A Straight Forward Approach Toward Antimicrobial Activity of Melia Azedarach (Bakayan) Plant (Aqueous) Extract Using Pathogenic Microorganisms from Patients of Islamabad and Rawalpindi

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ABSTRACT

Objectives: This study was conducted to determine the in vitro antimicrobial activity of plant extracts of Melia azedarach. Plants were taken from Islamabad region. Methods: Leaves of Melia azedarach were extracted by “Soxhlet apparatus” and sterilized by Millipore membrane filters. Diffusion and dilution methods were employed to check the antimicrobial activity of aforesaid extracts. In the diffusion method, the antimicrobial activity of extracts was observed by measuring the sizes of the zones of inhibition around the wells containing extracts on Nutrient agar plates. Measures zone sizes were statistically analyzed for significance against Streptomycin (control). In the dilution method, the antimicrobial activity was checked in two steps; in the first step, extracts were tested qualitatively for antibacterial activity and in the next step, these extracts were analyzed quantitatively by standard plate count method against control at 37°C and control at 4°C. Result: In the diffusion method, the leaf extract of Melia azedarach exhibited significant antimicrobial activity against all 9 bacterial species, but in the dilution method, the extract did not show its antibacterial significance against 08 clinically isolated pathogens. Only Pseudomonas growth was inhibited. On these bases, future prospects of plant medicines were discussed. Conclusion: In the new era this type of drug research will open the field for scientists to develop safe drugs and industry to serve not only the nation rather the humanity.

Keywords: Melia azedarach; Antibacterial activity; growth inhibition; leaf extracts

1. INTRODUCTION

During the past few years, a number of studies have been focused on the medicinal appraisal of plants used in traditional medicine. These include examples of Bonafousia species, Croton menthodororum and Heisteria acuminate which possess anti-inflammatory activity and are commonly used in pathologies related to inflammation\(^{(1)}\). Allium sativum possesses not only anti-thrombogenic activity but also contains anti-atherogenic effects along with antibacterial, antifungal and anticancer activity\(^{(2)}\). Studies also claimed that some plants, which are already used in traditional medicine, possess antimicrobial properties against bacteria, virus, and fungi; preparation of such plants considered to be effective against diseases of microbial etiology like Hepatitis B & C Tuberculosis, Typhoid, and Diphtheria, etc.\(^{(5)}\). When considering the antibacterial activity of preparations of plants, studies revealed that plants possess considerable antibacterial activity when compared with modern antibiotics like chloramphenicol and streptomycin\(^{(3)}\). Since diseases like typhoid fever and food poisoning are commonly treated with antibiotics like chloramphenicol and ampicillin, the extensive illogical use of these antibiotics have led to the problems of drug resistance\(^{(16)}\).

In third world countries where infectious diseases are more prevalent, there is a need to develop some medicines of plant origin against these persisting infectious diseases, which may be comparable to modern medicines and antibiotics. Medicinal plants used in the traditional medicine offer a great reservoir for the discovery of new plants having antimicrobial properties comparable to antibiotics of the modern medicine. Since almost all the antimicrobial agents are being imported and by considering the availability of medicinal plants in these countries, a lot of foreign exchange may be saved\(^{(5)}\). In addition to the cost of treatment is gradually increasing and it is becoming unaffordable by a common user. Therefore, the development of therapeutic agents from our own aboriginal resources will be of great help.

In alternate medicines, some of the homegrown medicinal plants, including Melia azedarach have been claimed to exert curative effects in the diseases caused by Salmonella species\(^{(11,15)}\). In another study ethnologic fruit extract of Melia azedarach was used to check the antibacterial activity against Salmonella typhimurium\(^{(1)}\). This plant is very common in Rawalpindi and Islamabad, and a very little work has been done as yet. It is commonly used in traditional and homeopathic medicines, and people in the countryside of Islamabad and Rawalpindi use the leaves extract of Melia azedarach to reduce the heat effect in summer despite its extremely bitter taste. I have studied the in-vitro antibacterial effects of Aqueous leaves extract of Melia azedarach (Bakayan). Plant extract was used in the aqueous form. Antibacterial assays were performed on a variety of clinically isolated Gram-positive and Gram-negative bacteria.

2. METHODS

Plant used in this research

Melia azedarach plant was used in the study. This plant is very common in Rawalpindi and Islamabad. Its leaves were collected from NIH colony; it was identified by the herbarium of the National Agriculture Research Council, Islamabad.

Extraction of active ingredients

Extraction is an important process in the preparation of medicine from plants. This process removes constituents from one phase bringing into contact with a second immiscible liquid phase\(^{(6)}\). In this experiment “Soxhlet extractor” was used. This extractor comprised of the flat bottom flask, chamber to which side arm and siphon tube are attached, along with a condenser\(^{69}\).

Sterilization of extract

Extracts were sterilized by 0.22 µ membrane filters (Millipore) under positive pressure and kept at 4°C until use\(^{(4)}\).

Media and reagents

Nutrient Agar (Difco.U.K), Nutrient Broth (Difco.U.K), Antibiotic Discs (Oxoid U.K)

Microbial isolates

Microorganisms used in this study were isolated in one of the famous Bacteriology Laboratory in Islamabad. These organisms were isolated from human blood, urine, throat, and pus, in the Laboratory and were re-identified and their antibiogram activity was determined. Streptomycin was found most suitable to be used as a control (Table 1).

Antimicrobial activity of plant extracts

Research in the past, on the anti-microbial activity of herbal medicinal plants, has been encountering several problems because of the variety of criteria and techniques employed for testing. The lipophilic
properties of some extracts such as oils make it very difficult to use an aqueous medium for the study of antimicrobial activity\(^{(2)}\). Among the several methods, which were employed in the plant research, following two conventional methods were adapted for this study: Diffusion method and Dilution method. These two methods are being described one by one.

\textit{Table1. Bacterial Isolates and their sources of Isolation}

<table>
<thead>
<tr>
<th>S. #</th>
<th>Bacteria</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Staphylococcus aureus</td>
<td>Pus</td>
</tr>
<tr>
<td>2</td>
<td>Proteus mirabilis</td>
<td>Pus</td>
</tr>
<tr>
<td>3</td>
<td>Salmonella typhi</td>
<td>Blood</td>
</tr>
<tr>
<td>4</td>
<td>Salmonella para-typhi A</td>
<td>Blood</td>
</tr>
<tr>
<td>5</td>
<td>Salmonella para-typhi B</td>
<td>Blood</td>
</tr>
<tr>
<td>6</td>
<td>Klebsiella pneun.</td>
<td>Throat</td>
</tr>
<tr>
<td>7</td>
<td>Strep. β</td>
<td>Throat</td>
</tr>
<tr>
<td>8</td>
<td>E. coli</td>
<td>Urine</td>
</tr>
<tr>
<td>9</td>
<td>Pseudomonas aeruginosa</td>
<td>Urine</td>
</tr>
</tbody>
</table>

\textbf{Diffusion method}

In this method, microbial culture is inoculated on the surface of agar medium using disk or hole as a reservoir for extracts or antibiotics. The same to be tested, present in the reservoir comes into contact with an inoculated medium, and after overnight incubation at 37ºC, the plates are observed for zones of inhibition surrounding the reservoirs. The zone of inhibition is the clear area around the reservoir, shows the inhibition of growth of microorganism by the diffused substance through the agar. The diameter of the clear zone around the reservoir (zone of inhibition) is measured \(^{(14,3)}\). Well method was used in this study.

\textbf{Material}

Nutrient agar plates, Streptomycin as a control (15µg/100µl) and Crude leaves extract.

\textbf{Procedure}

Dehydrated nutrient agar (23 grams) was mixed with one liter distilled water and boiled to dissolve the contents of the medium. It is sterilized by autoclaving at 121ºC for 20 minutes at 15 Lbs. pressure. When the temperature reached between 50 and 60ºC, the medium was poured in the Petri plates which were already washed and sterilized before the preparation of medium. The medium was poured aseptically in 30ml quantity in each plate; medium was allowed to solidify for 30 minutes, and after solidification, all plates were incubated at 37ºC for overnight to check the presence of contamination.

Borer, which was comprised of 6mm stainless steel tube attached to the arm of the conical flask and suction pump, which was attached to the mouth of the armed conical flask with a glass tube, assembled the components mentioned above of the borer aseptically. Total two holes were cut on the surface of agar medium in each of the 09 plates; these were used in each experiment—one plate for each bacterium. The holes were marked for Melia azedarach, and one for Control (Streptomycin). Bacterial cultures were inoculated using cotton swabs after standardization with McFarland standard solution\(^{(4)}\). Each hole was filled with 100ul corresponding product. The plates were kept in the refrigerator for one hour to allow the content of each hole to absorb in the medium. Plates were incubated at 37ºC for 18-20 hours. After incubation, the diameter of each zone of inhibition was measured at two different places, and the mean value was taken for the record. This procedure was repeated 03 times to confirm the size of zones of inhibition and antibacterial effect of extracts on each bacterium used in this study and to evaluate the results\(^{(4,12)}\).

\textbf{Dilution method}

This method is generally used for quantitative estimation of antimicrobial activities. It is also used in the preliminary screening purpose. In this method turbidity is the indication of growth, which is estimated by the colorimetric/spectrophotometric method for quantitative estimation whereas when there is no growth, the medium remains clear, due to anti-microbial activity of samples incorporated into the medium\(^{(14,17)}\). The standard plate count method was adopted in the study.

\textbf{Materials}

Media: Nutrient broth tubes and Nutrient Agar plates

\textbf{Controls}

1) Control 37ºC is the test tube containing 1 ml distilled water instead of plant extract and kept at 37ºC to compare the growth with the treated sample tubes.

2) Control +4ºC: Control +4ºC is the initial load of bacteria used in the test and during the test it was kept at +4ºC to compare the level of growth in the treated sample tubes.
**Procedure**

Nutrient broth tubes and Nutrient agar plates were prepared and checked for contamination and finally refrigerated until use. 24 hours before the start of experiments, the bacterial culture was freshly prepared by inoculating 9ml nutrient broth with 1ml bacterial culture and incubated at 37°C. After overnight incubation at 37°C, the nutrient broth was distributed in 20ml quantity into 100ml flasks. McFarland solution was used for standardization purpose. One ml bacterial culture freshly prepared was inoculated to this flask. After inoculation medium was distributed in the 4ml amount to three tubes. Tubes were marked for Melia azedarach, Control 37°C, and Control +4°C. In tubes marked for extract, the 1ml extract was added. Similarly, 1 ml of distilled water was added to each control tubes. All tubes were incubated at 37°C for 18-20 hours except the tube marked +4°C, was kept in the refrigerator at +4°C. After overnight incubation turbidity in each tube was checked. Serial dilutions were prepared from each tube up to 10\(^{-5}\). From each dilution, three plates of Nutrient Agar were inoculated for plate count and incubated for overnight. Colonies on each plate were counted and recorded. Each experiment was repeated three times. The same method was used to test other eight bacterial cultures.

### 3. RESULTS

**Physical characters and pH of crude extracts of Melia Azedarach**

The concentrated extracts (5.5 grams) of Melia azedarach in 50ml distilled water were used to check the color, turbidity, and pH (Table 2).

<table>
<thead>
<tr>
<th>Extract Parameters</th>
<th>Color</th>
<th>Turbidity</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melia azedarach</td>
<td>Dark brown</td>
<td>Turbid</td>
<td>5.78</td>
</tr>
</tbody>
</table>

**Diffusion method**

In order to show the effectiveness of extracts against each microbial datum of 11 experiments has been presented statistically in Table 3 and 4 in the form of the most commonly used assessment measures of position in statistics. These are Mean, Median, and Mode\(^{10,18}\). These three values show the complete picture of the effectiveness of extracts against each microorganism in the form of the zone size in millimeter (mm). Standard deviation, standard error T value, and probability were also calculated, showing significant values of the result.

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Mean</th>
<th>Median</th>
<th>Mode</th>
<th>Std. Dev.</th>
<th>Std. Err.</th>
<th>T. Test</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. Coli</td>
<td>23</td>
<td>23</td>
<td>23</td>
<td>0.6742</td>
<td>0.2033</td>
<td>46.38</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Pseud. aeroginosa</td>
<td>22</td>
<td>22</td>
<td>22</td>
<td>1.182</td>
<td>0.3402</td>
<td>40.166</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>S. typhi</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>0.8312</td>
<td>0.2506</td>
<td>46.483</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>S. typhi A</td>
<td>23</td>
<td>23</td>
<td>23</td>
<td>1.2136</td>
<td>0.3659</td>
<td>32.357</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>S. typhi B</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>1.1201</td>
<td>0.3377</td>
<td>54.327</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>S. aureus</td>
<td>19</td>
<td>19</td>
<td>19</td>
<td>0.7006</td>
<td>0.2113</td>
<td>16.716</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Kl. pneumoniae</td>
<td>20</td>
<td>20</td>
<td>19</td>
<td>1.0787</td>
<td>0.3252</td>
<td>12.867</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>23</td>
<td>23</td>
<td>24</td>
<td>1.4206</td>
<td>0.4283</td>
<td>8.3874</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>β Streptococci</td>
<td>20</td>
<td>21</td>
<td>21</td>
<td>1.206</td>
<td>0.3636</td>
<td>8.9721</td>
<td>P&lt;0.001</td>
</tr>
</tbody>
</table>
Table 4. Statistical analysis of zones of inhibition size (m.m.), showing the results of Control

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Mean</th>
<th>Median</th>
<th>Mode</th>
<th>Std. Dev.</th>
<th>Std. Err.</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. Coli</td>
<td>39</td>
<td>39</td>
<td>39</td>
<td>0.9244</td>
<td>0.2787</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>38</td>
<td>39</td>
<td>39</td>
<td>0.6876</td>
<td>0.2073</td>
</tr>
<tr>
<td>S.typhi</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>0.6742</td>
<td>0.2033</td>
</tr>
<tr>
<td>S.typhi A</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>1.2505</td>
<td>0.3777</td>
</tr>
<tr>
<td>S.typhi B</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>0.3015</td>
<td>0.0909</td>
</tr>
<tr>
<td>S.aureus</td>
<td>30</td>
<td>31</td>
<td>32</td>
<td>2.0671</td>
<td>0.6232</td>
</tr>
<tr>
<td>Kl.Pneumoniae</td>
<td>29</td>
<td>30</td>
<td>30</td>
<td>2.0538</td>
<td>0.6193</td>
</tr>
<tr>
<td>Prot. mirabilis</td>
<td>28</td>
<td>29</td>
<td>29</td>
<td>1.3751</td>
<td>0.4146</td>
</tr>
<tr>
<td>β Streptococci</td>
<td>28</td>
<td>29</td>
<td>30</td>
<td>2.7002</td>
<td>0.8141</td>
</tr>
</tbody>
</table>

Table 3 and figure 1 show the excellent antimicrobial activity of Melia azedarach against nine clinical isolates. Salmonella typhi was found most sensitive microorganism whereas Staphylococcus aureus showed the least susceptibility to this extract.

The extract of Melia azedarach showed significant antibacterial activity against each clinically isolated bacterial pathogen although it was in the crude form (Table 3). Standard deviation and T values show highly significant results (Table 3) as the probability is less than 0.001 in all cases of microorganisms.

Fig. 1. Statistical analysis of zone of inhibition size in Melia azedarach leaves extracts in the form of Mean, Median, And Mode.
**Dilution method**  
In this method, all the selected microorganisms were tested separately, and the sensitivity of each microorganism against the plant extract was checked thrice. But it was observed that the extract of Melia azedarach inhibited the growth of *Pseudomonas aeruginosa* only, and in other cases, the extract promoted the growth of all eight bacteria.

**4. DISCUSSION**  
In the diffusion method, Melia azedarach leaves extract showed its effectiveness against various pathogens. *Salmonella typhi* was found most sensitive microorganism against the antibacterial effects of the extract. *Salmonella paratyphi* A, *E. coli* and *Proteus mirabilis* were sharing the number position on the basis of their susceptibility to this extract, whereas the antibacterial activity of the extract was found least against *Staphylococcus aureus*.

If the results of Melia azedarach leaves extract are compared with the results of streptomycin (control) it seems encouraging in the sense that the antibacterial activity of this extract was above the level of that standard on which most of the commonly used antibiotics are considered as sensitive; although it was used in the crude form and may also contain growth promoting factors.

When the results of diffusion and dilution methods are compared, it is observed that in the diffusion method Melia azedarach extract was found more effective against all pathogens. But in the dilution method, it inhibited the growth of *Pseudomonas aeruginosa* only. The reason might be the solubility and diffuse-ability of active compounds present in the extracts which may either be growth promoting or inhibitory to microbes or sensitivity of microbes to these compounds(14).

**5. CONCLUSION**  
In the new era, this type of drug research will open the field for scientists to develop safe drugs from such types of plants, exist in the country and industry to serve not only the nation rather the humanity.

ACKNOWLEDGEMENT  
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CONFLICT OF INTEREST STATEMENT  
The authors declare that there is no conflict of interest regarding the publication of this paper.
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