ABSTRACT

*Sapindus trifoliates* L. is an extremely valuable medicinal plant distributed in tropical and subtropical regions of Asia. Plant extracts appear to be one of the better alternatives as they are known to have a minimal environmental impact and danger to consume in contrast to synthetic pesticides. The seed extracts of *Sapindus trifoliates* L. exhibited activity against *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The antimicrobial activity of each plant extract was tested against bacterial and fungal organisms i.e. *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *E. coli*. *Aspergillus niger*, *Aspergillus flavus* and *Fusarium* spp. The antimicrobial activity was done for Chloroform, Ethyl Alcohol, Petroleum Ether, Acetone and Aqueous extracts. The ethanol extract showed antifungal activity against *Aspergillus fumigates* and *Aspergillus niger* at 100% plant extract. The inhibitory activity of the various seed extracts like Chloroform, Acetone, Petroleum ether, Ethyl alcohol and aqueous were observed.

**Keywords:** Antimicrobial Activity, *Sapindus trifoliates* L., Soxlet Extraction.

INTRODUCTION

Medicinal plants are important sources of potentially useful new compounds for the development of chemotherapeutic agents. The genus Sapindus belongs to the family Sapindaceae. The major compounds isolated from genus sapindus are saponins, triterpenoids, fatty acids, and flavonoids are well known for their antimicrobial, antidiabetic, cytotoxic, molluscicidal, fungicidal and inflammatory activities. (Sharma A. et.al 2011.). The seeds of *Sapindus mukorossi* L. are used in Ayurvedic medicine to remove tan and freckles from the skin (Aparna Upadhyay & D.K. Singh 2012).
The first step towards this is the screening of plants used in medicine. Hence antibacterial research is going towards the discovery and development of antibacterial and antifungal agents. The screening of plants for their biologically active principles is done based on chemo–taxonomic investigations or ethno botanical knowledge of a particular disease (Sohni YRet.al., 1995).

Phytochemical work on different parts such as Fruit, Pericarp, Seeds, Leaves, Ripe Fruit, Roots, and Stems of *Sapindus mukorossi* L., *Sapindus saponaira* L. and *Sapindus trifoliatus* L. (Ibrahim 2015).

Due to increasing price of chemical drug, it is a need to find out cheaper drugs from natural resources. In recent years more species have been evaluated for their antimicrobial activity. The medicinal plant *Sapindus trifoliatus* L. is selected for the present study and is aimed at screening the antimicrobial properties against the selected pathogenic bacteria and fungal organisms. Dry seeds of *Sapindus trifoliatus* L. are one of the oldest cultivated medicinal plants in the world. In fact botanists traced it to the period of Vedas about 5000 years ago. (Aruna Pai 2014).

It is a medium size deciduous tree growing wild in South India. Fruits of *Sapindus trifoliatus* L. are rich in saponins. The seed is also used in Ayurvedic medicine to remove freckles and tan from the skin and for cleansing hair. The major compounds isolated from the plants are triterpenoids, fatty acids, steroids, alkaloids, carbohydrates, saponins, and flavonoids. They are also known for their antidiabetic, fungicidal, anti-inflammatory, antimicrobial and cytotoxic activities. (S.Priya1 and M. Mohanapriya 2021).

*Sapindus* is a genus of about five to twelve species of shrubs and small trees, native to warm temperate humid regions. The genus includes together deciduous and evergreen species. The members of the genus are commonly known as soap berries or soap nuts because the fruit pulp is used to make soap. The fruits are solitary globose and appear in the month of July-August. Experiments demon-starting the physiological, immunological and pharmacological properties of saponins have stimulated considerable clinical interest in these substances (Denise D. Pelegrini ET. Al 2008).

The *Sapindus trifoliatus* L. is a large, deciduous tree with a straight trunk up to 12 m in height, sometime attaining a height of 20 m and a girth of 1.8 m, with a globose crown and rather fine leathery foliage. Leaves are 30 to 50 cm long, alternate, paripinnate; common petiole very narrowly bordered, glabrous; leaflets 5-10 pairs, opposite or alternate, 5-18 by 2.5-5 cm, lanceolate, acuminate, entire, glabrous, often slightly falcate or oblique; petioles 2-5 m long. Seeds are 0.8 to 1.3 cm in diameter, globose, smooth, black and loosely placed in dry fruit (M. Rajeshwari et.al 2016).

The fruit is valued for the saponins (10.1%) present in the Pericarp which founds up to 56.5% of the drupe known for inhibiting tumor cell growth. (Varsha Parcha et. al. 2020). The fruit contains an active principle Saponin which ranges from 6-10 % of mass weight. The plant has been reported for its high content of Saponin and Sugar (Azhar I. et. al., 1994). Saponins are glycoside compounds and have many biological properties like hemolytic and antimicrobial effects. (Meltem
Mert Eren et al. 2021). It is used to treat various diseases by Ayurveda is traditional physicians. Knowledge on medicinal uses of Sapindus trifoliatus L. is scattered. (E.R.H.S.S. Ediriweera et. al. 2021). The present study focused on Antimicrobial activity and Antifungal activity.

MATERIALS AND METHODS

Collection of Plant Material:
Seeds of Sapindus trifoliatus L. were collected from the local market. The Pericarp of Fruits was shade dried at room temperature for 25-30 days and crushed into the grinder.

Preparation of plant extract:
25 gm powder of plant material was sequentially extracted with different solvents in the Soxlet apparatus. The solvent used for extraction included Chloroform, Acetone, Petroleum Ether, Water and Alcohol. The respective extract was dried in Petri plates using the electrical oven at 50ºC for 2-4 days to field solid/semi-solid residue working extract was prepared by adding it to DMSO solution at a concentration of 25gm/ml.

Soxlet Extraction of Sapindus trifoliatus L.:
For the extraction of Sapindus trifoliatus L., the crushed seeds Pericarp are used. In a Soxlet apparatus, the extraction thimble is fitted in between a round bottom flask at the bottom and a bulb condenser at the top. Inside the thimble holder solid matrix seed is wrapped within the packing. The packed bed is in contact with pure solvent for the extract to be transferred from the solid matrix to a fluid medium and the extract is leached out. The mass transfer occurs during solvent extraction in a packed column.

Antimicrobial assays:
The antimicrobial assays were carried out by Antibacterial activity of plant extracts were carried out against bacterial pathogens, such as E.coli and staphylococcus aureus using agar well diffusion method.

Disc diffusion method:
A sterile filter disc (diameter -4 mm, Whatmann paper No.-3) was placed in Petri dishes filled with Mueller Hinton agar and seeded with 0.3 ml of the test organism. The disc was impregnated with the test concentration of the compounds investigated dissolved in DMSO. The zones of growth inhibition around the disc were measured after 24 hrs of incubation at 37ºC. Each microorganism was tested in triplicate and solvent was used as a control.

Micro dilution Method:
The method micro dilution method/technique was used to obtain quantitative data for the compounds investigated. Bacterial species were cultured overnight at 37ºC in and LB medium. The inoculums suspension was adjusted with sterile saline to a concentration of approx. 1.0 x10^5 in a final volume of 100 µl /cell. The fungal spores were washed from the surface of agar plates with sterile 0.85% saline containing 0.1% in 80 (vol. /vol.) and adjusted with sterile saline to a concentration of 1.0 X 10^5 in a final volume of µl/ml. The inoculation was stored at 4ºC for further use. Dilution of the inoculate was cultured on solid MH for bacteria and MA for fungi to verify the absence of contamination and to check the validity of the inoculums.

Evaluated Method:
To suggest methodologists for screening the natural products’ antimicrobial activity, two different
qualitative methods were evaluated by the agar diffusion test and the Bio autographic method.

**Agar diffusion well method:**
The bacterial inoculums were uniformly spread using a sterile cotton swab on a sterile Petri dish. MH a garnon-sterile dilution yielded a concentration of 100, 80, 60, 40, 20, 10, 5, and 1.25 mg /ml for pure substances. 50 µl of natural products were added to each of the 5 wells. The system is incubated for 24 hrs at 36°C ± 1°C under aerobic conditions. After incubation confluent bacterial growth was observed. Inhibition of bacterial growth was observed and measured in mm. Agar diffusion disc method. Natural products were dissolved and diluted with solvents as mentioned previously. The same no. of subsequent diluted was performed as described above. However, natural products serial dilution was performed out of initial conc. 2.5 greater than the ones performed for the good method. 7mm filter paper dishes (No.3) were impregnated with 20ml of each of the different dilutions. The discs were allowed to remain at room temp unit for complete dilute evaporation and kept under refrigeration until ready to be used.

**Aseptic condition:**
An aseptic chamber that is laminar airflow was cleaned with 70% ethanol and irradiated with short-wave ultraviolet light and the empty Petri plates were sterilized in an autoclave. MH agar media were poured into plates in a sterile environment and allowed to cool at room temperature. At the same time spread 0.1 ml of a suspension of test organism on the surface of the sterile. MH agar with the help of a spreader in a zig-zag manner and wells were made with the help of a borer. Four well made in each other. Add 100 µl of different concentrations of plant extract in two well in each other and another one having DMSO solution and another antibiotic. Keep the plate at 37°C for incubation for at least two days to allow diffusion in extract through agar. Measure the diameter of the zone of inhibition of the test organism in presence of different plant extracts.

**RESULTS AND DISCUSSION**

**Antimicrobial activity:**
The antimicrobial activity of plant extracts was tested by the good diffusion method. The antimicrobial activity of each plant extract was tested against bacterial and fungal organisms i.e. *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *E.coli*, *Aspergillus niger*, *Aspergillus flavours* and *Fusarium spp.*

**Antibacterial activity:**
The seed extracts of *Sapindus trifoliatus* L. exhibited activity against *E. coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus*. The inhibitory activity of the various seed extracts like Chloroform, Acetone, Petroleum Ether, Ethyl Alcohol and Aqueous were also observed. The chloroform extracts of *Sapindus trifoliatus* L. showed less in *Pseudomonas aeruginosa* (6.03mm) and highest in *Bacillus subtilis* (8.06 mm) zones of inhibition at 100µl. The Ethyl alcohol extracts of *Sapindus trifoliatus* L. showed less in *Pseudomonas aeruginosa* (7.01mm) and highest in *E.coli* (9.09 mm) zones of inhibition at 100µl. The Petroleum ether extracts of *Sapindus trifoliatus* L showed less in *Pseudomonas aeruginosa* (6.09mm) and highest in *Staphylococcus aureus* (8.08 mm) zones of
inhibition at 100μl. The Acetone extracts of *Sapindus trifoliatus* L showed less in *Pseudomonas aeruginosa* (6.09mm) and highest in *Staphylococcus aureus* (8.08 mm) zones of inhibition at 100μl. The Aqueous extracts of *Sapindus trifoliatus* L showed less in *Pseudomonas aeruginosa* (7.03mm) and highest in *E.coli* (11.03 mm) zones of inhibition at 100μl.

**Table 1: Antibacterial activity of *Sapindus trifoliatus* L.**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Type of extract</th>
<th>Diameter of zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>E.coli</em></td>
</tr>
<tr>
<td>1</td>
<td>Chloroform</td>
<td>7.8</td>
</tr>
<tr>
<td>2</td>
<td>Ethyl Alcohol</td>
<td>9.9</td>
</tr>
<tr>
<td>3</td>
<td>Petroleum Ether</td>
<td>8.5</td>
</tr>
<tr>
<td>4</td>
<td>Acetone</td>
<td>8.3</td>
</tr>
<tr>
<td>5</td>
<td>Aqueous</td>
<td>11.3</td>
</tr>
</tbody>
</table>

Fig. No.01: Graphical Representation shows Antibacterial activity of *Sapindus trifoliatus* L. by using different extract

**Antifungal activity:**

The seed extracts of *Sapindus trifoliatus* L. exhibited activity against *Aspergillus niger*, *Aspergillus flavus* and *Fusarium* spp. The inhibitory activity of the various seed extracts like Chloroform, Ethyl Alcohol, Petroleum Ether, Acetone and Aqueous were also observed. The chloroform extracts of *Sapindus trifoliatus* L. showed less in *Aspergillus niger* (7.06 mm) and highest in *Aspergillus flavus* (9.09 mm) zones of inhibition at 100μl. The Ethyl alcohol extracts of *Sapindus trifoliatus* L. showed less in *Fusarium Spp.* (9.08 mm) and highest in *Aspergillus flavus* (11.02 mm) zones of inhibition at 100μl. The Petroleum ether extracts of *Sapindus trifoliatus* L showed less in *Aspergillus niger* (7.09mm) and highest in *Fusarium Spp.* (9.04 mm) zones of inhibition at 100μl. The Acetone extracts of *Sapindus trifoliatus* L. showed less in *Aspergillus niger* (8.01mm) and highest in *Aspergillus flavus* (9.03mm) zones of inhibition at 100μl. The Aqueous extracts of *Sapindus trifoliatus* L showed less in *Fusarium Spp.* (11.02 mm) and highest in *Aspergillus niger* (12.02 mm) zones of inhibition at 100μl.

**Table 2. Antifungal activity of *Sapindus trifoliatus* L.,**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Type of extract</th>
<th>Diameter of zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>Aspergillus niger</em></td>
</tr>
<tr>
<td>1</td>
<td>Chloroform</td>
<td>7.6</td>
</tr>
<tr>
<td>2</td>
<td>Ethyl Alcohol</td>
<td>10.9</td>
</tr>
<tr>
<td>3</td>
<td>Petroleum Ether</td>
<td>7.9</td>
</tr>
<tr>
<td>4</td>
<td>Acetone</td>
<td>8.1</td>
</tr>
<tr>
<td>5</td>
<td>Aqueous</td>
<td>12.2</td>
</tr>
</tbody>
</table>

Fig. No.02: Graphical Representation shows Antifungal activity of *Sapindus trifoliatus* L. by using different extracts
CONCLUSION:

Present study has concluded the fact that the antimicrobial activities are much more significant at *Sapindus trifoliatus* L. The seed extract of *Sapindus trifoliatus* L. species displayed inhibitory activities against the microorganisms involved in infections and skin diseases. Ethanol and Aqueous extracts of *Sapindus trifoliatus* L. Pericarp were investigated for in vitro antimicrobial screening by agar well method against *staphylococcus aureus, Bacillus subtilis, E.coli, Pseudomonas aeruginosa, Bacillus cereus, Aspergillus niger, Fusarium spp.* In the Agar well diffusion method, the highest imbibitions zone was found in aqueous extracts. It showed greater activity against the maximum tested bacteria and fungi followed by ethanol extract. The preliminary results of this study indicate that the aqueous seed extracts have the potential for antimicrobial activity.

REFERENCES


