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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, 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, 40% *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; Schempp *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 Asteracaea 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

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have been reported to exhibits 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 bacterial 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 *Staphyloccus*, *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 grinded into a fine powder with a laboratory mortal. 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 proved on to 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

Organism	A	AE	AEE	E	EE	STR	DMSO
Staphylococcus aureus	17	17	15	17	17	12	0
Non-coagulase Staphylococcus	10	10	10	0	0	15	0
Pseudomonas sp.	12	13	12	11	13	7	0
Klebsiella sp.	12	8	10	10	10	8	0
Proteus sp.	0	0	0	0	0	10	0

DMSO-Dimethylsulphoxide-Dissolvent; A-Aqueous extraction of *Tithonia diversifolia*; AE-Aqueous-Ethanolic extraction of *Tithonia diversifolia*; AEE-Aqueous-Ethanolic-Ethylacetate extraction of *Tithonia diversifolia*; E-Ethanolic 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

Organism	A	AE	AEE	E	EE	STR	DMSO
Staphylococcus aureus (n = 27)	13.40	13.44	13.93	13.88	14.55	13.88	-
Non-coagulase Staphylococcus (n = 7)	7.14	5.71	-	2.71	4.14	13.70	-
Klebsiella sp. (n = 20)	9.60	9.05	9.50	9.2	9.45	6.85	-
Pseudomonas sp. (n = 25)	10.44	11.00	10.60	10.12	10.24	6.72	-
Proteus sp. (n = 13)	-	-	-	-	-	6.5	-

DMSO-Dimethylsulphoxide-Dissolvent; A-Aqueous extraction of *Tithonia diversifolia*; AE-Aqueous-Ethanolic extraction of *Tithonia diversifolia*; AEE-Aqueous-Ethanolic-Ethylacetate extraction of *Tithonia diversifolia*; E-Ethanolic 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 extract. 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

Table 3: The MIC (μg mL^{-1}) result of the *Tithonia diversifolia* leaf extracts to the organisms

Organism	A	AE	AEE	E	EE
Staphylococcus aureus	250	250	250	250	125
Non-coagulase Staphylococcus	250	250	250	250	125
Pseudomonas sp.	250	250	250	250	125
Klebsiella sp.	250	250	250	250	125

A-Aqueous extraction of *Tithonia diversifolia*; AE-Aqueous-Ethanolic extraction of *Tithonia diversifolia*; AEE-Aqueous-Ethanolic-Ethylacetate extraction of *Tithonia diversifolia*; E-Ethanolic extraction of *Tithonia diversifolia*; EE-Ethylacetate Extraction of *Tithonia diversifolia*; STR-Streptomycin

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 aggress 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 82% 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.

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