STUDY COMPARATIVE ANTI-MICROBIAL ACTIVITY OF METHANOLIC EXTRACT OF FLOWERS OF CALOTROPIS GIGANTEA AND CALOTROPIS PROCERA

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ABSTRACT
Ayurveda is the oldest medicine system and includes numerous medicinal plants. Medicinal plants are massive source of biologically active compound and offering broad range of activity. The present study was aimed to evaluate and compare antimicrobial activity of methanolic extract of flowers of Calotropis gigantea and Calotropis procera which is also known as Rui. Its antimicrobial activity was performed by using gram negative bacteria Escherichia coli; gram positive bacteria Bacillus subtilis and fungi Aspergillus niger by cup plate method. Streptomycin and Itraconazole was used as a standard and to compare the antimicrobial activity of plants on pathogens. The antimicrobial activity was measured by different concentrations of methanolic extract of flowers of Calotropis gigantea and Calotropis procera with its minimum dose (20µg/ml) and maximum dose (100µg/ml) and Streptomycin (100µg/ml) and Itraconazole (100µg/ml). Antimicrobial activity was evaluated by measuring the zone of inhibition in millimeters. It can be concluded that methanolic extract of Calotropis gigantea and Calotropis procera was found to have antimicrobial activity against gram positive Bacillus subtilis; gram negative E. coli and fungi Aspergillus niger. It also indicated that methanolic extract of pants are potentially good for antibacterial resistant bacteria therapy.

KEYWORDS: Antimicrobial activity, Soxhlet extraction, Ultrasonication, cup plate method.

INTRODUCTION
In recent past, drug resistance to human pathogenic bacteria is being commonly reported from all over the world.[1] There are number antibiotics available in market but some of them are having adverse effects like hypersensitivity, gene toxicity, and depletion of Normal gut flora.[2] Ayurveda is the oldest system of medicines and includes numerous medicinal plants.[3] As per WHO medicinal plant is a plant in which one or more of its parts, contains substances that can be used for therapeutic purposes or which are substrates for the synthesis of various useful drugs.[4] Medicinal plants are used for the formation of various drugs and they have been used traditionally to cure various diseases.[5,6] These plants contain many phytochemical constituents like flavonoids, alkaloids, tannins, and phenols etc.[7] which show antimicrobial activity against the pathogens.[5,6] As per the WHO, almost more than 80% of world’s population depends upon the traditional medicine for primary care of health.[8] Calotropis R. Br. species belongs to family Asclepiadaceae and is distributed in northern Africa, Arabia, and tropical Asia.[9]

Calotropis procera also called as Sodom apple is a shrub about 6m high and is widely distributed in West Africa and other parts of the tropics. The plant is erect, large, tall, branched and perennial with milky latex throughout. It is used to treat various diseases. The secretion from the root bark is usually used for the treatment of skin diseases, enlargements of abdominal viscera and intestinal worms. It is also used to treat elephantiasis, toothache, asthma, leprosy, rheumatism.[5,10,11] It also possesses cardio tonic, hepatoprotective, antioxidant, analgesic and anticancer activities.[4,12] It contain cardenolides, triterpenoids, resins, proteolytic enzymes, flavonoids, tannins, sterol, gallic acid, phenols and terpenes.[12]

Calotropis gigantea also known as milkweed[13] has been used by tradition as an anthelmintic, carminative, astringent, digestive, stimulant, anti-inflammatory. It is also used to treat toothache, earache, sprain, anxiety, pain, epilepsy, diarrhea, mental disorders, cough, leprosy, and asthma.[6,9,13] It contain cardenolides, flavonoids, terpenes, ester of α-and β-calotropeols and a nonprotein amino acid etc.[9]

The aim of the present study was to prepare methanolic extract of flowers of Calotropis gigantea and Calotropis procera by using soxhlet and ultrasonication method of extraction and also to evaluate and compare their antimicrobial activity.
MATERIALS AND METHODS

● Selection and authentication of plant
The fresh flowers of plants *Calotropis gigantea* and *Calotropis procera* were collected from the Satara city, from Satara district of Maharashtra. The plants collected were authenticated botanically in department of Botany at Yashwantrao Chavan Institute of Science, Satara.

● Preparation of *C. gigantea* and *C. procera* plant extract
Fresh flowers were collected and washed in water. Flowers were air dried for 15-20 days and then powdered.

Soxhlet extraction
10 gm powdered plant material (flowers) of *Calotropis gigantea* and *Calotropis procera* were weighed and uniformly packed into two separate thimbles of Soxhlet extractor. The round bottom flasks were filled two-third with methanol and were adjusted to the extractor. The extraction process was allowed to run. After the extraction process completion, extract was filtered and collected in different petri plates. The petriplates were kept aside for evaporation at room temperature and collected extract was stored in refrigerator for further use.

Ultrasonic extraction
10gm powdered plant material (flowers) of *Calotropis gigantea* and *Calotropis procera* were weighed and taken in four different beakers respectively. After 50 ml methanol was added in each beaker, the beakers were kept in sonicator for sonication extraction at different time intervals 15, 30, 45, 60 minutes respectively. After completion of extraction process the extract was filtered and collected in different petri plates. The petriplates were kept aside for evaporation at room temperature and collected extract was stored in refrigerator for further use.

OBSERVATION

Antimicrobial activity by using soxhlet method
*E. coli* (gram negative)

Fig.1 *Calotropis gigantea* flower extract.

Fig.2 *Calotropis procera* flower extract.
bacillus subtilis (gram positive)

*Fig. 3 Calotropis gigantea flower extract.*

*Fig. 4 Calotropis procera flower extract.*

aspergillus niger

*Fig. 5 Calotropis gigantea flower extract.*

*Fig. 6 Calotropis procera flower extract.*

Antimicrobial activity by using ultra sonication method

*E. coli*

*Fig. 7 Calotropis gigantea flower extract.*

*Fig. 8 Calotropis procera flower extract.*

bacillus subtilis

*Fig. 9 Calotropis gigantea flower extract.*

*Fig. 10 Calotropis procera flower extract.*
aspergillus niger

Fig. 11 Calotropis gigantea flower extract. Fig. 12 Calotropis procera flower extract.

RESULTS
Gram positive: Bacillus subtilis.
Gram negative: Escherichia Coli.
Fungi: Aspergillus niger.

A. Antimicrobial Activity by Soxhlet Extraction Technique.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Pathogens</th>
<th>Zone of Inhibition in mm</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Flowers of C. gigantea</td>
<td>Flowers of C. procera</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Std. (100 µg/ml)</td>
<td>Min. (20 µg/ml)</td>
<td>Max. (100 µg/ml)</td>
<td>Std. (100 µg/ml)</td>
</tr>
<tr>
<td>1.</td>
<td>E. coli</td>
<td>26mm</td>
<td>30mm</td>
<td>25mm</td>
<td>16mm</td>
</tr>
<tr>
<td>2.</td>
<td>Bacillus subtilis</td>
<td>31mm</td>
<td>22mm</td>
<td>24mm</td>
<td>26mm</td>
</tr>
<tr>
<td>3.</td>
<td>Aspergillus niger</td>
<td>26mm</td>
<td>30mm</td>
<td>32mm</td>
<td>30mm</td>
</tr>
</tbody>
</table>

B. Antimicrobial Activity by Ultrasonic Extraction Technique.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Pathogens</th>
<th>Zone of Inhibition in mm</th>
<th></th>
<th></th>
<th></th>
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<tbody>
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<td></td>
<td></td>
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<td>Flowers of C. procera</td>
<td></td>
<td></td>
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<tr>
<td></td>
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<td>Min. (20 µg/ml)</td>
<td>Max. (100 µg/ml)</td>
<td>Std. (100 µg/ml)</td>
</tr>
<tr>
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<td>18mm</td>
<td>25mm</td>
<td>35mm</td>
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<tr>
<td>2.</td>
<td>Bacillus subtilis</td>
<td>30mm</td>
<td>28mm</td>
<td>26mm</td>
<td>32mm</td>
</tr>
<tr>
<td>3.</td>
<td>Aspergillus niger</td>
<td>30mm</td>
<td>32mm</td>
<td>26mm</td>
<td>34mm</td>
</tr>
</tbody>
</table>

DISCUSSION
The extracts obtained from Flowers of C. gigantea and C. procera exhibited activity against E. coli which is gram negative, Bacillus subtilis which is gram positive fungi Aspergillus niger. The finding of current study demonstrates that the methanolic extract of C. gigantea and C. procera showed better activity against gram positive and gram negative organisms and fungi Aspergillus niger.

Soxhlet extract of Calotropis gigantea flowers at minimum concentration shows better activity against E. coli; but it shows less activity as compared to standard against Bacillus subtilis; its maximum concentration shows better activity against fungi Aspergillus niger.

Ultrasoundation extract of Calotropis gigantea flowers shows better activity against E. coli at maximum concentration; but it does not shows activity superior than standard against Bacillus subtilis. Its minimum concentration shows better activity against Aspergillus niger.

Soxhlet extract of Calotropis procera flowers shows activity superior to the standard at maximum concentration against E. coli and Bacillus subtilis and at minimum concentration against Aspergillus niger.

Ultrasoundation extract of Calotropis procera flowers shows activity superior than standard at maximum concentration against E. coli but it is less effective than standard against Bacillus subtilis and Aspergillus niger.

CONCLUSION
Calotropis procera flowers extract by ultrasoundation method showed better activity against E. coli as compared to standard and Calotropis gigantea. Soxhlet extract of Calotropis gigantea flowers shows better
activity against *Bacillus subtilis* as compared to standard and *Calotropis procera*. Soxhlet extract of *Calotropis gigantea* and *Calotropis procera* shows better activity against *Aspergillus niger* than standard.

Therefore, *C. gigantea* and *C. procera* may be considered as plant having various health benefits. These plants showed good Antimicrobial activity. The methods of the extraction were easy. Their extracts showed Antimicrobial activity against *E. coli*, *Bacillus subtilis* and *Aspergillus niger*. The extracts possessing high Antimicrobial activity should be used further for therapeutic purpose. These plants are easily available and economically affordable and have many medicinal values. Hence these plants can be used to minimize health problems and for achieving healthy life.

**REFERENCES**

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