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Research Article

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Antifungal study of *Ailanthus excelsa* leaves

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ABSTRACT

The antifungal activity of Ailanthus excelsa was evaluated using human pathogenic organism (Aspargillus niger, Aspargillus flavus, Aspargillus fumigatus, and penicillium notatum). The Plant reported to contain chemical constituents like quassinoids excelsin, glaucarubin, ailanthone, glaucarubinone, glaucarubolone. This study involves the preliminary screening, quantitative determination and the qualitative thin layer chromatographic separation of secondary, metabolites from different part of plant. The chloroform fraction of methanol extract exhibited significant broad spectrum of inhibition at 500mg/ml concentration on fungi.

Keywords: *Ailanthus excelsa*, antifungal activity, methanol extract, and chloroform fraction of methanol extract (MECH).

INTRODUCTION

Ailanthus excelsa Roxb. (Simaroubiaceae) commonly known as Mahanimb. *Ailanthus excelsa* is a large tree originally from China, 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 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 [1]. The alcohol extract from leaf and stem bark exhibits remarkably high anti-implantation and early abortifacient activity [2]. The plant reported to contain chemical Constituents like Quassinoids, excelsin, glaucarubin, ailanthone, glaucarubinone, Glaucarubilone [3]. Quassinoids compounds were reported form Ailanthus excelsa leaves by Joshi Et al [4]. A new quassinoids, 13,18-dehydroexcelsin and glaucarubol have been isolated by Khan et al [3]. New dammarane-type triterpens, ailexcelone and ailexcelol, together with ocotillone, malabaricol, epoxymarabicol, lupeol were reported by Shrinivas et al [5, 6]. The present study is intended to explore the antifungal activity of Methanolic extract of Ailanthus excelsa. Whole plant was tested against selected human pathogenic fungi [7].

EXPERIMENTAL SECTION

Plant Materials:
The leaves of *Ailanthus excelsa* were collected from the University campus area during the month of August. The plant was botanically authenticated by the Botany department of Dr. Babasaheb Ambedker Marathawada University, Aurangabad. The leaves were dried under shaded condition in our laboratory for 7 days. The dried leaves were subjected to size reduction in multimill, powder size was kept at 40 mesh. Powder material was kept in an air tight plastic jar for further use at room temperature.

Preparation of extract:
Extract was prepared by continuous hot extraction using soxhlet apparatus with solvent petroleum ether, chloroform, methanol. Each extraction was continued for 70-80 hrs. About 2gm of accurately weighed homogenized drug was placed in glass stoppered conical flask. It was macerated with 100ml of solvent (water and alcohol for water extractive value and alcohol extractive value respectively) for 6 hour, shaking frequently and then was allowed to stand for 18 hours. Extract was filtered rapidly taking care not to loose any solvent [1]. 25ml of the filtrate was transferred to a tarred petri-dish and evaporated to dryness on a water bath. The residue was dried at 105 ^{O}c till its weight become constant, cooled in a desiccators for 30 minute and weighed immediately [8, 9].

Bioassay:
The efficacy of plant extract can be determined by inhibition of growth of test organism that are placed in contact with extract. Here qualitative antifungal screening was carried out using the agar well diffusion assay.

Inhibitory zone estimation:
Zone of inhibition was determined by 'Disk diffusion method' in which diffusion of an antifungal agent of specified concentration from disk into solid culture medium is determined and compared with standard. Saboured dextrose (3gm/100ml) and nutrient agar (2gm/100ml) dissolve in distil water to form nutrient medium. 20ml of nutrient medium was poured in each presterllised petriplate and allow to solidify. Plates seeded with 20µl fungal suspension. Cup of diameter 10mm was cut in solid medium which was loaded with sample solution (500mg/ml in chloroform) along with control. All plates were kept for incubation at 37^{0} c for 48 hrs, after incubation zone of inhibition was measured with vernier caliper in mm which is tabulated in table 1.

Determination of MIC:
Minimum inhibitory concentration (MIC) was regarded as the lowest concentration, which inhibit the growth of microorganism producing a visible zone of inhibition. MIC was determined by 'Broth dilution method', in which sterilized double strength saubourd dextrose medium (6gm/100ml) was added to presterllised test tubes. 1gm/ml sample was added to first test tube and by serial dilution method each test tube having concentration 500, 250, 125, 62.5 and 300, 400 mg was prepared. In above test tubes 20ugm/ml of fungal suspensions were added. All test tubes were incubated at 37^{o}c for 48hrs and turbidity was measured in colorimeter [10].

Phytochemical screening:
The plant may be considered as biosynthetic laboratory for multitude of compounds like alkaloids, glycosides, volatile oil, saponins, flavonoids etc [11]. These compounds are termed as secondary metabolites and are responsible for therapeutic effect [7, 12]. The presence of secondary metabolites in plants produces some biological activity in man and animal. They are the considerable tools, for the control of undesirable microorganism. Many plant contain nontoxic glycosides that can get hydrolyzed to release phenolics that are toxic to microbial pathogen. Therefore, the compounds detected may be responsible for the antifungal activity against the tested pathogen. To check the presence or absence of primary and secondary metabolites, all the extract were subjected to battery of chemical tests [13, 14, 15]. Positive tests along with results are tabulated in table 1.

Table 1: Phytochemical study of *Ailanthus excels*

Name of compounds	Preliminary Test	Pet. Ether extact	Unsaponifide fraction	Chloroform extract	Methanol extract	MECH
Sterols	Libermann- Burchard test	Present	Present	Present	Absent	Present
Terpens	Salkowaski test	Present	Present	Absent	Present	Present
Triterpens	Vanillin test	Present	Present	Absent	Present	Present

RESULTS AND DISCUSSION

The present work was carried out on different extract of *Ailanthus excelsa* leaves. The inhibitory effects of Ailanthus excelsa on different strains of fungi are shown in table 2. The inhibition level was measured with all microorganisms and it is clear that among fungi Aspergillus fumigatus exhibited significant level of inhibition when comparatively studied. MIC for MECH was 300, 250, 300, 400mg/ml for *A.niger, A.fumigatus, A. flavus, P.notatum* respectively.

Table 2: Inhibition of fungus by *Ailanthus excels*

Name of strain	**Zone of inhibition in mm**					
	Pet. Ether extract	Unsaponified fraction	Chloroform Extract	Methonol Exract	MECH	Control
A.flavus	0	0	0	2	4	0
P. notatum	0	0	0	4	5	0
A.niger	0	0	0	4	5	0
A.fumigatus	0	0	0	5.5	7	0

CONCLUSION

In the present work, attempt was made to study antifungal activity of *Ailanthus excelsa* and to isolate the constituent of active extract responsible for activity. Work has clearly proved that the chloroform fraction of methanol extract of plant have considerable antifungal activity, Which may due to presence of triterpens type 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 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 carried out on *Ailanthus excelsa* some useful drug may develop for treatment of fungal infection.

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