphimurium</i>	+ve	+ve	-ve

The Anti-fungal activity of extract (petroleum ether, chloroform and methanolic) of *Ailanthus excelsa* performed through a zone inhibition technique. The petroleum ether extract showed negative result against *A. flavus* and *A. fumigates* while showed positive result against *P. notatum*,

and *A.niger*. The chloroform extract showed positive result against all species *A. flavus* and *P. notatum*, *A. niger* and *A. fumigatus*. The methanolic extract showed negative result against *A. flavus* and *A. fumigatus* while showed positive result against *P. notatum* and *A. niger* (Table 3).

Table 3: Anti-fungal activity of petroleum ether, chloroform and methanol extract of *Ailanthus excelsa*.

S. No.	Fungus	<i>Ailanthus excelsa</i> Extract		
		Petroleum ether extract	Chloroform extract	Methanol extract
1	<i>A. flavus</i>	-ve	+ve	-ve
2	<i>P. notatum</i>	+ve	+ve	+ve
3	<i>A. niger</i>	+ve	+ve	+ve
4	<i>A. fumigatus</i>	-ve	+ve	-ve

CONCLUSION

The *Ailanthus excels* is an important medicinal used plant from ancient times. The results of phytochemical analysis suggested that *Ailanthus excels* possess bioactive phytochemicals of medicinal importance. The phytochemical screening initiated by TLC profiling and chemical test confirmed presence of Alkaloids, Flavanoids, Phenol, Terpenoids, Steroids and Saponins. The pharmacological evaluation of extracts was also performed. The petroleum ether, chloroform and methanolic extract of plant showed potential therapeutic response against bacteria and fungus. The study concluded that the bark of plant *Ailanthus excelsa* may be further recommended as potent antimicrobial agents.

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