ABSTRACT
The present study deals with the phytochemical investigation and therapeutic use importance of plant *spermacoce hispita* L. It is an important medicinal plant worldwide trend towards the utilization of natural plant remedies has created an enormous need for the use of medicinal plants. Phytochemical screening was evaluated with the methanolic and aqueous extract in presence of alkaloids, flavonoids, phenols, steroidal, glycosides, tannins and carbohydrate are found in the plant extracts. The ethanol extract of same plant was subjected to analysis the antimicrobial activity by disc method. The extract of the plant leaves showed antimicrobial activity against both gram(+) and gram(-) bacteria and fungi, Maximal antibacterial activity was observed against *streptococcus facials*. The maximal antimicrobial activity was against Diameter of zone inhibition exhibited by *spermacoce hispita* L ethanol extract in various concentrations.

Keywords: *spermacoce hispita*. bacteria and fungi.

INTRODUCTION
The medicinal properties of plant species have made an outstanding contribution in this origin and evolution of many traditional herbal therapies. Medicinal plants are important source for the therapeutic of various ailments [1]. It is a major symptom in many medical conditions and can significantly interfere with a person’s quality of life and general functioning [2]. The widespread use of herbal remedies and healthcare preparation has been traced to the occurrence of natural products with medicinal properties. Increasing reliance on
the use of medicinal plants in the industrialized societies has been traced to the extraction and
development of several drugs and chemotherapeutics from these plants as well as from
traditionally used rural herbal remedies [3]. The World Health Organization (WHO) estimated
that approximately 80% of world population relies mainly on traditional medicines, mostly
plant drugs in their health care [4]. Today, Ayurveda coexists with modern system of
medicine, and is still widely used and practiced. About 30% of the currently used therapeutics
is of natural origin [5].

There is increasing development of drug resistance in human pathogens as well as the
appearance of undesirable side effect of certain synthetic antimicrobial agents. It is this
background that necessitated the need for the extensive and intensive screening of plants for
more safe, selective and efficacious natural products [6]. Many reports have shown that some
Spermacoce hispita. L contain anti-microbial, bioactive compounds, particulary Spermacoce
hispita. L. Today natural products derived from plants are being tested for presence of new
drugs with new modes of pharmacological action [7]. The aim of the present study is to
investigate phytochemicals constituents and anti microbial studies of Spermacoce hispita. L.

MATERIALS AND METHODS

Collection of plant materials

Fresh parts of plant Spermacoce hispita. L whole plants were collected at Kolli hills,
Namakkal district, Tamil Nadu. The plant materials were identified by botanically. The plant
materials were shaded and dried until all the water molecules evaporated and plants became
well dried for grinding. After drying, the plant materials were grinded well using mechanical
blender into fine powder and transferred into the sealed container with proper labeling.

Preparation of plant extracts

Crude plant extract was prepared by Soxhlet extraction method. About 100 g of powdered
plant material was uniformly packed into a thimble and extracted with 500 ml of methanol
solvents are used separately. The process of extraction continues for 22 hours or till the
solvent in siphon tube of an extractor become colorless. After that the extract was taken in a
beaker and kept on hot plate and heated at 30-40ºC till all the solvent got evaporated using
the vacuum evaporator so as to regenerate the ethanol [8]. Dried extract was stored in
refrigerator at 4ºC.
ANTIMICROBIAL STUDIES
The dried extract was analyzed. The antimicrobial activity was determined using disc diffusion method \cite{12} by measuring zone of inhibition in mm and comparing with standard drugs Ciprofloxin and fluconazole for bacteria and fungi respectively \cite{4}. The extract was tested for the presence of bioactive compounds by qualitatively using standard methods. Medicinal plants were used as excellent antimicrobial agents because it poses a variety of chemical constituent is nature recently much attention has directed towards extracts and biologically active compounds isolated from popular plant species \cite{13}.

Qualitative phytochemical analysis

Test for Alkaloids (Mayer’s Test)
The extract of *Spermacoce hispita* L was evaporated to dryness and the residue was heated on a boiling water bath with 2% hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Mayer’s reagent. Yellow colour was observed. It indicates that the presence of alkaloids.

Test for Tannins
0.5 ml of extract solution 1 ml of distilled water and 1-2 drops of ferric chloride solution was added. Blue colour was observed for gallic tannins and green black for catecholic tannins \cite{9}.

Test for Terpenoid and Steroid
4 ml of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of $\text{H}_2\text{SO}_4$ acid was added slowly and red violet colour was observed for terpenoid and green bluish colour for steroids\cite{8}.

Test for reducing sugars
0.5 ml of extract solution 1 ml of distilled water and 5-8 drops of Fehling’s solution was added and heated. The brick red precipitate was formed. Hence reducing sugar was identified.

Test for Glycoside
The plant extract 5ml is mixed with glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid are added, and observed for reddish brown colouration at the junction of two layers and the bluish green colour in the upper layer was formed. It indicates the presence of glycosides.
Test for saponins
The plant extract 50ml was diluted with 20 ml of distilled water and it was agitated in a graduated cylinder for 15 minutes. The formation of 1 cm layer of foam showed the presence of saponins.

Test for Flavonoids
1 ml of the plant extract and a few drops of dilute sodium hydroxide was added. An intense yellow colour was produced which become colorless on addition of a few drops of dilute acid indicates the presence of flavonoids [7].

Test for Phenolic compounds
The plant 5ml was dissolved in distilled water. Then few drops 1% lead acetate was added. A bulky white precipitate was formed, which indicates that the presence of phenolic compounds.

RESULTS AND DISCUSSION

Preliminary phytochemical analysis
Qualitative preliminary screenings of extracts were performed initially with different chemical reagents to detect the phytochemical constituents present in methanol and aqueous extracts.

*Spermacoce hispita* L was evaluated for preliminary phytochemical screening standard procedure. Preliminary phytochemical screening showed the presence of various classes of secondary metabolites such as alkaloids, flavonoids, phenols, steroid, glycosides, tannins and carbohydrates. The results are presented in table – 1.

Antimicrobial studies
Antimicrobial activity of methanol, chloroform, ethanol and aqueous extracts of whole extracts showed significant activity against various pathogens [14].

Methanolic extract of *Spermacoce hispita* L shows antimicrobial activity against the tested organisms in the order of *Klebsiella pneumoniae*, *Listeria monocytogenes*, *Vibrio cholerae*, *Bacillus megaterium*, *Yersinia enterocolitica*, *Bacillus subtilis*, *Salmonella typhi*, *Staphylococcus aureus*, *Aspergillus niger*, *Candida albicans*. In case of the maximal antibacterial activity was observed against *Vibrio cholerae*. Table - 3.
Table 1: Preliminary phytochemical analysis of *Spermacoe hispita. L*

<table>
<thead>
<tr>
<th>S. No</th>
<th>Phytochemical Constituents</th>
<th>Name of the Test</th>
<th>Methanol Extract</th>
<th>Aqueous Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloid</td>
<td>Mayer’s test</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dragondraff test</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wagner Test</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Carbohydrate</td>
<td>Molish Test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fehling Test</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Benedicts Test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Steroidal Glycosides</td>
<td>Libermann’s test</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Salkowski test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Saponin</td>
<td>Foam Test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Tannin</td>
<td>Lead Acetate test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Pseudo tannins</td>
<td>Ferric chloride</td>
<td>Condensed tannin</td>
<td>Condensed tannin</td>
</tr>
<tr>
<td>7</td>
<td>Chlorogenic acid</td>
<td>Ammonia test</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Flavones</td>
<td>Shinoda’s Test</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>Flavonoid</td>
<td>Ammonia test</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>Coumarin</td>
<td>Sodium chloride test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>Anthocyanin</td>
<td>H₂SO₄ test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>Anthracene Glycoside</td>
<td>Borntrager’s test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>Terpenes</td>
<td>H₂SO₄ test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>Phenols</td>
<td>Ferric chloride</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>15</td>
<td>Glycosides</td>
<td>Libermann test</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 2: Antimicrobial Activity Of Methanolic Extract *Spermacoe Hispita. L*

<table>
<thead>
<tr>
<th>S. No</th>
<th>Name of the organisms</th>
<th>Concentration of methanolic extract added and Zone of inhibition (mm/ml)</th>
<th>50 µl</th>
<th>100 µl</th>
<th>150 µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vibrio cholerae (-)</td>
<td>40</td>
<td>7</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>Klebsiella pneumoniae (-)</td>
<td>31</td>
<td>9</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>Listeria monocytogenes (+)</td>
<td>29</td>
<td>9</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>4</td>
<td>Bacillus megaterium (+)</td>
<td>28</td>
<td>8</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>5</td>
<td>Yersinia enterocolitica (-)</td>
<td>38</td>
<td>9</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td>6</td>
<td>Bacillus subtilis (+)</td>
<td>28</td>
<td>10</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td>7</td>
<td>Salmonella typhi (-)</td>
<td>38</td>
<td>9</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>8</td>
<td>Staphylococcus aureus (+)</td>
<td>34</td>
<td>12</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>9</td>
<td>Aspergillus niger</td>
<td>20</td>
<td>11</td>
<td>13</td>
<td>16</td>
</tr>
<tr>
<td>10</td>
<td>Candida albicans</td>
<td>25</td>
<td>9</td>
<td>12</td>
<td>13</td>
</tr>
</tbody>
</table>
CONCLUSION
The ethanol and aqueous extracts of plant contains many bioactive chemical constituents alkaloids, flavonoids, phenols, steroidal, glycosides, tannins and carbohydrate. Spermacoce hispita. L. was effective against both gram positive, gram negative bacteria. Therefore it can be concluded that antimicrobial activity of Spermacoce hispita. L. against bacteria shows its medicinal value and supports the widespread use of the plant as local remedy for a variety of ailments ranging from ulcers to bronchitis.
REFERENCES


