Viji. M. O*, Neeba Wilson and Iyna Bastian

*Department of Biotechnology, St. Joseph’s College Irinjalakuda, Thrissur, Kerala, India.

ABSTRACT

The present study was conducted to evaluate the antibacterial and cytotoxic properties of chloroform, ethyl acetate and methanolic extract of leaves of *Nerium indicum, Ichnocarpus frutescens, Tabernaemontana alternifolia, Tabernaemontana divaricata, Catharanthus roseus*. The test gave positive results regarding antibacterial activity with inhibition zone diameter ranging from 2mm-17mm. Methanolic extract of the leaves of *Tabernaemontana divaricata* showed highest activity. In the brine shrimp lethality test all the extract showed significant results. Percentage of lethality was concentration dependent. Percentage of mortality was assessed as LC50 value of the plant extracts which were found to be ranging from 68.2µg/ml to 11.3µg/ml. The study confirmed antimicrobial and cytotoxic property of the plants and its potential as source of plant based drugs.

KEYWORDS: *Nerium indicum, Ichnocarpus frutescens, Tabernaemontana alternifolia, Tabernaemontana divaricata, Catharanthus roseus*, antibacterial activity, cytotoxic activity.

INTRODUCTION

World is endowed with a rich wealth of medicinal plants and man cannot survive on this earth for long healthy life without the plant kingdom because the plant products and their active constituents played an important role in maintaining health. [1] There are well known drugs that are directly developed from plant species, for example Vinblastine and Vincristine from *Catharanthus roseus*, the first cures in human cancer. Beside the cytotoxic drugs as Aspirin (Analgesic,anti-inflammatory) from *Filipendula ulmavia*. Benzoin (Oral disinfectant) from Slyrax tonkinensis, Morphine (Analgesic) from *Papaver somniferum* and Quinine (for malaria prophylaxia) from *Cinchona pubescens*. [2]
Infectious diseases are leading cause of premature death. Even after introduction of new antimicrobial agents for clinical use an alarming increase in bacterial resistance to existing agents demands that a effort is to be made against bacterial resistance to current antimicrobials. \cite{3} The studies showed that several alcoholic extracts of various traditional medicinal plants exhibit antibacterial activity. The selected plants are used in ayurveda for the treatment of various diseases.

**MATERIALS AND METHODS**

**Sample Preparation**

The leaves of the plants were collected and washed thoroughly in tap water, cleaned with deionized water and dried at shade for a week. Leaf extracts (concentration: 0.25g/ml) were prepared in solvents namely, chloroform, ethyl acetate and methanol. (Powdered samples of leaves were mixed with solvents and kept in shaker and centrifuged at 85 rpm for 24 hours maintained. This process of extraction was repeated for 2 times and the extracts were filtered using Whatman filter paper No.1 and evaporated to dryness). It was then mixed well with 5ml methanol and transferred to vials.

**Growth and Maintenance of Test Microorganism for Antimicrobial Studies**

The bacterial test performed using agar-well diffusion assay with different plant extracts. Microbial pathogens used in the determination of antibacterial activities of different plant extracts were *Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumoniae, Escherichia coli, Lactobacilli, Proteus*. The stock cultures were maintained in nutrient agar slant at 4°C and sub-cultured monthly. Working cultures were prepared by inoculating a loop full of each test microorganism in 3ml of nutrient broth from nutrient agar slants. Tubes incubated at 37°C for 12 hours. The suspension was diluted with sterile distilled water to obtain approximately 100 CFU/ml.

**Screening Test for Antibacterial Activity**

The antibacterial assay was performed using agar-well diffusion assay with different plant extracts. Agar plates were swabbed with test bacteria and 30µl of plant extract was added to the well. Tetracycline in the concentration 1mg/ml serves as control. Plates were incubated overnight at 37°C.
**Brine Shrimp Leathality Assay**

Brine shrimp lethality test (BSLT) was used to predict the cytotoxic activity in the plant extracts. DMSO solutions of the samples were applied against Artemia salina in a one day in vivo assay. For the experiment, plant extracts were dissolved in DMSO and solutions of varying concentrations (50, 40, 30, 20, 10, 5, 2.5, 1.25, 0.625 µg/ml) were prepared by serial dilution technique using DMSO for each extract. \(^{[4]}\)

**RESULT AND DISCUSSION**

**Screening Test for Antibacterial Activity**

*In vitro* screening tests with crude extracts of the dried leaves conducted against *Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumoniae, Escherichia coli, Lactobacilli, Proteus* demonstrated positive result against all pathogenic organisms. Among the different solvents used (chloroform, ethyl acetate and methanol extracts of leaf sample), the methanol leaf extract showed significantly (P<0.050) higher activity. Chloroform leaf extract showed the maximum inhibitory zone against *Proteus* and *Escherichia coli*. Ethyl acetate leaf extract was found be inhibitory for *Lactobacilli* and *Bacillus subtilis*. Methanolic leaf extract had an inhibitory effect on *Klebsiella pneumonia* and *Staphylococcus aureus*. The maximum inhibition zone diameter was shown by methanolic extract of *Tabernaemontana divaricata* (17.00±0.11). The antimicrobial activity might be due to the presence of alkaloids, flavonoids, tannins, phenolic compounds, steroids, saponins and triterpenoids, whose presence may be attributed to the medicinal properties of plants. \(^{[5,6,7]}\)
Table I: Antibacterial activity of dried leaf extracts of medicinal plants of Apocynaceae family

<table>
<thead>
<tr>
<th></th>
<th>Extracts</th>
<th>Lactobacillus</th>
<th>Klebsiella pneumoniae</th>
<th>Proteus</th>
<th>Staphylococcus aureus</th>
<th>Eschericia coli</th>
<th>Bacillus subtilis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C</td>
<td>0</td>
<td>0</td>
<td>9.02±0.23</td>
<td>5.00±0.25</td>
<td>10.00±0.52</td>
<td>5.04±0.28</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>10.09±0.25</td>
<td>6.05±0.52</td>
<td>6.03±0.49</td>
<td>6.02±0.52</td>
<td>7.01±0.32</td>
<td>4.02±0.31</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>5.03±0.31</td>
<td>12.01±0.37</td>
<td>9.01±0.18</td>
<td>4.06±0.61</td>
<td>4.03±0.56</td>
<td>4.03±0.20</td>
</tr>
<tr>
<td>2</td>
<td>C</td>
<td>0</td>
<td>0</td>
<td>5.00±0.42</td>
<td>5.02±0.19</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>12.00±0.13</td>
<td>5.04±0.62</td>
<td>4.04±0.38</td>
<td>6.01±0.32</td>
<td>4.00±0.71</td>
<td>3.09±0.37</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>0</td>
<td>7.05±0.11</td>
<td>6.00±0.25</td>
<td>6.00±0.40</td>
<td>6.01±0.63</td>
<td>6.00±0.54</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>0</td>
<td>0</td>
<td>5.04±0.34</td>
<td>9.04±0.48</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>5.04±0.43</td>
<td>5.00±0.72</td>
<td>4.03±0.60</td>
<td>3.02±0.53</td>
<td>4.04±0.56</td>
<td>3.03±0.66</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>6.00±0.54</td>
<td>4.01±0.47</td>
<td>5.01±0.52</td>
<td>6.04±0.29</td>
<td>4.08±0.27</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>C</td>
<td>4.01±0.56</td>
<td>0</td>
<td>8.00±0.61</td>
<td>0</td>
<td>5.01±0.61</td>
<td>4.04±0.46</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>8.05±0.62</td>
<td>6.03±0.33</td>
<td>6.04±0.41</td>
<td>3.00±0.41</td>
<td>3.02±0.22</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>17.00±0.11</td>
<td>2.07±0.65</td>
<td>2.09±0.70</td>
<td>4.04±0.58</td>
<td>4.01±0.50</td>
<td>3.02±0.45</td>
</tr>
<tr>
<td>5</td>
<td>C</td>
<td>8.00±0.17</td>
<td>0</td>
<td>9.01±0.51</td>
<td>7.00±0.11</td>
<td>9.01±0.18</td>
<td>5.02±0.32</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>7.04±0.53</td>
<td>5.04±0.44</td>
<td>4.02±0.39</td>
<td>4.02±0.62</td>
<td>3.02±0.65</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>9.02±0.64</td>
<td>4.00±0.56</td>
<td>0</td>
<td>16.00±0.21</td>
<td>4.00±0.35</td>
<td>4.01±0.60</td>
</tr>
<tr>
<td>Tetra cycline</td>
<td>C</td>
<td>14.52±0.32</td>
<td>13.01±0.59</td>
<td>16.68±0.46</td>
<td>17.04±0.22</td>
<td>18.57±.19</td>
<td>23.00±0.42</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>12.03±0.59</td>
<td>18.09±0.11</td>
<td>16.52±0.46</td>
<td>17.05±0.55</td>
<td>12.51±0.35</td>
<td>19.00±0.01</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>17.00±0.19</td>
<td>18.00±0.29</td>
<td>17.02±0.37</td>
<td>20.03±0.01</td>
<td>20.01±0.3</td>
<td>19.93±0.11</td>
</tr>
</tbody>
</table>

Inhibition zone diameter mm±SD

1- Nerium indicum
2- Ichnocarpus frutescens
3- abernaemontana alternifolia
4- Tabernaemontana divaricata
5- Catharanthus roseus

C-Chloroform
E- Ethyl acetate
M- Methanol
Antibacterial activity of the chloroform extracts of leaves of medicinal plants in Apocynaceae family

1- Nerium indicum  
2- Ichnocarpus frutescens  
3- Tabernaemontana alternifolia  
4- Tabernaemontana divaricata  
5- Catharanthus roseus

C - Control

- Lactobacillus
- Klebsiella pneumoniae
- Proteus
- Staphylococcus aureus
- Escherichia coli
- Bacillus subtilis

Antibacterial activity of the ethyl acetate extracts of leaves of medicinal plants in apocynaceae family

1- Nerium indicum  
2- Ichnocarpus frutescens  
3- Tabernaemontana alternifolia  
4- Tabernaemontana divaricata  
5- Catharanthus roseus

C - Control

- Lactobacillus
- Klebsiella pneumoniae
- Proteus
- Staphylococcus aureus
- Escherichia coli
- Bacillus subtilis
To confirm the medicinal property of the plants, as well as to give a scientifically validated report for their traditional medicinal use, detailed experimental studies have to be conducted. The brine shrimp lethality bioassay has been shown to be a useful for predicting toxicity of plant extracts and guiding their photochemical fraction.\textsuperscript{[8]} The presence of saponins, alkaloids and glycosides may be responsible for the observation.\textsuperscript{[9]}

**Brine Shrimp Lethality Assay**

The presence of saponins, alkaloids and glycosides may be responsible for the observation.\textsuperscript{[9]}

**Table II: Effect of different extracts of medicinal plants under study on brine shrimp lethality**

<table>
<thead>
<tr>
<th>Medicinal plants</th>
<th>Chloroform extract (µg/ml)</th>
<th>Ethyl acetate extract (µg/ml)</th>
<th>Methanol extract (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Nerium indicum</em></td>
<td>14.70</td>
<td>18.80</td>
<td>11.30</td>
</tr>
<tr>
<td><em>Ichnocarpus frutescens</em></td>
<td>13.20</td>
<td>18.40</td>
<td>15.40</td>
</tr>
<tr>
<td><em>Tabernaemontana alternifolia</em></td>
<td>25.10</td>
<td>23.80</td>
<td>18.90</td>
</tr>
<tr>
<td><em>Tabernaemontana divaricata</em></td>
<td>26.80</td>
<td>29.30</td>
<td>30.40</td>
</tr>
<tr>
<td><em>Catharanthus rosea</em></td>
<td>23.60</td>
<td>68.20</td>
<td>18.01</td>
</tr>
</tbody>
</table>
The results of brine shrimp lethality assay are expressed in percentage lethality assessed as LC50 values of the plant extracts. The values were obtained by plotting extract volume against log concentration of the shrimp naupli killed. The percentage of mortality increased with increase in concentration of extract. The lowest value was found to be most potent. Maximum cytotoxicity was shown by *Nerium indicum* (11.30µg/ml) and lowest by ethyl acetate extract of *Catharanthus roseus* (68.20µg/ml).

The LC50 values ranging from 68.2 to 11.30µg/ml. The cytotoxicity activity is an indicator of wide range of pharmacological activities such as anticancer, antiviral, insecticidal, pesticidal etc. [10]

**CONCLUSION**

Our results offers a scientific basis for the traditional use of plant extracts of *Nerium indicum*, *Ichnocarpus frutescens*, *Tabernaemontana alternifolia*, *Tabernaemontana divaricata*, *Catharanthus roseus* which were found active against *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Escherichia coli*, *Lactobacilli*, *Proteus*. All the plant extract show significant cytotoxic activity. Maximum cytotoxicity was shown by *Nerium indicum*. The results obtained in this screening justify continuing with the purification of crude extracts and isolation of active components for improving their potential as antibacterial, anticancerous, antitumor drugs.

**AKNOWLEDGEMENTS**

The authors gratefully acknowledge Dept. of Biotechnology St. Joseph’s College, Irinjalakuda, Kerala, India for providing the lab facilities.

**REFERENCES**


