**In-vitro** Antibacterial Activity of *Acacia Nilotica* against *Lactobacillus casei*

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Authors’ contributions

This work was carried out in collaboration among all authors. Authors AK, MOI and MTB designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SSK, AJ, SS and US managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

**Background:** The role of Ethno-pharmacology is important to discover the new biologically active compounds. The process usually starts with searching of useful plants from different records to the development of methods for the industrial production of drugs. World Health Organization (WHO) states that more than 80% of population of the world makes the use of plants for the treatment of diseases. The extensive use of plants for therapeutic purposes has the history of centuries. Herbal pharmaceuticals have treated many diseases and confirmed the importance of medicinal plants on curative ground. The aim of this study was to evaluate in-vitro antibacterial activity of traditionally used *Acacia nilotica* by aqueous and Ethanolic extraction, against *Lactobacilli casei*.

**Methodology:** Disc diffusion method for antimicrobial susceptibility testing was carried out according to the Kirby-Bauer method to assess the presence of antibacterial activities of Ethanolic
1. INTRODUCTION

Lactobacilli, among adults, are not only found to present in the caries of tooth root [1-2] but also in caries of dentine and are associated with pulpitis. Hahn et al. [3] suggested the presence of two important types of carious lesions: high level lactobacilli lesions and low level lactobacilli lesions. In former, the progression of these bacteria is very varied from necrotic superficial dentine to the deep level. In a study of van Strijp et al. [4], both intact and entirely demineralized dentine, were employed together in the fabricated partial prosthetic appliances of 8 individuals to investigate the influence of the type of substrate on the composition of the bacterial flora. They found that lactobacilli in the dentine specimens were positively associated to the depth of lesion (10.2 ± 13.9 %). As per research of McGrady et al. [5], authors guided a clarification of observation of the existence of lactobacilli species affinity and its possible correlation to collagen of dentine (90 % of the organic phase), which stayed undamaged even after the demineralization has taken the place.

The extensive use of plants for therapeutic purposes has the history of centuries [6]. Herbal pharmaceuticals have treated many diseases and confirmed the importance of medicinal plants on curative ground [7]. The chemistry of natural product, particularly phyto-chemistry has attracted the interest of the researchers because of advantages of the plant extracted compounds by the old-style ways of using herbal plants. The role of Ethno-pharmacology is important to discover the new biologically active compounds. The process usually starts with searching of useful plants from different records to the development of methods for the industrial production of drugs [8]. World Health Organization (WHO) states that more than 80% of population of the world makes the use of plants for the treatment of diseases [9].

Rural people reserve the knowledge and the importance of plants, its medicinal parts, processing and its development to herbal medicines for the treatment of diseases. They have the knowledge from their elders about the important parts of plants during reproductive or vegetative stages. These different parts of plants, sometime is eaten directly or with other appropriate additives [10]. In the system of traditional healing health system of our country Pakistan, approximately 400–600 medicinal plants have been identified [11]. This traditional system of is mainly based on home-produced herbal healings. Pakistan is gifted with different climates, topographical region and ecological zones, having numerous medicinal plants [12], and the Thar desert of province of Sindh is yet lacking the exploration concerning the ethnobotanical surveys of medicinal plants [13]. The aim of this study was to evaluate antibacterial activity of traditionally used Acacia nilotica against Lactobacillus casei. The most commonly used traditional antibacterial plant was selected on basis of published ethnobotanical survey for medicinal plants of Thar Desert [13], Sindh.

2. METHODOLOGY

In this study, Lactobacillus casei (ATCC 393) was used to test the antimicrobial activity of Ethanolic and Aqueous extracts of Acacia nilotica collected from Thar Desert, Sindh Pakistan This has been most commonly used in traditional medicines as an antibacterial plant; on basis of published ethnobotanical survey for medicinal plants of Thar Desert [13], Sindh.

3. COLLECTION OF PLANTS

The plant was obtained from the Nangarparker, Thar Desert of Sindh-Pakistan from October 2019 to October 2020. The scientific names of the plant species was verified using online sources. The selection of plant species was based on literature data on their traditional medicinal uses for the treatment of ailments caused by microbial agents [14].

3.1 Identification of Plants

The plant specimen was authenticated and identified with the help of Chairperson
Department of Botany, Federal Urdu University of Arts, Sciences and Technology Karachi, Pakistan. Voucher specimens were also submitted [15-17].

3.2 Antibiotic Discs

Sterile paper disks (6 mm diameter; oxoid, Cambridge, UK) were impregnated with 40 µL of each of the plants extract and standards and controls, respectively.

3.3 Controls

In this study the negative controls were Water and DMSO while positive controls were 2.5% Sodium hypochlorite and 2% Chlorhexidine.

3.4 Preparation of Ethanolic Extract of Acacia nilotica

The collected Plant material of Acacia nilotica was washed thoroughly with distilled water at room temperature and shade-dried for 3 days. The dried whole plant material was uniformly ground using an electric grinder. With the help of weight balance (CQT 202, Adam), each powdered plant material (1000 g) was soaked for 48 hours in 2.5 L 100% ethanol with continuous stirring through a flask shaker (SF1, Biby Scientific Ltd., UK) at room temperature. Extracts were subsequently filtered through Whatman No. 1 filter paper using filtration assembly (GFL3005, Burgwedel, Germany) and concentrated in vacuo using a rotary vacuum evaporator R-200 (Buchi, Flawil, Switzerland) at 25 degree Celsius. Then it was scooped out into a vial that was previously autoclaved. This was then labeled and kept in -21°C in refrigerator [15,18]. The same method was repeated for extraction with water.

3.5 Disc Diffusion Method

Disc diffusion method for antimicrobial susceptibility testing was carried out according to the standard method by Bauer et al. (1966) to assess the presence of antibacterial activities of Ethanolic and Aqueous extracts of Acacia nilotica. The blank discs were impregnated with both the extracts and both the positive control and negative control. The fresh bacterial culture of Lactobacilli casei was lawn on MHA plates evenly using a sterile swab under sterile conditions in laminar flow cabinet. The plates were dried for 15 minutes and then used for the sensitivity test. The discs which had been impregnated with plant extract were placed on the Mueller Hinton Agar (MHA) surface. All the discs were placed about equidistance to each other. The properly labeled plates were then incubated at 37°C for 24 hours. After the incubation, the plates were examined for inhibition zone. The inhibition zones were then measured using Vernier Caliper and were recorded in proper tables. All the tests were repeated three times to ensure reliability [16-17].

3.6 Measurement of Zone of Inhibition

Presence of a clear area on the MHA plate around any disc, placed on the plate, represents the zone of inhibition which signifies the antibacterial activity of the antibiotic as well as of the plant extract. The diameter of the clear zone was measured three times in millimeter (mm) with a vernier caliper and the average value of zone of inhibition for each extracts was calculated and recorded [16-17].

The same procedure was repeated for both ethanolic and aqueous extracts of Acacia nilotica and results were recorded accordingly.

4. RESULTS AND DISCUSSION

The higher yields were obtained through Ethanolic extract of Acacia nilotica (7.2%) while with Aqueous extraction the yield was very low (3.6%). This may be due to less solubility of the contents present in Acacia nilotica. Table 1 shows that the zone of inhibition of Ethanolic extract of Acacia nilotica (20.33 ± 2.33 mm) was found to be lesser than that of 2.5% Sodium hypochlorite (24.15 ± 2.76 mm) and 2% Chlorhexidine (29.17 ± 1.23 mm) against test organism; Lactobacilli casei Table 2.
Table 1. Comparison of Zone of Inhibition of Ethanolic extract of *Acacia nilotica* with 2.5% Sodium hypochlorite and 2% Chlorhexidine against *Lactobacilli casei*

<table>
<thead>
<tr>
<th></th>
<th>Zone of Inhibition by Ethanolic Extract of <em>Acacia nilotica</em> (mm)</th>
<th>Zone of Inhibition by 2.5% Sodium hypochlorite (mm)</th>
<th>Zone of Inhibition by 2% Chlorhexidine (mm)</th>
<th>Zone of Inhibition by Control (1 % DMSO) (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>Valid</td>
<td>Missing</td>
<td>Mean</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0</td>
<td>20.33</td>
<td>0</td>
</tr>
<tr>
<td>Mean</td>
<td>2.33</td>
<td>2.76</td>
<td>1.35</td>
<td>1.55</td>
</tr>
<tr>
<td>Std. Error of Mean</td>
<td>2.04</td>
<td>0.95</td>
<td>1.35</td>
<td>2.75</td>
</tr>
</tbody>
</table>

Table 2. Comparison of Zone of Inhibition of Aqueous extract of *Acacia nilotica* with 2.5% Sodium hypochlorite and 2% Chlorhexidine against *Lactobacilli casei*

<table>
<thead>
<tr>
<th></th>
<th>Zone of Inhibition by Aqueous Extract of <em>Acacia nilotica</em> (mm)</th>
<th>Zone of Inhibition by 2.5% Sodium hypochlorite (mm)</th>
<th>Zone of Inhibition by 2% Chlorhexidine (mm)</th>
<th>Zone of Inhibition by Water (mm)</th>
</tr>
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<tbody>
<tr>
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<tr>
<td></td>
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<tr>
<td>Mean</td>
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<td>2.17</td>
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<td>0.53</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>1.40</td>
<td>1.30</td>
<td>1.23</td>
<td>1.65</td>
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</tbody>
</table>

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5. CONCLUSION

It is concluded that Plants have been used extensively for medicinal purposes for decades. Herbal pharmaceuticals have successfully treated a variety of diseases, demonstrating the therapeutic value of medicinal plants. It was found that Ethanolic extracts have antibacterial activity which was close to the positive controls (standards) of the study. Ethanolic extract of Acacia nilotica, possesses antibacterial activity against Lactobacillus casei.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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