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PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY (STAPHYLOCOCCUS AUREUS AND PSEUDOMONAS AERUGINOSA) OF THESPESIA POPULNEA LEAF EXTRACTS BY DIFFERENT SOLVENTS

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Abstract: *The objective of the study is preliminary phytochemical screening and antimicrobial activity. The Thespesia populnea extract was made by using different solvents.*

Method: *The collected leaves were shade-dried and ground into a coarse powder. The powdered plant material was subjected to successive extraction using ethanol, chloroform, and ethyl acetate. The extracts were then analyzed for preliminary phytochemical constituents and tested for antimicrobial activity.*

Conclusion: *Thespesia populnea leaves have antibacterial activity against pathogenic microorganisms like Staphylococcus aureus and Pseudomonas aeruginosa. The given ethanol extract shows good antibacterial activity when compared to the chloroform and ethyl acetate*.

Key words: Thespesia populnea(L), Soxhlet method, phytochemical screening, anti-microbial activity.

I. INTRODUCTION

Thespesia populnea (L.) Soland. Ex.Correa, commonly referred to as the 'Portia tree' or 'Indian tulip tree,' belongs to the family Malvaceae. It is a small- to medium-sized tree typically found along coastal regions. The tree attains a considerable height during its 15-metre growth [1].

The stem bark, leaves, flowers, and fruits have been traditionally used in the treatment of cutaneous infections, including scabies, psoriasis, eczema, ringworm, and guinea worm [2].

The plant was found to be rich in diverse phytochemicals such as alkaloids and glycosides, flavonoids, terpenoids, carbohydrates, proteins, steroids, and colouring agents. [3].

The present study was designed to determine the antimicrobial activity of ethanol, chloroform, and ethyl acetate leaf extract of Thespesia populnea to estimate MHA of the selected extract against Streptococcus aureus and Pseudomonas aeruginosa and to compare its antimicrobial activity with the well-known antibiotic chloramphenicol [4].

Fig; 01 Thespesia populnea leaf

Taxonomical classification:

Taxonomy: Thespesia populnea

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Magnoliidae

Order: Malvales

Family: Malvaceae

Genus: Thespesia

Species: Populnea

Vernacular Name:

English: Indian tulip tree, portia tree, cork tree, umbrella tree.

Tamil: puvarasam, puvaraassu, poris, kallal, karvarachu.

Hindi: parasapeepala

Telugu: Ganga rari chettu

II. MATERIALS AND METHODS

Leaves and materials:

The Thespesia populnea leaves used in the study are collected from the Collectorate, Dharmapuri. The collected samples were carefully cleaned and rinsed two to three times with distilled water. The leaves were then shade-dried under ambient conditions for seven days. Their dead leaves are coarsely ground and make coarse talc.

Materials:

- Soxhlet extractor
- Extraction thimble
- Solvents [ethanol, chloroform, ethyl acetate]
- Sample [coarse powder]
- Heating mantle
- Condenser

Preparation of extracts:

The preparation of extracts from the powdered leaves of Thespesia populnea is done by using the following solvent:

1. Ethanol extract
2. Chloroform extract
3. Ethyl acetate extract

Ethanol extract

The shade-dried coarse powder of fifty grams of leaves was packed well in a Soxhlet apparatus and subjected to continuous hot extraction with 500 ml of ethanol for 18 hours. The extract was distilled in a vacuum under pressure in order to remove the solvent completely. The extract was dried and kept in a desiccator until use. The dried extract was then weighed, and the percentage yield was determined based on the air-dried crude drug.

Chloroform extract:

Fifty grams of shade-dried leaf powder were coarsely ground and packed into a Soxhlet apparatus for continuous hot extraction using 500 ml of chloroform for 18 hrs. The obtained extract was concentrated using vacuum distillation under reduced pressure to remove the solvent completely. The dried extract was subsequently stored in a desiccator until further use. The extract was weighed, and the percentage yield was calculated with respect to the air-dried powdered crude drug.

Ethyl acetate extract:

Fifty grams of shade-dried coarsely powdered leaves were tightly packed into a Soxhlet apparatus and extracted continuously with 500 ml of ethyl acetate for 18 hrs under hot conditions. The resulting extract was concentrated by vacuum distillation under reduced pressure to completely remove the solvent. The dried extract was kept in a desiccator until further use. The extract was then weighed, and the percentage yield was calculated relative to the air-dried powdered crude drug.

III. PHYTOCHEMICAL ANALYSIS OF THESPESIA POPULNEA

Test for alkaloids:

Dragendorff's test:

One mL of the extract was mixed with 1 mL of Dragendorff's reagent (potassium bismuth iodide solution). The formation of an orange precipitate indicated the presence of alkaloids.

Test for glycosides:

Test sol: It was prepared by dissolving the sample in alcohol.

Killer Killiani test:

To test the solution, a few drops of ferric chloride solution were added to concentrated sulphuric acid. This resulted in the formulation of two distinct layers a reddish- brown lower layer and a bluish-green upper layer.

Test for carbohydrates:

Mulish test:

To 2-3 ml of the test solution, a few drops of Molisch reagent were added, and the mixture was shaken. Concentrated sulfuric acid was then carefully added along the side of the test tube, resulting in the formation of a violet ring at the junction of the two liquids.

Test for terpenoids:

1 ml of extract was taken in a test tube, and 2 ml of chloroform was added to it, followed by the addition of 3 ml of con. The formation of a reddish-brown layer at the interface of the two solutions upon the addition of sulfuric acid confirms the presence of terpenoids.

Test for Flavonoids:

A small quantity of the extract was treated with lead acetate, resulting in the formation of a yellow precipitate, which indicates the presence of flavonoids.

Test for Tannins:

Test solution: the test solution was prepared by dissolving the extract in a mixture of water and alcohol.

Ferric chloride solution test:

To 1 ml of the extract, ferric chloride was added, resulting in the formation of a dark blue or greenish-black coloration.

Test for Saponins:

Test solution: The solution was prepared by dissolving the extract in water to obtain an aqueous solution.

Foam test:

The dry extract was vigorously shaken with water. The absence of persistent foam indicates the absence of saponins.

Test for proteins:

Test sol: The extract was dissolved in water to obtain an aqueous extract.

Biuret test:

To 2-3 ml of the extract solution, sodium hydroxide and a few drops of copper solution were added, resulting in the formation of a blue color.

Test for Fat:

Solubility test: Fats are insoluble in water and ethanol but soluble in chloroform, benzene, and ether in the presence of fat.

Test for cholesterol:

Add 2 ml of chloroform with 10 drops of acetic anhydride and add 2-3 drops of conc. sulphuric acid, which appears rose-red in color and shows the absence of cholesterol.

Test for steroids:

Add extract solution, a few drops of chloroform, and a few drops of conc. sulphuric acid; it gives a reddish-brown ring interface.

TABLE 01: Phytoconstituents of ethanol, chloroform, and ethyl acetate of dried leaves of Thespesia populnea (L).

S.NO	PHYTOCONSTITUENTS	ETHANOL	CHLOROFORM	ETHYL ACETATE
1	ALKALOIDS	+	+	+
2	GLYCOSIDES	+	+	+
3	CARBOHYDRATES	+	+	+
4	TERPENOIDS	+	+	+
5	FLAVANOIDS	+	+	+
6	TANNIS	+	_	+
7	SAPONINS	+	+	_
8	PROTEINS	+	+	+
9	FATS	_	+	+
10	CHOLESTROL	_	+	_
11	STEROIDS	+	+	+

IV. ANTIMICROBIAL ACTIVITY OF THESPESIA POPULNEA

Test microorganisms:

A total of one bacterial strain was used throughout the investigation. Bacterial cultures were obtained from the Microbial Type Culture Collection, the Institute for Microbial Technology, and Chandigarh, India. The bacteria used were Staphylococcus aureus and Pseudomonas aeruginosa.

ANTI-BACTERIAL ASSAY:

1. **Preparation of inoculum:**

   Stock cultures were maintained at 4°C on slopes of nutrient agar. Active bacterial cultures were prepared by transferring a loop full of cells from the stock cultures into a test tube containing Muller-Hinton broth (MHB). The culture was incubated at 37°C for 24 hours. The bacterial cultures were diluted using fresh Muller-Hinton broth to obtain a density equivalent to 2.0 X 106 colony-forming units (CFU/ml) for bacteria (Smith & Doe 2018).

2. **Preparation of sterile swabs:**

   Cotton wool swabs made on wood or plastic shafts were prepared and sterilized either by autoclaving or by dry heat (the latter method was used only for the wooden swabs). Sterilization was carried out by packing the swabs into culture tubes, wrapping them in paper, and placing them in tins, as appropriate.

3. **Sterilization of forceps:**

   Sterilization of forceps can be achieved by dipping them in alcohol followed by flaming to remove the alcohol.

Antibacterial assay using the agar well diffusion method:

The antibacterial activity of crude drug extracts was determined by the well diffusion method.

In this study, the antibacterial activity of the test sample was assessed using Mueller-Hinton agar (MHA), a standard nutrient medium recommended for antimicrobial susceptibility testing. Muller-Hinton agar (MHA) obtained from Himedia (Mumbai) was prepared by pouring approximately 50 mL of molten medium into sterile petri dishes and allowing it to solidify.

A 0.1% bacterial inoculum was prepared and uniformly spread over the agar surface using a sterile cotton swab to ensure even microbial growth. The plate was kept aside for a few minutes to allow the inoculum to dry.

Sterile wells of equal diameter were made on the agar using a cork borer, and 100 µl of the test extract was carefully added to each well. A standard antibiotic, chloramphenicol, was used as the positive control to compare the activity of the sample diffused into the medium and acting against the bacteria. After incubation, antibacterial activity was evaluated by measuring the zone of inhibition.

The antimicrobial activity was assessed using the agar well diffusion method. The extract exhibited antibacterial activities against the tested microorganism.

Table 02: Antibacterial activity of ethanol, chloroform and ethyl acetate with 100 µl concentration

S.NO	MICROORGANISM	EXTRACT	ZONE OF INHIBITION	
			100µL	STANDARD
1.	STRETTOCOCCUS AUREUS	EHANOL	20	25
		CHLOROFORM	17	25
		ETHYL ACETATE	16	25
2.	PSEUDOMONAS AURUGINOSA	ETHANOL	21	24
		CHLOROFROM	16	24
		ETHYL ACETATE	14	24

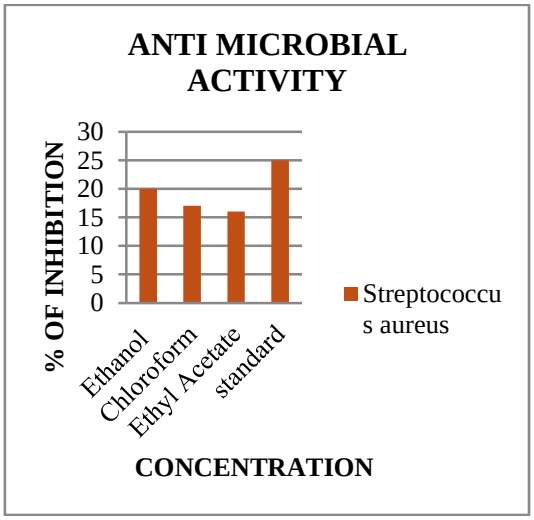

Fig. 02: Concentration vs. Inhibition

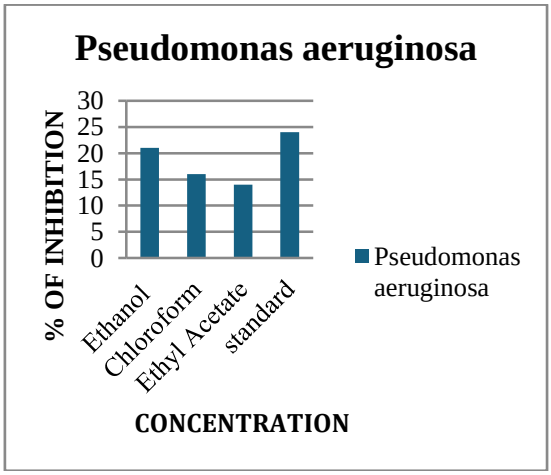

Fig. 03 Concentration vs Inhibition

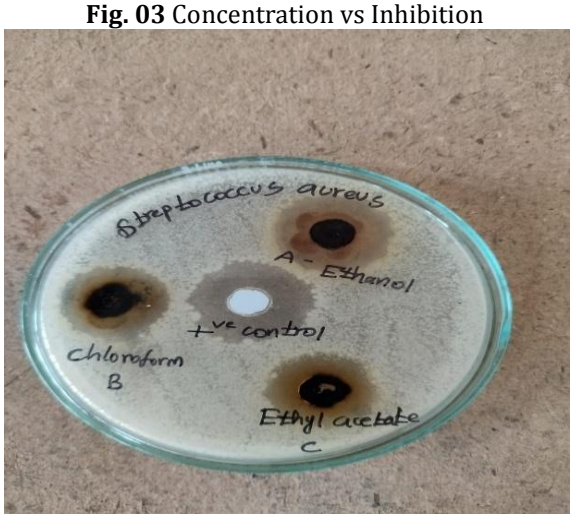

Fig. 04 Streptococcus aureus (Ethanol, Chloroform, Ethyl Acetate)

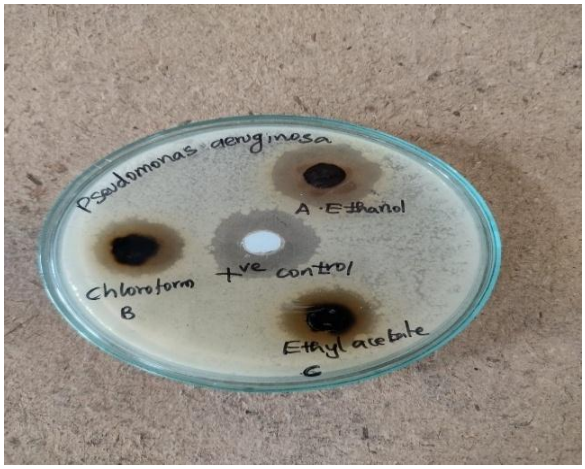

Fig. 05 Pseudomonas aeruginosa (Ethanol, Chloroform, Ethyl Acetate)

V.CONCLUSION

The given sample shows the antibacterial activity against pathogenic microorganisms, such as Staphylococcus aureus (20 mm) and Pseudomonas aeruginosa (21 mm). The ethanol extract demonstrated significant antimicrobial activity against both Staphylococcus aureus and Pseudomonas aeruginosa compared to the chloroform and ethyl acetate extract. Among the extracts tested, the ethanol extract showed the highest efficacy, while the ethyl acetate extract. Extract exhibited the least antimicrobial activity.

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