5.1 INTRODUCTION

For many centuries, higher plants are used as remedies for human diseases. Medicinal plants are used for the treatment of infections by bacteria, fungi, virus and parasites. Many infectious diseases are known to treat by herbal remedies throughout mankind history.

The critical use of commercial antimicrobial drugs which are used to treat infectious diseases was developed because of multiple drug resistance. To treat infectious diseases there is necessity to develop an alternative antimicrobial drugs from medicinal plants. The pathogenic microorganisms built side effects and resistance against antibiotics, recently researches are increasing their attentions for plant extracts and biologically active compounds used in herbal medicine.

Plant produced antimicrobials have much therapeutic potential. Compounds produced by plant are safer and more effective and it is a good substitute for synthetically produced antimicrobial agents. Botanist, ethnopharmacologist, microbiologist and natural product chemist combined the medicinal flora for biological substances that are developed to treat infectious diseases.

Plants possessing antimicrobial activity should be tested against appropriate microbial strains to confirm the activity and for ascertainment of parameters associated with it. In different parts of the world, effect of plant extracts on bacteria were studied by various researchers. In India most of the work are done on ethnomedicinal plants.
5.2 MATERIALS AND METHODS

5.2.1 Preparation of plant extract

Decalepis hamiltonii root was collected from herbal suppliers in Chennai, India. The roots were air dried under shade and powdered to 40 meshes coarse and stored in airtight container. 100 g of Decalepis hamiltonii root powder were subjected to successive extraction in increasing polarity by various solvents (petroleum ether, benzene, chloroform, ethyl acetate, acetone, methanol, ethanol and distilled water) by using soxhlet apparatus. The solvents in plant extracts were evaporated under reduced pressure and extracts were stored in desiccators at 4°C.

5.2.2 Microorganisms used

Microbial strains were obtained from National Chemical Laboratory (NCL), Pune, India. The organisms were maintained at 4°C in Nutrient agar (Hi Media, India) slope and subculture before use. Among 8 microorganisms used, 6 Gram positive bacteria were Escherichia coli, Klebsiella pneumonia, Salmonella typhi, Proteus mirabilis, Vibrio cholera, Serratia sp and 2 Gram negative bacteria were Staphylococcus aureus and Bacillus subtilis.

5.2.3 Antibacterial activity

The screening of antibacterial activity for various extracts of Decalepis hamiltonii root was performed by Disc diffusion method\textsuperscript{12}. The Mueller Hinton Agar (Hi Media) was used as a bacteriological medium. Mueller Hinton Agar plates were prepared by pouring 15 ml of molten media to sterile petriplates. Petriplates were allowed for solidification for 15 minutes and 0.1% inoculum
suspensions were swabbed uniformly and inoculums were allowed to dry for 5 minutes. Under aseptic conditions, 6 mm diameter (Whatman no 1) filter paper disc were impregnated with 10 μl (contains 5 mg/ disc) of various extracts of Decalepis hamiltonii dissolved in DMSO. The discs were overlaid on MHA plates and incubated for 24 hours at 37°C. The diameter of zone of inhibition produced by extracts was compared with standard drugs (Gentamycin 10 μg/ disc and Streptomycin 10 μg/ disc). The controls were maintained for each bacterial strain, whereas DMSO was used instead of extracts. To minimize the error, experiments were performed thrice and mean values are presented and reported.

5.3 RESULTS

The potentially useful structures of the plant were the important source for development of new chemotherapeutic agents. In vitro antibacterial activity was the first step towards development of new chemotherapeutic agents. The extracts from higher plants were the better source of antibiotics against different bacterial pathogens13. Antibacterial activity of extracts against 8 bacterial strains was presented in Table 5.1 and Plate 5.1. All the extracts were found to possess different degrees of antibacterial activities. Petroleum ether extract showed a broad spectrum antibacterial activities may be due to the presence of steroids and glycosides than other extracts. Methanolic extract showed antibacterial activity to all microorganisms except Staphylococcus aureus. Methanolic extract showed antibacterial activity to all microorganisms except Klebsiella pneumonia and Serratia sp. Acetone extract showed antibacterial activity to all bacteria. In this study some of extracts were ineffective due to absence of antibiotic properties or
presence of antibacterial constituents of plant extracts not in effective concentration.

Table 5.1: Antibacterial activity of various extracts of Decalepis hamiltonii root against various bacterial strains by disc diffusion method

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>PE</th>
<th>H</th>
<th>A</th>
<th>M</th>
<th>Et</th>
<th>S</th>
<th>G</th>
<th>DMSO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>25</td>
<td>30</td>
<td>12</td>
<td>20</td>
<td>15</td>
<td>21</td>
<td>20</td>
<td>_</td>
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<tr>
<td>Bacillus subtilis</td>
<td>20</td>
<td>22</td>
<td>12</td>
<td>15</td>
<td>12</td>
<td>16</td>
<td>16</td>
<td>_</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>21</td>
<td>19</td>
<td>14</td>
<td>15</td>
<td>14</td>
<td>11</td>
<td>11</td>
<td>_</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>20</td>
<td>19</td>
<td>12</td>
<td>16</td>
<td>10</td>
<td>15</td>
<td>10</td>
<td>_</td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
<td>18</td>
<td>18</td>
<td>12</td>
<td>11</td>
<td>_</td>
<td>14</td>
<td>13</td>
<td>_</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>23</td>
<td>19</td>
<td>18</td>
<td>14</td>
<td>12</td>
<td>16</td>
<td>15</td>
<td>_</td>
</tr>
<tr>
<td>Vibrio cholera</td>
<td>22</td>
<td>23</td>
<td>14</td>
<td>12</td>
<td>11</td>
<td>11</td>
<td>12</td>
<td>_</td>
</tr>
<tr>
<td>Serritia spp</td>
<td>17</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>20</td>
<td>17</td>
<td>_</td>
</tr>
</tbody>
</table>

Values include cup border diameter (6 mm). Values are mean of three replicates PE-Petroleum ether; H- Hexane; A- Acetone; M- Methanol; Et- Ethanol; S-Streptomycin; G - Gentamycin and DMSO- Dimethyl sulfoxide.
Plate 5.1: Antibacterial activity of various extracts of Decalepis hamiltonii root against various bacterial strains by disc diffusion method

(a) Staphylococcus aureus

(b) Bacillus subtilis

(c) Escherichia coli
(d) Salmonella typhi

(e) Klebsiella pneumonia

(f) Proteus mirabilis
(g) Vibrio cholera

(h) Serratia sp.
5.4 DISCUSSION

The present study was done to assess the antibacterial activity of Decalepis hamiltonii against several bacterial strains. Various workers already studied that gram positive bacteria were more susceptible against plant extracts than gram negative bacteria\textsuperscript{14,15}. The difference was due to cell wall in gram positive bacteria were single layered where as gram negative cell wall were multi layered structure\textsuperscript{16}. Alternatively, flow of active compounds through Gram negative cell wall may be inhibited. Plant based antimicrobials have numerous therapeutic potential because of lesser side effects.

Tannins were well known to have antimicrobial properties\textsuperscript{17}. Tannins were extremely resistant to microbial attack and familiar for inhibition of growth of some microorganisms. Antimicrobial activity of tannins slowed down the rate of biodegradation of soil organic matter. Antimicrobial agents can damage pathogens by several ways viz., protein synthesis inhibition, interference with nucleic acid synthesis, interference with cell wall synthesis and metabolic pathway inhibition\textsuperscript{18}.

Flavonoids were hydrolyzed compounds of phenol that were synthesized by plants in response to microbial infection and very effective against vast range of microorganisms in in vitro study. Saponin antimicrobial activity may be due to ability of leakage of proteins and certain enzymes from cell. Steroids may also exerted antibacterial activity by specific association with membrane lipids leads to leakage of lipids from liposomes\textsuperscript{19}. 
The potential to development of antimicrobial agents from higher plants gives satisfaction because it leads to development of phytomedicine against microbes. Plant-based antimicrobial agents have numerous therapeutic potential because of lesser side effects than synthetic antimicrobials\textsuperscript{20}. However, in vitro antimicrobial evaluation of Decalepis hamiltonii brings primary platform for further pharmacological studies. The results supported that traditional usage of Decalepis hamiltonii root against infectious disease caused by pathogens.
5.5 REFERENCE


