In vitro Antimicrobial Activity of Tithonia diversifolia Leaf Extracts on Bacterial Isolates from Wound Infections from a Nigerian Hospital

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Abstract: The in vitro antimicrobial activity of the Aqueous (A), Aqueous-Ethanol (AE), Aqueous-Ethanol-Ethylacetate (AEE), Ethanol (E) and Ethylacetate (EE) extracts of the Tithonia diversifolia leaf on bacterial isolates from wound infections was studied. These isolates include Staphylococcus aureus, non-coagulase Staphylococcus, Pseudomonas, Proteus and Klebsiella sp. The Mean Zone Diameter (MZD) of inhibition to 0.06 mL of each of the extract to Staphylococcus sp., 14, 14, 14, 14 and 15 mm, non-coagulase staphylococcus sp., 7, 6, 0, 3 and 4 mm, Pseudomonas sp. 10, 11, 11, 10 and 10 mm, Klebsiella sp. 10, 9, 10, 9 and 10 mm and Proteus sp. has no mean zone diameter. The MZD for 10 μg Streptomycin (control) disk shows 14, 14, 7 and 7 mm for Staphylococcus sp., non-coagulase staphylococcus sp., Pseudomonas sp., Klebsiella sp., Proteus sp. About 82% Staphylococcus aureus have Zone Diameter (ZD) of inhibition above 10 mm to A extract, 82% to AE, 79% to AEE, 82% to E and 85% to EE extracts. Non-Staphylococcus aureus shows no zone diameter of inhibition above 10 mm, 49% Pseudomonas sp. shows zone diameter of inhibition above 10 mm to A, 44% to AE, 36% to AEE, 40% to E and 32% to EE extracts. About 15% Klebsiella sp. shows zone diameter of inhibition above 10 mm to A, 15% to AE, 25% to AEE, 4% to E, 4% to EE. In comparison, 82% of Staphylococcus aureus shows zone diameter of inhibition above 10 mm to 10 μg streptomycin (control) disk. 10% for Klebsiella sp., 85% for non-coagulase Staphylococcus aureus and none for Pseudomonas sp. and Proteus sp. When compared with the control drug, 0.06 mL of each of the leaf extract possesses the same antistaphylococcal activity but possesses twice the effect on gram negative bacteria isolated. However, 0.06 mL of each of the extracts has an appreciable antibacterial effect except for Proteus sp. compared to 10 μg streptomycin disk. Tithonia diversifolia leaf extract shows a promising broad spectrum antibacterial effect on human pathogens. The minimum inhibitory concentration of all the extracts to the organism except for Proteus sp. ranges from 125-250 μg mL⁻¹ while the minimum bactericidal concentration ranges from 500-1000 μg mL⁻¹. Tithonia diversifolia leaf showed promising broad spectrum antibacterial effect on human pathogens.

Key words: Tithonia diversifolia, Mean Zone Diameter (MZD), spectrum, antibacterial effect, gram negative, concentration

INTRODUCTION

The abundance of plants on the earth’s surface has led to an increasing interest in the investigation in different extracts obtained from traditional medicinal plants as potential sources of new antimicrobial agent (Bonjar and Farrokhi, 2004). Plants and plant extracts have been used for the treatment of skin disorders for centuries (Augustin and Hoch, 2004; Avalos and Maibach, 2000; Schepm et al., 1999). Because of increasing resistance to antibiotics of many bacteria, plant extracts and plant compounds are of new interest as antiseptics and antimicrobial agents in dermatology (Augustin and Hoch, 2004; Norton, 2000). Tithonia diversifolia (Hemsley) Gray is a plant belonging to the family Asteraceae commonly called Mexican sunflower is a common shrub native to central America but has become naturalized in many tropical countries including Nigeria. It is found in Nigeria on road sides, crop fields and waste areas. Extracts of the various parts of the plant

Corresponding Author: O. Ogunfolakan, Department of Biomedical Sciences, Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, P.M.B. 4000 Osogbo, Osun State, Nigeria
have been reported to exhibit antimalarial properties (Madureira et al., 2002), anti-inflammatory (Rungeler et al., 1998), anti-proliferation (Gu et al., 2002), insecticidal (Hongshanich et al., 1979) analgesic and anti-inflammatory and anti-bacterial (Bork et al., 1996) activities. Wound infection can be defined as the presence of rapidly multiplying bacteria in the tissue with associated signs of redness, swelling, warmth, pains as sometimes by the presence of pus or formation of an abscess (Mandel et al., 1989).

Bacterial wound infection can be quantitatively defined as presence of 1,00,000 or more bacterial per gram of tissue or per mL of exudates. The skin which provides protection against the external environment has the largest surface area of all the body organs and being the most exposed organ is most vulnerable to infection. This study deals with the antimicrobial activity of the Tithonia diversifolia leaf extracts in vitro against wound bacterial pathogens.

**MATERIALS AND METHODS**

**Bacterial isolates:** The bacterial organisms were fresh isolates from wound swabs from various wards at Obafemi Awolowo University Teaching Hospital Complex (OAUTHC) Ile-Ife. The specimen was routinely processed at the Microbiology sub unit laboratory of Biomedical Science department, Ladoke Akintola University, Osogbo and identified using standard microbiology methods. The bacteria isolates are Staphylococcus, Proteus, Pseudomonas and Klebsiella sp.

**Preparation of Tithonia diversifolia leaf extract.** Sunflower leaf: Tithonia diversifolia was plucked from various locations in Osogbo and Ile-Ife metropolis in Nigeria and was identified by the botany department in Obafemi Awolowo University, Ile-Ife, South Western Nigeria. The leaves were air dried at room temperature and ground into a fine powder with a laboratory mortar. The dried powder was then extracted using aqueous solvent (distilled water), Ethanol and Ethylacetate, respectively. About 40 g of the powder was weighed and then added into 400 mL of distilled water.

The powder solvent mixture was stirred for 2 h using hot plate stirrer and then allowed to stay for 24 h. The extract was then filtered and the filtrate was allowed to evaporate in the oven. The residue of the aqueous extract was used for the sequential extraction of ethanol and ethylacetate while 20 g of a fresh powder was used for ethanol and ethylacetate extraction separately.

**Extract susceptibility testing procedure:** Susceptibility testing was done on each of the isolates for each of the extract and streptomycin using the single disc. Agar-well diffusion method was used in the sensitivity testing using 4 mg mL⁻¹. The bacterial isolates were first grown in nutrient broth for 18 h before use. The inoculum suspensions were standardized with 0.5 McFarland standards. The bacterial inoculum was then plated onto a freshly prepared DST agar plate and excess fluid decanted into a disinfectant jar. With the aid of a sterile Kahn tubes, well was made and each of the extract was then seeded into the wells on the DST plate together with the control antibiotics streptomycin and incubated at 37°C overnight. The zone of inhibition of each of the extract as then measured using a calibrated ruler and recorded. The antimicrobial activity of each extract was inferred from the zone diameter of inhibition of each organism and this was compared with that of streptomycin.

**Determination of minimum inhibitory concentration:** The Minimum Inhibitory Concentration (MIC) was determined using broth dilution. The minimum inhibitory concentration is defined as the lowest concentration of a drug that will inhibit the visible growth of an organism after on overnight incubation. The plant extract was dissolve in dimethylsulfoxide and different concentration ranging from 0.01-2 mg mL⁻¹ were prepared. About 2.5 mL of the concentration from each of the dilution was added to 2.5 mL of molten sterile nutrient broth aseptically and the organism was added in a drop in 10 μL. The mixture was then incubated overnight at 37°C. The lowest concentration preventing visible growth of organism i.e., turbidity of the broth was taken as the minimum inhibitory concentration.

**RESULTS AND DISCUSSION**

Patient with clinical evidence of wound infections were studied. About 94 isolates cultured from the wound swabs are Staphylococcus, pseudomonas, Klebsiella and Proteus sp. The antibacterial effects of different extract of Tithonia diversifolia and 10 μg Streptomycin disc were tested against 94 bacterial isolated from the wound infection. Table 1 shows the zone diameter of the plants extracts to the organism where Staphylococcus aureus has 17 and 12 mm for klebsiella sp.

The Mean Zone Diameter (MzD) of inhibition according to Table 2 for 0.06 mL of the extracts to Staphylococcus aureus ranges between 0-15 mm for all the extracts. While for non-coagulase Staphylococcus aureus ranges between 0 and 7 mm, Pseudomonas sp. ranges between 0 and 11 mm, Klebsiella sp. ranges
Table 1: Antimicrobial screening of crude extract of *Tithonia diversifolia* leaves

<table>
<thead>
<tr>
<th>Organism</th>
<th>A</th>
<th>AE</th>
<th>AEE</th>
<th>E</th>
<th>B</th>
<th>STR</th>
<th>DMSO</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>17</td>
<td>17</td>
<td>15</td>
<td>17</td>
<td>17</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Non-coagulase</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>Staphylococcus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudomonas sp.</td>
<td>12</td>
<td>13</td>
<td>12</td>
<td>11</td>
<td>13</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Klebsiella sp.</td>
<td>12</td>
<td>8</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Proteus sp.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>

DMSO-Dimethylsulphoxide-Dissolvant, A-Aqueous extraction of *Tithonia diversifolia*, AE-Aqueous-Ethanol extraction of *Tithonia diversifolia*, AEE-Aqueous-Ethanol-Ethylacetate extraction of *Tithonia diversifolia*, E-Ethanol extraction of *Tithonia diversifolia*, EE-Ethylacetate Extraction of *Tithonia diversifolia*, STR-Streptomycin; DMSO-Dimethylsulphoxide

Table 2: Mean zone diameter of the bacterial isolates by the plant extracts and Streptomycin

<table>
<thead>
<tr>
<th>Organism</th>
<th>A</th>
<th>AE</th>
<th>AEE</th>
<th>E</th>
<th>B</th>
<th>STR</th>
<th>DMSO</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em> (n = 27)</td>
<td>13.40</td>
<td>13.44</td>
<td>13.93</td>
<td>13.88</td>
<td>14.55</td>
<td>13.88</td>
<td>-</td>
</tr>
<tr>
<td>Non-coagulase</td>
<td>7.14</td>
<td>5.71</td>
<td>-</td>
<td>2.71</td>
<td>4.14</td>
<td>13.70</td>
<td>-</td>
</tr>
<tr>
<td>Staphylococcus (n = 7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Klebsiella sp.</td>
<td>9.60</td>
<td>9.05</td>
<td>9.50</td>
<td>9.2</td>
<td>9.45</td>
<td>6.85</td>
<td>-</td>
</tr>
<tr>
<td>(n = 20) Pseudomonas sp.</td>
<td>10.44</td>
<td>11.00</td>
<td>10.60</td>
<td>10.12</td>
<td>10.24</td>
<td>6.72</td>
<td>-</td>
</tr>
<tr>
<td>(n = 25) Proteus sp.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>6.5</td>
<td>-</td>
</tr>
</tbody>
</table>

DMSO-Dimethylsulphoxide-Dissolvant, A-Aqueous extraction of *Tithonia diversifolia*, AE-Aqueous-Ethanol extraction of *Tithonia diversifolia*, AEE-Aqueous-Ethanol-Ethylacetate extraction of *Tithonia diversifolia*, E-Ethanol extraction of *Tithonia diversifolia*, EE-Ethylacetate Extraction of *Tithonia diversifolia*, STR-Streptomycin; DMSO-Dimethylsulphoxide

between 0 and 10 mm. Proteus shows resistant to all the extracts. The MZD for 10 µg streptomycin (control) disk for *Staphylococcus aureus*, non-coagulase *Staphylococcus aureus, Pseudomonas, Klebsiella* and *Proteus* sp. between 0 and 14 mm. About 79-82% *Staphylococcus aureus* have Zone Diameter (ZD) of inhibition above 10 mm to all the extracts. Non-*Staphylococcus aureus* shows no ZD of inhibition above 10 mm, 32-40% *Pseudomonas* sp. shows ZD of inhibition above 10 mm to all the extracts. About 4-15% *Klebsiella* sp. shows ZD of inhibition above 10 mm to all the extracts. In comparison, 82% of *Staphylococcus aureus* shows ZD of inhibition above 10 mm 10 µg streptomycin (control) disk. About 10% for *Klebsiella* sp., 85% for non-coagulase *Staphylococcus aureus* and nil for *Pseudomonas* and *Proteus* sp. When compared with the control drug, 0.06 mL of each of the leaf extract possesses the same antistaphylococcal activity but possesses twice the effect on gram negative bacteria isolated.

However, 0.06 mL of each of the extracts has an appreciable antibacterial effect except for *Proteus* sp. compared to 10 µg streptomycin disk. The minimum inhibition concentration of the plants extracts to the organisms ranges from 125-500 µg mL⁻¹ and the minimum bactericidal concentration ranges from 500-1000 µg mL⁻¹. This study shows that *Tithonia diversifolia* extracts has a greater antimicrobial effect on gram positive pathogens than gram negative pathogens. This disagrees with the findings done by other researcher (Taiwo et al., 2007) who reported that *Tithonia diversifolia* extracts using disk diffusion method has no appreciable antimicrobial activity while on the other hand, it agrees with another researcher (Obafemi et al., 2006) that *Tithonia diversifolia* extracts has an appreciable antimicrobial activity considering ZD>10 mm as a mean value for susceptibility, *Tithonia diversifolia* inhibits 82% *Staphylococcus* sp. using each of the different extract, respectively (Table 3). Each of the Gram negative isolates shows different percentage to each of the extract, respectively. *Pseudomonas* sp. shows 40, 44, 36, 40 and 32% for the extracts, respectively *Klebsiella* sp. shows 15, 15, 25, 4 and 4%, respectively while *Proteus* shows resistant to all the extracts. When the antimicrobial effect of 0.06 mL of each of the extracts was compared with 10 µg Streptomycin on gram positive pathogens, 82-84% of the pathogens showed ZD of inhibition >10mm for *Tithonia diversifolia* and 78% for 10 µg Streptomycin. For gram negative pathogens, it ranges from 4-44% shows ZD of inhibition to *Tithonia diversifolia* and 10-14% to 10 µg Streptomycin. These shows that *Tithonia diversifolia* extracts have antimicrobial effect against human pathogens.

CONCLUSION

This study does show that *Tithonia diversifolia* leaf extract has a great antimicrobial effect on bacterial isolates used with MZD>10 mm implying that each of the extract can be used as a broad spectrum antibacterial agent. Studies on the other properties showed be studied and analyzed for the development of traditional medicine.

REFERENCES
