

Phytochemical Analysis and *In Vitro* Antifungal Activity in Leaves Extract of *Santalum Album L., Dalbergia Sisso Roxb. and Madhuca Indica L*

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ABSTRACT: - Plants historically have served as models in drug development. The production of Medicines and the pharmacological treatment of diseases began with the use of herbs. The aim of the present study to investigate the phytoconstituents and antifungal Potential from the natural source i.e. from the leaves of *Santalum album*, *Dalbergia sisso* and *Madhuca indica*. Aqueous and Methanolic extracts were screened for Preliminary phytochemical analysis and antifungal activity using Disc diffusion method against different plant pathogenic organisms. The results revealed that the methanolic extract exhibited significant antifungal activity of concentration of 100-500 µg/ml respectively against tested organisms.

KEYWORDS: - Phytoconstituents, *Santalum album*, *Dalbergia sisso*, *Madhuca indica*, antifungal.

I. INTRODUCTION: -

Medicinal plants are finding use as pharmaceuticals, nutraceuticals, cosmetics and food supplements^[2]. Plant derived products have been used for medicinal purposes for centuries. In the past few decades, a worldwide increase in the incidence of fungal infections has been observed. The majority of clinically used anti-fungals have various drawbacks in terms of toxicity, efficacy and cost, and their frequent use has led to the emergence of resistant strains. With an increase in the antibiotic-resistant strains of microorganisms, traditional plants are being investigated for their antifungal and medicinal values. Traditional uses of plants have led to investigating their bioactive compounds, which have resulted in the detection of a significant number of therapeutic properties^[1]. In 1998, the WHO estimated that 80% of the people living in developing countries almost exclusively use traditional medicine^[3].

II. MATERIAL AND METHODS

Fresh and disease free leaves of *Santalum*, *Madhuca* and *Delbergia*. Were collected from the campus of Government Institute of Science, Aurangabad. Collected leaves were washed with distilled water and then put it for drying in shade. After drying the dried leaves were grinded in fine powder using mechanical mixer.

III. PREPARATION OF METHANOLIC EXTRACT AND AQUEOUS EXTRACT

The air dried and powdered parts of *Santalum*, *Madhuca* and *Delbergia Madhuca indica* (250 gm) were extracted in soxhlet with Aqueous and Methanolic (2 each) and subsequently was concentrated to a small Volume. Different conc. were prepared for methanolic and aqueous extract viz; 5%, 10%, 15% in 80% methanol. The concentrated extracts were tested for preliminary phytochemical assay and antifungal activity.

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IV. PHYTOCHEMICAL TESTS

Identification of Plant Constituents by Phytochemical Tests -

Methanolic extract was subjected to various preliminary phytochemical analysis to test for the presence or absence of various phytochemical constituents by the following tests. The results were mentioned in table 1.

V. ANTIFUNGAL ASSAY

DISC DIFFUSION METHOD -

6mm fungal mycelium discs were prepared using cork borer. Mycelium discs were inoculated on dried PDA plates. 10µl of the extraction was placed on the disc. These plates were incubated at 37°C. Zone of inhibition was noted around the disc at 24 and 48 hrs. The positive control used in the study was 99.9% methanol. The zone of Inhibition of fungal growth was measured and compared with standard antibiotics. Each experiment was performed in triplicate; their mean of linear growth of zone of inhibition was recorded and calculated as follows: (Alves *et al.*, 2000; Janaki *et al.*, 1998).

$$\% \text{ inhibition of fungal growth} = \frac{100 - \text{Linear growth in test (mm)}}{\text{Linear growth in control (mm)}} \times 100 \text{ tests (mm)}$$

VI. RESULTS

1) TEST FOR ALKALOIDS -

To the extract dilute hydrochloric acid (HCl) was added and filtered. The filtrate was treated with various alkaloid reagents.

- A) **Mayer's test** - The filtrate was treated with Mayer's reagent: appearance of cream color indicated the presence of alkaloids.
- B) **Dragondroff's test** - The filtrate was treated with dragondroff's reagent: appearance of reddish brown color precipitate indicated the presence of alkaloids
- C) **Hager's test** - The filtrate was treated with Hager's reagent: appearances of yellow color indicate the presence of alkaloids.

2) TEST FOR CARBOHYDRATES AND REDUCING SUGARS

- A) **Molish's test** - To a small portion of filtrate add a little amount of Molish's reagent and Sulfuric Acid (H₂SO₄): Formation of a violet ring indicated the presence of carbohydrates.
- B) **Fehling's test** - To a small portion of filtrate add a little amount of Fehling's Reagent A and Fehling's Reagent B: formation of a reddish brown indicated the presence carbohydrates

3) **TEST FOR STEROIDS - LIEBERMANN BURCHARD'S TEST** - The extract was treated with 3ml of acetic anhydride, few drops of glacial acetic acid followed by a drop of concentrated H₂SO₄.

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4) TEST FOR PROTEINS –

A) Biuret test - To the extract add small amount of copper sulphate solution followed by addition of NaOH solution. Formation of a violet colour indicated the presence of proteins.

5) TEST FOR TANNINS- The extract was treated with 10% lead acetate solution. Formation of a white color indicated the presence of tannins.

6) TEST FOR PHENOLIC COMPOUNDS -

A) Extract was treated with neutral ferric chloride: formation of a violet color indicated the presence of phenolic compounds.

B) Extract was treated with 10% NaCl solution formation of a cream colour indicated the presence of phenolic compounds.

7) TEST FOR FLAVONOID - A) 5ml of extract was hydrolyzed with 10% of H₂SO₄ and cooled, then it was extracted with diethyl ether and divided into three portions in 3 separate test tubes, 1ml of diluted sodium carbonate, 1ml of 0.1 N sodium hydroxide and 1ml of strong ammonia solution was added to the first, second and third test tube respectively in each test tube. Development of yellow color demonstrated the presence of flavonoids.

B) Shinoda's Test - The extract was dissolved in alcohol. To which few magnesium turnings were added followed by concentrated HCl drop wise and heated. The appearance of magenta color indicated the presence of flavonoids.

8) TEST FOR GLYCOSIDES -When a pinch of the extract was treated with glacial acetic acid and few drops of ferric chloride solution followed by addition of concentrated H₂SO₄.formation of a ring at junction of two liquids.

9) TEST FOR SAPONINS -FOAM TEST -- About 1ml of the extract was diluted to 20 ml with distilled water and shaken well in a test tube. The formation of foam in upper part of test tube indicated presence of Saponin.

Table 1: Preliminary Phytochemical screening of *Santalum*, *Madhuca* and *Dalbergia* leaves extracts

Constituent	Alkaloid	Carbohydrate	Reducing Sugar	Steroid	Protein	Tannin	Phenolic	Flavonoids	Glycosides	Saponin
<i>Santalum album</i> L.	++	+	+	-	+	-	+++	+++	-	+
<i>Madhuca Indica</i> L.	++	++	+	--	++	++	+++	++	+	-
<i>Dalbergia sisso</i> Roxb.	+	-	-	+	++	-	+++	++	+	-

Photoplates – Antifungal activity against targeted pathogens

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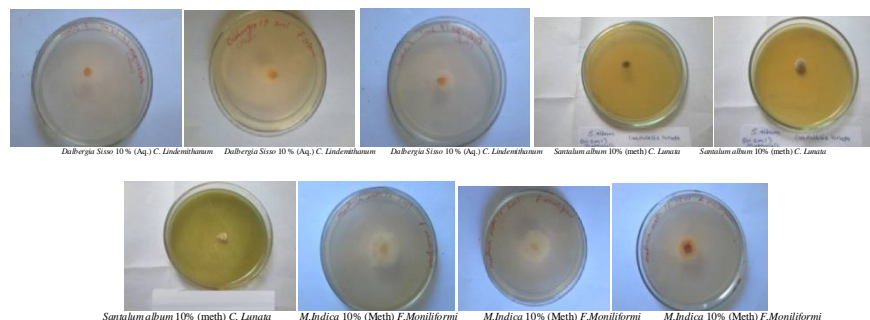


Table 2:-Antifungal activity of selected plant extract

Sr.No.	Plant Name→ Tested Pathogen ↓	<i>Santalum album,L.</i>	<i>Dalbergia sissoo,roxb.</i>	<i>Madhuca indica,L.</i>
		1	<i>Colletotrichum lindemithanum</i>	++
2	<i>Curvularia lunata</i>	+++++	++	+
3	<i>Fusarium moniliformi</i>	+	+	+++++

VII .DISCUSSION

In the present studies phytochemical analysis of aqueous and methanolic leaf extract of selected plants showed phenolic and alkaloid constituent's. *Dalbergia sissoo* aqueous extract showed highest inhibition against *collectotrichum lindemithanum* at 10 % concentration in compare to methanolic extract against *F.Moniliformi* and *C. Lunata*. *Santalum album* methanolic extract showed highest inhibition against *Curvularia lunata* and *Madhuca indica* showed highest inhibition against *F.monoliformi*. Extract of *Madhuca indica* showed good potency in terms of inhibition zones against all the tested fungal strains. It was observed that the zone of inhibition varies from one organism to another at different concentrations.

The successful management of new and emerging resistant fungal pathogens of humans in the present and future depends on the discovery and/or development of new antifungal agents. The plant secondary metabolism is a natural source of a wide range of classes of substances with antimicrobial and antifungal properties, fostered by the intense pressure exerted by microbial pathogens in the environment (Silva et al., 2009).

Further studies are needed to determine the chemical identity of the bioactive compounds responsible for the observed antifungal activity. Natural plant-derived fungicides may be a source of new alternative active compounds, in particular with antifungal activity. The high proportion of active extracts in the assayed species, selected according to available ethnobotanical data, corroborates the validity of this approach for the selection of plant species in the search for a specific activity.

VIII. CONCLUSION

In the preliminary screening, it was observed that the leaves of all plants possess antifungal activity at different levels. The antifungal activity of each plant extract was influenced by the solvent used for extraction, Process condition for extraction and test microorganisms. Therefore, this plant extract has a great potential for further research to discover the activity of isolated compounds against other microorganisms. In addition, this new antifungal compounds could be used for the development of new antifungal drugs to treat infectious diseases. It is concluded that

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in co ordinance of the chemical literature finding resistant strains of organism plant biodiversity may lead to unexpected research findings.

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