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STUDY OF TOTAL PHENOLICS, TOTAL TANNINS ANTIOXIDANT POTENTIAL AND ANTIMICROBIAL ACTIVITY OF *AILANTHUS EXCELSA* L

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ABSTRACT: *Ailanthus excelsa* leaves were evaluated for antimicrobial activity against clinical isolates obtained from patients with eye and skin infections and also for its antioxidant potential. The bacterial isolates obtained were identified to be *S. aureus, P. aeruginosa, E. coli* and the fungal isolates were identified as *Aspergillus niger and Penicillium.* The extract showed a maximum zone of inhibition against *A. niger* followed by *P. aeruginosa*. But it did not exhibit any inhibitory activity against *S. aureus.* There was a remarkable inhibitory activity recorded against *E. coli* and *Penicillium*. The extracts were found to possess 0.7954 µg/g of total Tannins and 1.41µg/g of total Phenols. The extract also exhibited 12.5% radical scavenging activity in FRAP assay and 8.5% activity in CUPRAC assay.

INTRODUCTION: Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, In fact, plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. The relatively lower incidence of adverse reactions to plant preparations compared to modern conventional pharmaceuticals, coupled with their reduced cost, is encouraging both the consuming public and national health care institutions to consider plant medicines as alternatives to synthetic drugs. Due to increased awareness of the limited ability of synthetic pharmaceutical products to control major diseases and the need to discover new molecular structures as lead compounds from the plant kingdom has forced our researchers to screen scientifically various traditional claims.

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Antibiotics are undeniably one of the most important therapeutic discoveries of the 20th century that had effectiveness against serious bacterial infections. However, only one third of the infectious diseases known have been treated from these synthetic products. This is because of the emergence of resistant pathogens that is beyond doubt the consequence of years of widespread indiscriminate use, incessant and misuse of antibiotics. Antibiotic resistance has increased substantially in the recent years and is posing an ever increasing therapeutic problem.

One of the methods to reduce the resistance to antibiotics is by using antibiotic resistance inhibitors from plants. Plants are known to produce a variety of compounds to protect themselves against a variety of pathogens. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resistant pathogens. Hence, researchers have recently paid attention to safer phyto-medicines and biologically active compounds isolated from plant species used in herbal medicines with acceptable therapeutic index for the development of novel drugs [1]. Thus, a proper scientific evidence or

assessment has become the criteria for acceptance of herbal health claims.

Infections caused by bacteria and fungal organisms on various body parts of the human beings are a very common event. Among these eyes and skin are more prone to infections as they are more exposed to the outer environment. Even though different types of medicines such as Allopathy are used in various treatments, due to the increased infectious conditions noticed in the population makes researchers search for alternate herbal therapies.

Plants with possible antimicrobial activity have been studied by a very large number of researchers in different parts of the world. Much work has been done on medicinal plants in India. It has been suggested that aqueous and ethanolic extracts from plants used in allopathic medicine are potential sources of antiviral, antitumor and antimicrobial agents. The selection of crude plant extracts for screening programs has the potential of being more successful in initial steps than the screening of pure compounds isolated from natural products [2].

Free radicals are generated in our body as results of oxidation of biomolecules. Formations of such free radicals are known to cause injury to cells and leads to inflammatory disorders and others. Natural antioxidants are the chemical agents which have the ability to quench the hazardous free radicals and to neutralize their potential to attack the cells [3].

Ailanthus excelsa Roxb is a tree belonging to family Simaroubaceae, which consist of 32 genera and over 170 shrubby species. It is indigenous to Central and Southern India and commonly it is known as a plant of Heaven. The traditional claims, phytochemical investigations and pharmacological evaluation and some Ayurvedic formulations provide the backbone to make this tree as a plant of Heaven. *Ailanthus excelsa* is also known as Mahanimba due to its resemblance with the Neem tree (*Azadirachita indica*) and Maharuksha due to its large size.

It is distributed in Western Peninsula, Rajasthan, Bihar, Orissa, Bundelkhand, throughout Madhya Pradesh, Broach and Panchamal district of Gujarat, in dry deciduous forests of Maharashtra, forest of Tamil Nadu, Scarce in Deccan and Karnataka. It is often planted along the roads. It is exotically found in Sudan. The plant is known for its high economical and commercial importance. Tribal in Nilgiris region traditionally use it in anti-fertility. [4] Studies on its extract has shown to reduce labour pain, febrifuge and presence of anticancer, antimicrobial, anti-amoebic and anti-protozoan activities [5].

The plant contains a rich source of β-sitosterol, Vaitexin, 13-18 dehydroglucarabol-15 and Glucarabol-15. The bark contains several Quassinoids including Ailanthone derivatives. The other compounds reported from this plant are Glaucarubinone, Ailanthin, beta-sitosterol, Malanthin, 1-*p*-deoxy-13-formyl ailanthinol, 13, 18-dehydro excelsin, Glaucarubin, Glaucarbol, 13-18-dehydro 15-*iso*-valearte and Trihydroxy tirucal 7-ene.

Methonolic extract of stem barks of *Ailanthus excelsa* Roxb was evaluated for anti-asthamatic activity by employing *in-vivo* and *in-vitro* screening models in Guinea pigs and reported that extract produced significant dose-dependent anti-asthamatic activity [6].

The study on Hepato protective activity of alcoholic leaf extract of *Ailanthus excelsa* root against Swiss Albino rats with liver damage induced by carbon tetra chloride ($CC1_4$), biochemical studies of blood samples of carbon tetrachloride treated rats showed significant increase in the levels of serum enzyme, reflecting liver injury and blood sampled from the animals treated with ethanolic root extracts of *Alianthus exelsa* has showed significant decrease in the levels of serum markers, indicating the protection of hepatic cell.

MATERIALS AND METHODS:

Extraction of Plant Material:

The aerial parts of the plants were collected and leaves were separated. Physical purity of the leaves was maintained. 100 gm of shade dried leaves were weighed and grounded using miller. The coarsely powdered leaves were packed in filter paper and kept for extraction in Soxhlet apparatus. 250 ml of ethanol was used for extraction. The extract

collected, evaporated in the water bath at 50°C and the resultant slurry was used for further studies.

Isolation of Clinical Samples:
The skin and eye infection samples were collected from Bowring Hospital, Bangalore. The collected samples were inoculated on nutrient broth and Sabouraud's dextrose broth. After 16 hrs of enriching, the cultures were spread on respective agar plates and incubated.

Identification of Microorganisms:
The organisms grown in nutrient agar media were subjected to biochemical tests and gram staining for identification. The bacterial organisms were also sub-cultured on selective media such as Mannitol Salt agar (MSA) media, Eosin Methylene Blue (EMB) agar media and Cetrimide agar media for identification. The fungal organisms grown on Sabouraud's dextrose agar media were identified based on colony characteristics, colour, texture, Microscopic features like hyphae and spore using lacto phenol cotton blue staining.

Antimicrobial activity:
The ethanol extract of *A. excelsa* leaf was subjected to antimicrobial test using agar well diffusion method. For the antibacterial activity Nutrient agar plates were used and for fungal cultures potato dextrose agar was used. Overnight enriched cultures were swabbed on the plates and wells of uniform size were bored using sterile borer. 1g of plant extract was dissolved in 10 ml of 80% ethanol. 20µl, 40µl, 60µl and 80µl of the extract were loaded into each well and the plates were incubated at respective temperatures.

Estimation of Phyto-constituents:
The concentrated residue was diluted and subjected to estimation of tannins and phenolic compounds. For estimation of tannins, Folin-Dennis reagent and sodium carbonate method was used with tannic acid as standard the absorbance was recorded at 700 nm against a blank. For phenolics, Folin-ciocalteau reagent was used and with caffeic acid as standard the absorbance was recorded at 650nm against a blank.

Antioxidant Activity: The extract was also subjected to evaluate its antioxidant potential. FRAP (Ferric Reducing Antioxidant Power) assay and CUPRAC (Cupric ion reducing antioxidant capacity) assay was carried out. In FRAP assay to 0.2 ml of extract, 3.8 ml of FRAP reagent (0.1mM acetate buffer pH 3.6, 0.25 ml of 0.3mM 2,4,6-tri pyridyl-s tri-azine (TPTZ) solution and 0.25ml of 10mM $FeCl_3$) was added and the tubes were incubated for 30 min at 37°C. Absorbance was recorded at 593 nm against a blank.

In CUPRAC assay, 1ml of 0.01M $CuCl_2$ was added into a clean test tube followed by the addition of 1ml of 7.5mM Neocuproine alcohol solution and 1 ml of ammonium acetate buffer of pH 5.66. After mixing thoroughly, 1ml of the extract was added followed by addition of 0.1 ml of deionised water. The tubes were mixed well and incubated for 30 minutes under room temperature. The absorbance was measured at 450nm against a blank.

RESULTS AND DISCUSSION:
Extraction of leaves:
Out of the 100 gm leaves extracted with 250 ml of ethanol, after 30 complete cycles, about 150 ml of residual solvents containing secondary metabolites was collected which after evaporation in the water bath gave about 8.23gms of thick slurry.

$$\text{Total percentage of yield} = \frac{\text{Weight of the residue}}{\text{Weight of the raw material}} \text{ X } 100$$

Thus the yield was 6%.

ANTIMICROBIAL ACTIVITY OF *AILANTHUS* EXTRACTS:
The plant *Ailanthus excelsa* extract was screened for its biological activity against the bacteria and fungi isolated from infected eye and skin clinical isolates using agar well diffusion method.

The antimicrobial activity of the plant extract was compared with the CONTROL 80% ethanol. The ethanolic plant extract produced zone of inhibition against both the fungal clinical isolates *Aspergillus niger* and *Penicillum*, two bacterial isolates *Pseudomonas aeruginosa* and *E coli* was susceptible to the plant extract while the ethanolic plant extract was found not to be effective against

Staphylococcus aureus. The maximum zone of inhibition was observed against *Aspergillus niger*.

Quantitative Estimation of Phytocontituents:

Estimation of Tannin:

0.5 ml of the unknown test sample corresponds to 0.21 OD at 670nm, from the standard graph plotted 0.5ml of the unknown test sample contains 48.5µg of Tannic acid and 10ml contains 970 µg. The total yield of the extract obtained from 100g of leaves is 8.2g.

Therefore 100g of the leaves contains 970×8.2 = 79.54. µg of Tannin

1g of the slurry contains 79.54/100 = 0.7954 µg of Tannin.

Estimation of Total Phenolics:

0.5ml of the unknown test sample corresponds to 0.34 OD at 650nm, from the standard graph plotted 0.5ml of the test sample contains 0.86 µg of Caffeic acid and 10ml contains 17.2µg.The total yield of the extract obtained from 100g of leaves is 8.2g. Therefore

100g of the leaves contains 17.2×8.2 = 141.04 µg of Caffeic acid.

1g of the slurry contains 141.04/100 = 1.41 µg of Caffeic acid.

Estimation of Antioxidant Potential of the Plant Extract:

FRAP Assay (ferric reducing antioxidant power)

The antioxidant potential of the test sample was determined using the standard graph. 0.4ml of the test sample corresponds to 0.14 OD at 570nm. From the graph 0.4ml of the test sample contains 96 µg of ascorbic acid.

$$\text{Scavenging Effect} = \frac{(\text{Control absorbance} - \text{Test absorbance})}{\text{Control Absorbance}} \times 100$$

$$= \frac{(0.16 - 0.14)}{0.1} \times 100$$

$$= 12.5\%$$

Test sample shows 12.5% of radical scavenging activity.

Cuprac Assay: (Cupric ion reducing antioxidant capacity assay)

The antioxidant potential of the test sample was determined using standard graph. 0.5ml of the test sample corresponds to 0.32 OD at 450nm. From the graph 0.2ml of the test sample contains 57 µg of ascorbic acid.

$$\text{Scavenging Effect} = \frac{(\text{Control absorbance} - \text{Test absorbance})}{\text{Control Absorbance}} \times 100$$

$$= \frac{(0.35 - 0.32)}{0.35} \times 100$$

$$= 8.57$$

Test sample shows 8.57% of radical scavenging activity.

Identification of the Clinical Isolates:

Three distinctive bacterial colonies were observed in plates inoculated with the clinical eye and skin samples which were identified based on the following:

TABLE 1: IDENTIFICATION OF CLINICAL ISOLATES

	Gram Staining	Growth On Selective And Different-Ial Media	Biochemical Test				
			Oxidase	Catalase	Indole	Methl Red	Voges Proskauer
Organism 1	Gram Positive cocci, in clusters.	On MSA showed yellow characteristic colony.	Negative	Positive	Negative	Positive	Negative
Organism 2	Gram negative small rods, in single.	On Cetrimide agar showed blue-green characteristic colony.	Positive	Positive	Negative	Negative	Negative
Organism 3	Gram negative bacilli.	On EMB agar showed characteristic purple coloured colonies with green metallic sheen.	Negative	Positive	Positive	Positive	Negative

On the basis of the above records three bacterial isolates isolated from infective eye and skin samples was identified to be *Staphylococcus aureus, Pseudomonas aeruginosa*, *Escherichia coli* respectively.

Based on the morphological characteristic observation the two fungi isolated from the infected skin samples were found to be *Penicillium* and *Aspergillus niger.*

CONCLUSION: Based on the above study we can conclude that the plant extract of *Ailanthus excelsa L* has considerable antioxidant activity which protects from toxic and harmful effects of free radicals and also shows anti-microbial against selective pathogenic organisms.

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