IN-VITRO ANTILEPROTIC ACTIVITY OF PETROLEUM ETHER EXTRACTS OF DALBERGIA SISSOO BARK

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ABSTRACT

Leprosy is a chronic infectious disease mainly affecting skin and peripheral nerves in patients. The present study investigated activity of ethanolic extracts of Dalbergia sissoo bark against Mycobacterium leprae using agar-well diffusion method. Zone of inhibition was determined by taking Dapsone as the positive control and ethanol as the negative control. Dalbergia sissoo was found effective against M. leprae. Material and Method: Plant material: Dalbergia sissoo Bark was collected from riverside of Loni KD. Dist. Ahmednagar (Maharashtra). The taxonomical identification and authentication was done by Dr. Medakkar Head department Botany Arts, Science and commerce college Rahuri. Test Organism: The test microorganism used in the study was Mycobacterium leprae. The bacterial strain was maintained in sterile conditions and was grown on nutrient agar medium. Standard bacterial suspension of 108 (CFU)/ml was prepared and used. Preparation of extracts: Bark of plant of Dalbergia sissoo was washed with distilled water further it was separated and air dried at room temperature under shade. 250 gm of the dried bark was extracted with Petroleum ether by soxhlation method. Phytochemical investigation of the extracts was conducted. These extracts were used to study the in-vitro antileprotic activity. Method: Cup-plate agar diffusion method using Nutrient agar: In a radial or 2D technique, Petri dishes of agar were prepared by pouring melted agar media previously inoculated with Mycobacterium Leprae microorganism. After the solidification agar cups are made with the help of borer and cups are filled with solution of suitable concentration of sample and standard respectively and are inoculated at 37°C for 24 hrs. Result and Discussion: Petroleum ether extract of Dalbergia
sissoo bark was used to determine its antileprotic activity via an Agar diffusion method. The antileprotic activity ranges from 4.55 mm to 6.22 mm for *Mycobacterium Leprae* in comparison with std. Antileprotic drug Dapson (100 μg /ml); it was found that the Petroleum ether extract of β-Sitosterol (100 μg /ml) has antileprotic activity almost equivalent to standard compound. **Conclusion:** The Petroleum ether extracts shows the presence of β sitosterol as active compound. The Preliminary Phytochemical studies of this extracts shows the presence of steroids, Alkaloids, Flavonoids, Saponins, Tannin compounds. Which are responsible for Antileprotic activity.

**KEYWORDS:** *Dalbergia sissoo* bark, Petroleum ether extracts, β sitosterol, *Mycobacterium Leprae*.

**INTRODUCTION**

Leprosy is commonly known as Hansen's disease (HD)\(^1\) is a long-term infection by the bacterium *Mycobacterium leprae* or *Mycobacterium lepromatosis*.\(^2\) Initially, in the starting period infection are without symptoms and naturally remain by this way for mostly 5 to 20 years. Symptoms that occur include granulomas of the nerves, respiratory tract, skin, and eyes this causes further in a lack of ability to feel pain, which can lead to the loss of parts of sensitivity due to repeated injuries and infection due to ignorance towards wounds\(^3\) there can be serious weakness of important body parts and it affects the visibility of the eyes.

Leprosy is spread commonly between people and possibly from insectivorous animals. The spreading of infection generally occurs by cough and the fluid coming from the nose of infectious persons due to its contact it spreads rapidly. People of less income group affected very recently with Leprosy.\(^4\) *Dalbergia sissoo* are cultivated by seeds and suckers. The trees are sometimes smaller but can grow upto 27 m in height and upto 4 m in diameter\(^3,\)\(^5\) sometimes the trees are smaller. Leaves are about 16 cm long these are alternate, leathery, and pinnately compound. Barks are 2.5 cm in thickness, sheds in narrow strips. Plant contains large number of upper branches which supports spreading crown.\(^6\)

**EXPERIMENTAL METHODS**

Antileprotic activity is determined based on the in-vitro activity in pure cultures. *In-vitro* susceptibility tests are done by the following methods.
Agar diffusion method\(^7\)

In this technique petri dishes of agar are prepared by pouring method. The microorganisms were inoculated in agar. In the agar dilution method different antibiotic concentrations are incorporated into an agar medium. The plates are incubated at a temperature of 37\(^0\)C for 24 hrs. A clear zone of inhibition was produced in agar due to diffusion of the antimicrobial substances. The diameter of this zone of inhibition can be measured and an estimation of the degree of activity of the antimicrobial substance can be obtained.

Materials Used

1) **Culture:** *Mycobacterium leprae* used for activity

2) **Apparatus:** Sterile petri plates, sterile cotton swabs, sterile cork borer, sterile test tubes, 1ml syringes, micropipette, Inoculating loop, Spirit lamp, etc.

3) **Media:** From Hi-Media nutrient agar was used with composition

*Composition of nutrient agar media*

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptic digest of animal tissue</td>
<td>5.00 gm/lit</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.00 gm/lit</td>
</tr>
<tr>
<td>Beef extract</td>
<td>1.50 gm/lit</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>1.50 gm/lit</td>
</tr>
<tr>
<td>Agar</td>
<td>15.00 gm/lit</td>
</tr>
</tbody>
</table>

In 1000 ml of distilled water dissolve 28 gm of media by heating, sterilized by autoclaving at 121\(^\circ\)C temperature and 15 lb/Inch\(^2\) pressure for 15 minutes.

Preparation of Inoculums

One day prior to activity testing, inoculations of the above bacterial cultures were made in the Nutrient agar media and incubated at 370\(^\circ\)C for 18-24 hrs.

Preparation of test solutions

Each test compound (1mg) was dissolved in Dimethylformamide (10 ml) to give stock solution of concentration 100 \(\mu g\) /ml. Then 0.1 ml of this solution was used for testing.

Preparation of standard solution

Standard drug Dapson was used at the concentration of 100\(\mu g\) /ml.
Method of testing
Nutrient agar plates were prepared by pouring 15-20 ml of the medium into each sterilized petri dish and were allowed to set at room temperature. The cell suspension was standardized to the density of 530 nm using spectrophotometer and was inoculated over the surface of agar medium using sterile cotton swab. The cups were scooped in each plate using a sterile cork borer of 6 mm diameter.

By using micropipettes test solutions of compounds about 0.10 ml were added in cups after this these plates were incubated at 37°C for 48 hrs. The zone of inhibition was measured in mm for each organism.

OBSERVATION
Plates were observed within 20 to 24 hrs and may be continued to incubate for 48 hrs. Zone of inhibition of the compound were measured and compared with the standard compound.

Evaluation of Antileprotic potential
Method: Cup-plate agar diffusion method using Nutrient agar.

In a radial or 2D technique, Petri dishes of agar were prepared by pouring melted agar media previously inoculated with *Mycobacterium Leprae* microorganism. After the solidification agar cups are made with the help of borer and cups are filled with solution of suitable concentration of sample and standard respectively and are inoculated at 37°C for 24 hrs.

The anti-microbial agents diffuses through the agar around its cup and produces a characteristic zone of inhibition of the microorganism sensitive to the sample, the diameter of which can be measured.

Zone of inhibition of the extract of *Dalbergia sissoo* bark against *Mycobacterium Leprae*.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Zone of inhibition (mm) 100μg/ml Mean±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pet. Ether</td>
<td>6.22±0.516</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>4.55±0.33</td>
</tr>
<tr>
<td>Methanol</td>
<td>5.66±0.300</td>
</tr>
<tr>
<td>Ethanol</td>
<td>5.83±0.307</td>
</tr>
<tr>
<td>Standard (Dapsone)</td>
<td>8.83±0.47</td>
</tr>
<tr>
<td>Pet. Ether</td>
<td>6.22±0.516</td>
</tr>
</tbody>
</table>
Zone of inhibition of the petroleum ether extract of *Dalbergia sissoo* bark against *Mycobacterium Leprae*

The Petroleum ether extracts shows the presence of β sitosterol as active compound. The Preliminary Phytochemical studies of this extracts shows the presence of steroids, Alkaloids, Flavonoids, Saponins, Tannin compounds. Which are responsible for Antileprotic activity.

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REFERENCES


