EVALUATION OF IN VITRO ANTIMICROBIAL ACTIVITY OF THAZHAMPOO MATHIRAI – A SIDDHA HERBO MINERAL FORMULATION

C. Mary Sharmila1*, R. Chithra Devi2, A. Sureka3, N. J. Muthu Kumar4 and V. Banumathi5

1*Resident Medical Officer, National Institute of Siddha, Tambaram Sanatorium.
2House Officer, National Institute of Siddha, Tambaram Sanatorium.
3Emergency Medical Officer, National Institute of Siddha, Tambaram Sanatorium.
4Associate Professor, Hospital Superintendent, National Institute of Siddha, Tambaram Sanatorium.
5Director, National Institute of Siddha, Tambaram Sanatorium.

*Corresponding Author: C. Mary Sharmila
Resident Medical Officer, National Institute of Siddha, Tambaram Sanatorium.

ABSTRACT
In the present scenario of increasing antibiotic drug resistance, and in the search of newer antimicrobial agents, Thazhampoo mathirai is a traditional siddha herbo mineral formulation in the siddha literature “siddha vaidhya thiruttu” specifically indicated to treat nerikatti suram and the associated andu veekam (lymphadenitis and the associated scrotal swelling). The aim of the present study is to evaluate the antimicrobial activity of the siddha drug Thazhampoo mathirai. The antimicrobial activity evaluation was carried out by the cup plate method (Indian pharmacopoeia., 1996) against various strains of gram positive, gram negative bacteria and fungi. The test drug was diluted to various concentrations and the diameter of the zone of inhibition produced was measured against the standard ampicillin 10mg/ml. The obtained results reveal that the test drug produced a maximum zone of inhibition of 29 mm with pseudomonas aeruginosa, 19 mm with salmonella typhimurium and candida albicans. We can conclude that the test drug possesses effective antimicrobial activity against gram positive, gram negative bacteria and fungal strain.

KEYWORDS: Antimicrobial activity, Thazhampoo mathirai, Siddha, Herbo mineral drug.

INRODUCTION
In view of the health impact due to communicable diseases, there is an increased outbreak of new infectious diseases caused by different microbial pathogens. The increasing multidrug resistant microbial pathogens pose a challenging hurdle in the management of infectious diseases.[1] Researches are undertaken to discover newer compounds which target the microbes through mechanisms which is different and distinct from the existing available drugs in the market.[2] Accelerated researches are being carried out to identify new antimicrobial agents to provide an infection free future to the upcoming generations.

The implementation of metallic drugs for therapeutic purposes is a renowned division of pharmacology in Siddha system of medicine. Scientifically, the metals are known to perform important biological functions and to coordinate some essential chemical reactions in the body.[3] Studies suggest three strategies to effectively combat the drug resistance in microbes, firstly to create a reversed mechanism of microbial resistance, secondly a new drug to act in a mechanism unknown to the pathogens and thirdly to reduce the toxicity of the metal ion in the form of a complex.[3]

Thazhampoo mathirai is a siddha herbo-metallic drug which consists of Veeram (Mercury chloride), Lingam (Red sulphide of mercury), Rasa chenduram (Red sulphide of mercury, natural), Gandhagam (Sulphur), Nervalam (Croton tigillum) and is prepared with the extracts of Thazhampoo (Pandanus odoromatissus) [4] In this study the author has made an attempt to scientifically validate the antimicrobial activity of the siddha drug Thazhampoo mathirai.

MATERIALS AND METHODS
Thazhampoo mathirai was obtained from IMPCOPS pharmacy; Chennai, Tamilnadu and the test organisms used in the study were obtained from MTCC, IMTECH, Chandigarh (The microbiology department of IISC, Bangalore).

Preparation of test and standard solutions
The stock solution of the test drug Thazhampoo mathirai was prepared by dissolving the dried extract in DMSO at various concentrations from 50 mg/ml, 25 mg/ml, 12.5
mg/ml to 0.19mg/ml. The stock solution of the reference standard Ampicillin was prepared with sterile water at a concentration of 10mg/ml.

**Anti Bacterial and Anti Fungal Assay**

The microbial activity evaluation was carried out by the cup plate method as in the Indian pharmacopeia 1996. The test organisms used in the study were *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Staphylococcus aureus* which were bacteria strains and *Candida albicans* for fungal strain. The micro-organisms chosen for the study were inoculated in a 100ml sterile nutrient broth and incubated. 0.1 ml of the inoculum was spread over the plate containing the agar medium with wells of 6mm diameter. 0.06 ml of the test drug solution of each concentration and the reference standard were added to the cups with a micropipette. All the plates were then refrigerated at 8°C for a period of 2 hours for effective diffusion of test compounds and standards. Later they were incubated for 24 hours at 37°C. The presence of definite zone of inhibition of any size around the cup indicated antimicrobial activity. The solvent control was run simultaneously to assess the activity of drug vehicles. The experiments were performed three times. The diameter of the zone of inhibition was measured and recorded.\(^5\)

**RESULTS AND DISCUSSION**

The antimicrobial activity of the test drug was evaluated against eight microbial strains, i.e. five gram positive, two gram negative bacteria and one fungal strain. The results of the minimum inhibitory concentration of Thazham poo mathirai required to be effective against the various microbial strains and the zone of inhibition produced are given in table 1. The test drug was effective against pseudomonas aeruginosa at a concentration of 0.09 mg/ml and produced an inhibitory zone of 27mm at 50mg/ml. At a concentration of 0.78mg/ml the drug was effective against proteus vulgaris and at 50mg/ml concentration produced an inhibitory zone of 15mm against the same. Maximum zone of inhibition at a drug concentration of 50mg/ml against Bacillus cereus, *Staphylococcus aureus* and salmonella typhimurium was measured to be 15mm, 17mm and 19 mm respectively. The drug concentration to be effective against Candida albicans was 0.39mg/ml and the zone of inhibition produced against the fungal strain was 19mm at concentration of 50 mg/ml. Thazhampoo mathirai was found not to be effective against two bacterial strains namely *Escherichia coli* and klebsiella pneumoniae. An increased shelf-life, low dose of the medicine, easy storage and continuous availability of the drug makes herbal-metallic preparations more preferable than the herbal drugs.

### Table 1: Zone of Inhibition of the test drug against the microbes.

<table>
<thead>
<tr>
<th>S.NO</th>
<th>ORGANISM</th>
<th>ZONE OF INHIBITION - THAZHAMPOO MATHIRAI (60µL/WELL)</th>
<th>Amp (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DRUG CONCENTRATION mg/ml</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>ORGANISM</td>
<td>50</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td><strong>GRAM POSITIVE BACTERIA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>1. <em>Bacillus cereus</em></td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>2. <em>Staphylococcus aureus</em></td>
<td>17</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>3. <em>Escherichia coli</em></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>4. <em>Klebsiella pneumonia</em></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td><em>Proteus vulgaris</em></td>
<td>16</td>
<td>14</td>
</tr>
<tr>
<td>6.</td>
<td><em>Salmonella typhimurium</em></td>
<td>19</td>
<td>16</td>
</tr>
<tr>
<td>7.</td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>27</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td><strong>GRAM NEGATIVE BACTERIA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td><em>Candida albicans</em></td>
<td>19</td>
<td>18</td>
</tr>
</tbody>
</table>

\(www.ejbps.com\)
Figure 1: Agar well diffusion method.

Drug concentration (60µl/well)
1. 50mg/ml
2. 25mg/ml
3. 12.5mg/ml
4. 6.25mg/ml
5. 3.12mg/ml
6. 1.56mg/ml
7. 0.78mg/ml
8. 0.39mg/ml
9. 0.19mg/ml
10. 0.09mg/ml

CONCLUSION
In Siddha medicine Thazhampoo mathirai is used to treat the spectrum of diseases affecting the lymphatic system. From the above study results we can conclude that the siddha medicinal drug Thazhampoo mathirai is a potent antimicrobial agent against gram positive, gram negative bacteria and fungal strains.

ACKNOWLEDGEMENT
I would like to express my sincere gratitude to Regional Research Institute of Unani Medicine, Royapuram for providing the facilities needed for the research work.

REFERENCES