ANTIFUNGAL ACTIVITY OF ZIZIPHUS MAURITIANA AGAINST CANDIDA ALBICANS AND ASPERGILLUS NIGER

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ABSTRACT
Ziziphus mauritiana Lam. is a medium sized tree (Rhamnaceae) grows in almost all parts of the country. It is a potent medicinal plant with many biologically active components such as phenolics, flavonoids, triterpenic acids, polysaccharides and saponins. It majorly possesses anti cancer, anti oxidant, wound healing, antifertility, anti inflammatory, antilulcer, antidiaphoreal and antifungal activities. In the present study, anti fungal activity of Ziziphus mauritiana was tested with methanol extracts against various pathogenic fungi such as Candida albicans. The invitro anti fungal activity was performed by agar well diffusion method. Of the leaf extracts, methanol extract from Z. mauritiana leaves exhibited significant antifungal activity. In anti fungal studies, methanol leaf extract showed promising results against Candida albicans.

KEYWORDS: Ziziphus mauritiana, Methanol extract, antifungal activity, Candida albicans, Aspergillus niger.

1. INTRODUCTION
Ziziphus mauritiana a tropical fruit tree species. It is a spiny, evergreen shrub or small tree up to 15 m high, with trunk 40 cm or more in diameter; spreading crown; stipular spines and many drooping branches. The fruit is of variable shape and size. It is oval, obovate, oblong or round, and it can be 1-2.5 in (2.5- 6.25 cm) long, depending on the variety. The flesh is white and crisp. When slightly unripe, this fruit is a bit juicy and has a pleasant aroma. The fruit's skin is smooth, glossy, thin but tight. It is the most commonly found in the tropical and sub-tropical regions. Originally native to India it is now widely naturalized in tropical region from Africa to Afghanistan and China, and also through Malaysia, Australia and in some pacific regions. It can form dense stands and become invasive in some areas, including Fiji and Australia and has become a serious environmental weed in Northern Australia. It is a fast growing tree with a medium life span that can quickly reach up to 10–40 ft (3 to 12 m) tall.

Vernacular Names
English: Chinee apple, Chinese date, cottony jujube, Indian cherry, Indian jujube, Indian plum, jujube
Fijian: baer
French: jujubier, massonnier
Hindi: baher, bahir
Spanish: azufaifo africano

In traditional medicine of Ayurveda, unripe fruits are used to pacify “Vata”, the leaves, fruits, bark & even roots are used to treat a variety of ailments including cold, flu and malnutrition related diseases in children, convulsions and indigestion. The leaves are applied as poultices and are helpful in liver troubles, asthma, fever and to treat sores and the roots are used to cure and prevent skin diseases. All the parts of this plant are very effective against different types of diseases. Its leaves are useful in the treatment of diarrhoea, wounds, abscesses, swelling and gonorrhoea. The leaves of Z.mauritiana are also used in the treatment of liver diseases, asthma and fever. The fruit has been used as anodyne, sedative, tonic anticancer and potent wound healer. The fruit, leaves and seed extracts have been shown to exhibit antioxidant activity, where as bark is reported to have cytotoxicity against different cancer cell lines.
Classification

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Rosales
Family: Rhamnaceae
Genus: Ziziphus
Species: Z. mauritiana
Binomial name: Ziziphus mauritiana Lam.

Chemical Constituents
It is a rich source of cyclopeptide alkaloids lupane and triterpenes. Cyclopeptidmacrocycles of Ziziphus species showed interesting biological properties, including sedative, analgesic, antibacterial, antifungal and, antiplasmodial activity etc. It have 14-membered ring cyclopeptides to be the largest subgroup of alkaloid obtained, whereas only one 13-membered macrocyclic alkaloid isolated from this plant. These included the 4(14)-membered ring class: mauritine C, amphibine F and frangufoline the 5(14)-membered ring type: mauritines A and B. It also contain protein, carotene and vitamin C. The leaves are readily eaten by camels, cattle and goats and are considered nutritious.

Collection of Plant Materials
Fresh leaves of Z.mauritiana free from disease were collected from the village of lonkheda, and the plant was identified and authenticated by Dr. S. K. Tayade, Dept. of Botany, P. S. G. V. P. Mandal’s, Shahada, Dist-Nandurbar.

2. MATERIALS AND METHOD
Preparation of extract
Fresh leaves of Z.mauritiana were rinsed with distilled water to remove any dust and particulate matter. Leaves were spread on a filterpaper sheet in well ventilated room. The dried leaves were ground with the help of food processor (Singer, FP-500) into a fine powder. The powder was passed through sieve(0.25 mm). Sieved powdered material was stored in tightly packed polyethylene bags. Extraction was carried out as with slight modification. Briefly, 8g of leaf powder was extracted with 80 mL methanol on an orbital shaker at 350 rpm for 5 hrs. The excess solvent from the filtrate was evaporated under vacuum using a rotary evaporator. The crude concentrated extract was transferred to dark brown colored sample vial and stored at room temperature for further uses.

Chemical Test

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>TEST</th>
<th>OBSERVATION</th>
<th>INFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>ALKALOIDS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a)</td>
<td>Mayer’s test:</td>
<td>Yellow cream ppt observed</td>
<td>Present</td>
</tr>
<tr>
<td>b)</td>
<td>Wagner’s test:</td>
<td>Brown reddish ppt observed</td>
<td>Present</td>
</tr>
<tr>
<td>2.</td>
<td>FLAVONOIDS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a)</td>
<td>Lead acetate test:</td>
<td>Yellow ppt observed</td>
<td>Present</td>
</tr>
<tr>
<td>b)</td>
<td>H2SO4 test:</td>
<td>Orange colour observed</td>
<td>Present</td>
</tr>
<tr>
<td>3.</td>
<td>STEROIDS</td>
<td>Colour change does not occur</td>
<td>Absent</td>
</tr>
<tr>
<td>4.</td>
<td>TERPENOIDS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a)</td>
<td>Salkowski test:</td>
<td>Reddish brown colour of inner phase observed</td>
<td>Present</td>
</tr>
<tr>
<td>5.</td>
<td>ANTHRAOQUINONES</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a)</td>
<td>Borntrager’s test:</td>
<td>Pink colour does not observed</td>
<td>Absent</td>
</tr>
<tr>
<td>6.</td>
<td>PHENOLS</td>
<td></td>
<td></td>
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<tr>
<td>-----</td>
<td>-------------------------</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>a)</td>
<td>Lead acetate test</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yellow colour observed</td>
<td>Present</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>SAPONINE</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Creamy texture observed</td>
<td>Present</td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>TANNINS</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Formation of dark green colour</td>
<td>Present</td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>OILS AND RESINS</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Transparent appearance on filter does not observed</td>
<td>Absent</td>
<td></td>
</tr>
<tr>
<td>10.</td>
<td>GLYCOSIDES</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a)</td>
<td>Legal test:</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pink to red colour does not appear</td>
<td>Absent</td>
<td></td>
</tr>
</tbody>
</table>

**Determination of antifungal activity**

**Antimicrobial screening**
The leaf extracts of *Z. mauritian* were tested for antifungal activity against the selected fungi. Nutrient agar media were used for fungal growth. The media were prepared and then autoclaved at 121°C for 15 minutes and were poured on petriplates and allowed to solidify.

**Well Diffusion Method**
Well diffusion method is used to evaluate antifungal activity. The prepared culture plates were inoculated with selected strains of fungus using spread plate method. The wells were made on the agar surface with sterile cork borer. The extracts were poured into the well using micropipette. Ketoconazole was used as positive reference standard to determine the sensitivity of each fungal species tested. The fungal culture plates were incubated at 37°C for 48 hours. The zone of inhibition was calculated by measuring the diameter of the zone around the well in millimeters (mm).

**Reagents and Requirements**

**Nutrient agar plate**
It is prepared by dissolving 2.8gm of nutrient agar in 100ml of distilled water and into that 2gm of agar agar powder added for solidification. The dissolve medium was autoclave at 151bs pressure at 121 C for 15 min. The autoclave medium was mixed well and poured into petriplate up to 100mm while still.

3. **RESULTS AND DISCUSSION**

| Zone of inhibition over fungi candida albicans (Extract) |   |

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**Procedure**

1) Four sterile petri plate containing 25 to 30 ml of nutrient medium were seeded with 48hr culture bacterial strain.
2) The procedure is taken sterile petri plate into the nutrient medium were added in aseptic area or aseptic condition to avoid microbial contamination.
3) After adding of nutrient medium the fungal strain are incorporated into that sterile petri plate
4) This plate then kept into an incubator for growth of fungi.
5) After 48 hours remove the plate from incubator and in aseptic condition or in between two burner bore the plate.
6) Into that bore add sample that is from bear leaf
7) Each plate of nutrient agar is prepared according to this manner and sample were added
8) In plate 1 and 2 the fungal strain is taken i.e. *candida albicans* and into the bore of that plate extract and standard drug were added the plate were kept into incubator for 48 hours for the growth of microbes. The growth is observed and noted zone of inhibition this plate are nutrient agar plate for albicans candida.
9) In plate 3 and 4 the fungal strain is taken i.e. *Aspergillus niger* and into the bore of that plate extract and standard drug were added the plate were kept into incubator for 48 hours for the growth of microbes.
Antifungal activity of leaf extract of Ziziphus mauritiana Lam.

<table>
<thead>
<tr>
<th>Test Organism</th>
<th>Zone of inhibition (mm) (Methanol extract)</th>
<th>Standard (Ketoconazole)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida albicans</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>-</td>
<td>8</td>
</tr>
</tbody>
</table>

Note: - (-) = No Zone of inhibition.
4. CONCLUSION
The results demonstrate that antifungal activity of these plant against also dermatophytes correlates well with the claims of traditional uses for infection since some of these plant appeared to have broad spectrum of activity and are cheap they could be useful in antifungal formulation.

5. ACKNOWLEDGEMENT
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6. REFERENCES
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