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ANTIMICROBIAL ACTIVITY OF ACACIA NILOTICA AGAINST SOME BACTERIAL ISOLATES ASSOCIATED WITH WOUND INFECTIONS

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ABSTRACT
The search for biologically active compounds extracted from traditionally used plants is relevant due to the increasing resistance of bacteria to synthetic antibiotics and the occurrence of fatal opportunistic infections. Acacia nilotica, of the family leguminosae, is one of the oldest existing plant species having various therapeutic, biological and ethno-botanical claims and has diverse medicinal properties. This study was designed to determine the antimicrobial activity of leaf and stem-bark extract of Acacia nilotica against some bacterial isolates associated with wound infections. Leaf and stem-bark of Acacia nilotica plant was collected, identified, dried and extracted with 100% ethanol. The antimicrobial activity of the extracts and the fractions were evaluated against Staphylococcus aureus (SA), Bacillus subtilis (BS), Pseudomonas aeruginosa (PA) and Escherichia coli (EC) using agar well diffusion technique. Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of the leaf and stem-bark extract was determined using microbroth dilution method. Phytochemical screening was carried out on the extracts and the leaf extract was subjected to liquid-liquid fractionation where n-hexane, ethyl acetate and residual aqueous fractions were obtained. The leaf and stem-bark extracts of Acacia nilotica were both active against the test organisms, but the leaf extract was more active. Antimicrobial activity against SA was the highest (at diameter zone of inhibition of 32.00±0.00 mm); and was observed in with the leaf extract. The MICs of the extracts against the organisms were 15.6-31.3 mg/ml (leaf) and 125 mg/ml (stem-bark); and the MBCs were 31.3 mg/ml (leaf) and 250 mg/ml (stem-bark). Tannins, flavonoids, phenols and terpenoids were present while alkaloids, saponins and glycosides are only present in the leaf extract. This study revealed that the leaf and stem-bark extracts of Acacia nilotica have antibacterial activity due to the presence of phytochemicals, and justify the use of Acacia nilotica in traditional medicine for wound healing.

Keywords: *Acacia nilotica*, Honey, Antifungal activity, Phytochemicals

INTRODUCTION

The commonest bacteria often associated with infected wounds are *Staphylococcus aureus, Streptococcus pyogenes Escherichia coli*, *Pseudomonas aeruginosa* and other enteric bacilli (Hay and Morris-Jones, 2016). *Acacia nilotica*, of the family leguminosae, is one of the oldest existing plant species with various therapeutic, biological and ethno-botanical claims and has diverse medicinal properties (Meresa *et al.,* 2017). The emergence of antimicrobial resistance in wound bacterial pathogens is a serious challenge (Mandal and Mandal, 2011).

In Nigeria, many plants used in traditional medicine for wound healing have not been investigated to determine their efficacy, safety and active principles (Alves-Silva *et al*., 2017). Eighty per cent of the populations rely on traditional herbal remedies for their primary healthcare needs (Organization, 2017). The search for biologically active compounds extracted from traditionally used plants is relevant due to the increasing resistance of bacteria to synthetic antibiotics and also occurrence of fatal opportunistic infections (Cheesman *et al*., 2017). Hence, the aim of this research is to determine the antimicrobial activity of leaf and stem-bark extracts of *Acacia nilotica* against some bacterial isolates associated with wound infections.

MATERIALS AND METHODS

Collection and Identification of Plant material

Acacia nilotica leaves and stem-bark were collected at the botanical medicinal garden of the Department of Pharmacognosy and Ethnopharmacy, Usmanu Danfodiyo University, Sokoto, Sokoto State, Nigeria. The plant leaves and stem-bark were identified and authenticated at the Herbarium Unit of the department, assigned Voucher number (PCG/UDUS/legn/0008) and deposited as specimens at the Herbarium.

Extraction of Plant material

The leaves and stem-bark of *Acacia nilotica* were dried at room temperature and were powdered using mortar and pestle. Fifty gram (50) grams of powdered leaf and one hundred gram (100) grams of powdered stem-bark were weighed. Two hundred and fifty (250) ml and 350 ml of 100 % ethanol were added to the powdered leaf and stem-bark respectively. The beaker was covered with foil paper and allowed to macerate for 24 hours and then filtered through filter paper. The filtrates were transferred into an evaporating dish and dried in an oven at 40ºC. The crude extracts were weighed, labeled and stored in dedicator until ready to be used.

Fractionation of *Acacia nilotica* leaf extract

The crude ethanolic leaf extract was partitioned using different solvents based on their polarity. The solvents used were n-hexane and ethyl acetate. The fractionation was done using liquid-liquid partition method. Fifteen (15) grams of the crude ethanolic leaf extract was first suspended in 100 ml of distilled water and was successfully extracted with organic solvents of increasing polarity (100 ml five times each) yielding n-hexane, ethyl acetate and residual aqueous fractions respectively. The

obtained fractions were dried. The weight of the dried fractions was taken, the percentage yield was calculated and the fractions were kept for subsequent test.

Preparation of Samples

The extract (1000) mg was dissolved in 1 ml of 10 %v/v Dimethyl Sulphoxide (DMSO). This stock solution was again diluted, thus six concentrations of the extract were prepared that is 1000 mg, 500 mg, 250 mg, 100 mg, 50 mg, and 25 mg/ml. Honey was diluted in distilled water and thus six concentrations were obtained; 100 %, 50 %, 25 %, 12.5 %, 6.25 %, and 3.125 % v/v. The solutions of the extract and honey were used for test control. Standard antibiotic disc, tinidazole/ciprofloxacin and 10% DMSO were used for positive and negative control respectively.

Preparation of Media

Nutrient agar medium was prepared by dissolving 28g/1000ml of distilled water according to the manufacturer's instructions and was autoclaved at 121ºC for 15 min. Fifteen (15) ml of the nutrient agar was poured into each sterile petri dish (20 Petri dishes in all) and allowed to solidify. The plates were seeded with 2 ml of overnight standardized culture of each bacterial isolate.

Collection of Test Organisms

Four clinical isolates of bacteria, two Gram positive (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*), were collected from the microbial strain bank at the Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, Sokoto State, Nigeria. The organisms were maintained on nutrient agar medium at 4ºC after standardization.

Standardization of Inoculum

The organisms were harvested from 24-h culture using normal saline. The turbidity of the harvested culture was adjusted to 0.5 McFarland standard spectrophotometrically (OD at 625nm = 0.10)(Wiegand *et al*., 2008) .

Antimicrobial Testing of crude Extracts and Fractions

Agar well diffusion technique as described by (Cheesbrough and Kolattukudy, 1984) was used to determine the antibacterial activity of the extracts. Bacteria were seeded into nutrient agar in petri dishes while seeded plates were allowed to set after uniform distribution of the bacterial isolates following slow rotation of the Petri dish. Uniform wells were bored on the surface of the agar using a standard sterile cork borer of 8 mm in diameter. The wells were sealed with 0.1 ml of nutrient agar then filled with 0.1 ml of extract using micropipette. One of the well in each plate was filled with 0.1 ml of 10% DMSO as a negative control. Standard antibiotic disc; tinidazole/ciprofloxacin was placed on the nutrient agar as a positive control. The plates were then allowed to stand for 1 hour at room temperature to allow extract diffusion. All the plates were incubated at 37 ºC for 24 h and observed for zones of inhibition. The diameter zone of inhibition was measured in millimeter (mm) using a meter rule and was repeated in triplicate.

Determination of Minimum Inhibitory Concentration (MIC)

The MICs of the extracts against the test organisms were determined using the microbroth dilution method. Cultures of each tested bacterium were diluted in normal saline and the turbidity of the inoculums was adjusted to 0.5 McFarland standard spectrophotometrically (OD at 625nm = 0.10)
(Wiegand *et al*., 2008)Double strength nutrient broth was prepared according to manufacturer's instructions.

The extracts were diluted with 10% DMSO. Exactly 50 µl aliquot of the extract was added into wells of microtitre plate containing 100 µl nutrient broth and was serially diluted to concentrations ranging from 1000 mg/ml to 0.5 mg/ml. Exactly 50 µl of the adjusted culture was added to each well and the plates were allowed to stand for 1hour to allow extract diffusion. The plates were incubated at 37ºC for 24 h. Negative control and blank control was maintained for each test. After 24 h incubation, the absorbance of the wells was taken using microplate spectrophotometer. The lowest concentration of the agent that inhibited the visible growth of the test organisms was taken as the MIC.

Determination of Minimum Bactericidal Concentration (MBC)

Exactly 20 µl of sample were collected from those wells which did not show any growth and inoculated on a sterile nutrient agar. The plates were then incubated overnight at 37ºC. The lowest concentration of *Acacia nilotica* which did not show any visible growth on agar plate was taken as the MBC.

Phytochemical Screening

The methods described by Evans, (2009) were used to test for the presence of active ingredients in the extracts.

RESULTS

Yield of crude Extracts and Fractions

The yields of crude extracts and fractions of *Acacia nilotica* are as given in Table 1. The percentage yield of the crude leaf extract was higher than that of stem-bark extract; and that of ethyl acetate fraction of leaf extract was the highest.

Table 1: Yield of crude extracts and fractions of *Acacia nilotica*

Plant Parts	**Yield**	
	g	**%**
Leaf Extract	33.02	86.04
Stem Bark Extract	2.22	2.22
n-Hexane Fraction	2.06	13.73
Ethyl acetate Fraction	6.48	43.20
Residual Aqueous Fraction	5.24	34.93

Antimicrobial Activity of crude Extracts and Fractions

The antimicrobial activities of the crude leaf and stem-bark extracts of the plant are as shown in Table 2 and 3 respectively. The activities against all the test organisms were concentration dependent; and were greater against Gram positive than Gram negative test organisms. The antimicrobial activities of the fractions are as given in Table 4. Ethyl acetate and residual aqueous fractions have antibacterial activity against the test organisms while n-hexane has no activity against all test organisms.

The MICs and MBCs of the crude extracts are as presented in Table 5 (leaf) and Table 6 (stem bark). Growth of most of the test organisms were inhibited at concentration ranging from 15.631.3 mg/ml and killed at concentration of 31.3 mg/ml by the leaf extract; but inhibited at concentration of 125 mg/ml by the stem-bark extract.

Phytochemical Screening

The preliminary phytochemical screening shows that *Acacia nilotica* contains flavonoids, Alkaloids, tannins and carbohydrates while amino acid is absent as presented in the Table 7.

Table 2: Antibacterial activity of crude leaf extract of *Acacia nilotica*

Bacteria	Diameter of Zone of Inhibition of Leaf Extract (Mean ± SD) (mm)					TCF (50/5 µg)	DMSO (10%)
	1000 mg/ml	500 mg//ml	250 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml	
SA	32.00±0.00	28.67±0.58	23.67±0.58	22.33±0.58	22.00±0.00	17.33±0.58	32 00 0.00±0.00
BS	26.00±0.00	24.33±1.15	24.00±1.00	22.67±0.58	20.00±0.00	16.00±0.00	32.00	0.00±0.00
PA	27.00±0.00	22.00±0.00	18.67±0.58	17.33±0.58	14.67±0.58	13.00±0.00	40.00	0.00±0.00
EC	25.67±0.58	22.00±0.00	19.33±0.58	18.00±0.00	15.00±0.00	14.33±0.58	31.00	0.00±0.00

Key: SA= *Staphylococcus aureus*; PA= *Pseudomonas aeruginosa*; EC= *Escherichia coli*; BS= *Bacillus subtilis*; TCF: Tinidazole/Ciprofloxacin; MSO: Dimethyl sulphoxide.

Table 3: Antibacterial activity of crude stem-bark extract of *Acacia nilotica*

Bacteria	Diameter of Zone of Inhibition of Leaf Extract (Mean ± SD) (mm)					TCF (50/5 µg)	DMSO (10%)
	1000 mg/ml	500 mg//ml	250 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml	
SA	20.00±0.00	19.00±0.00	15.33±0.58	12.33±0.58	11.67±0.58	11.33±0.58	32.00 0.00±0.00
BS	18.00±0.00	13.33±0.58	13.00±0.00	12.00±0.00	11.33±0.58	0.00±0.00
PA	16.67±0.58	15.00±0.00	13.33±0.58	13.00±0.00	0.00±0.00	0.00±0.00
EC	14.33±0.58	13.00±0.00	12.67±0.58	13.00±1.00	0.00±0.00	0.00±0.00

Key: SA= *Staphylococcus aureus*; PA= *Pseudomonas aeruginosa*; EC= *Escherichia coli*; BS= *Bacillus subtilis*; TCF: Tinidazole/Ciprofloxacin; DMSO: Dimethyl sulphoxide.

Table 4: Antibacterial activity of fractions obtained from crude ethanolic leaf extract of *Acacia nilotica*

Bacteria	Diameter of Zone of Inhibition of fractions from crude ethanolic leaf extract (Mean ± SD) (mm)			TCF (50/5 µg)	DMSO (10%)
	n-Hexane (100 mg/ml)	Ethyl acetate (100 mg/ml)	Residual Aqueous (100 mg/ml)		
SA	0.00±0.00	27.33±0.58	12.33±0.58	32.00	0.00±0.00
BS	0.00±0.00	30.00±0.00	14.00±0.00	31.00	0.00±0.00
PA	0.00±0.00	22.67±0.58	16.67±0.58	32.00	0.00±0.00
EC	0.00±0.00	27.00±0.00	13.00±0.00	40.00	0.00±0.00

Key: SA= *Staphylococcus aureus*; PA= *Pseudomonas aeruginosa*; EC= *Escherichia coli*; BS= *Bacillus subtilis*; TCF: Tinidazole/Ciprofloxacin; DMSO: Dimethyl sulphoxide

Table 5: Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of Crude ethanolic leaf extract of *Acacia nilotica*

Organism	Concentration of Leaf extract (mg/ml)	
	MIC	**MBC**
Staphylococcus aureus	31.30	31.30
Bacillus subtilis	31.30	31.30
	15.60	31.30
Pseudomonas aeruginosa		
Escherichia coli	15.60	+

Key: MIC: Minimum Inhibitory Concentration; MBC: Minimum Bactericidal Concentration; +: growth

Table 6: Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of rude ethanolic stem-bark extract of *Acacia nilotica*

Organism	Concentration of Stem-bark extract (mg/ml)	
	MIC	**MBC**
Staphylococcus aureus	125	+
Bacillus subtilis	125	+
Pseudomonas aeruginosa	125	250
Escherichia coli	125	+

Key: MIC: Minimum Inhibitory Concentration; MBC: Minimum Bactericidal Concentration; +: growth

Table 7: Phytochemical screening of crude ethanolic extracts of *Acacia nilotica*

Constituents	Leaf Extract	Stem-bark Extract
Carbohydrate	+	+
Flavonoids	+	+
Alkaloids	+	+
Phenols	+	+
Tannins	+	+
Saponins	+	
Diterpenes	+	+
Cardiac glycosides	+	
Triterpenoids	+	+
Amino acids		

DISCUSSION

Acacia nilotica is one of the oldest existing plant species with various therapeutic, biological and ethno-botanical claims and has diverse medicinal properties (Meresa *et al*., 2017). This study investigated the antimicrobial activity of leaf and stem-bark extracts of *Acacia nilotica* against some bacterial isolates associated with wound infections.

The higher yield obtained for the leaf compared with the stem-bark extract is in line with a study by Okoro *et al*., (2014) which demonstrated a higher yield for leaf extract than the stem-bark extract. The highest yield of ethyl acetate fraction of the crude ethanolic leaf extract suggests that the bioactive constituents are mostly moderately polar.

The study showed that *Acacia nilotica* leaf and stem bark extract were effective inhibitors of bacterial growth. The extracts

showed varying degrees of activity against Gram positive and Gram negative bacteria. The higher antibacterial activity observed for the crude leaf extract of *Acacia nilotica* against all test organisms is in contrast with the study by Okoro *et al*. (2014) which showed that the stem-bark and root extracts had better activity than the leaf extract.

The observed decrease in antibacterial activity of both crude leaf and stem-bark extracts against all test organisms with decrease in extract concentration can be explained as possible reduction in concentration of the bioactive compounds with decrease in extract concentration.

The observation that large inhibition zone diameters obtained with leaf extract at concentration of 1000 mg/ml against *Staphylococcus aureus* was comparable with inhibition zones produced by Tinidazole/Ciprofloxacin is indication of comparable antibacterial activity.

The observed zero antibacterial activity for n-hexane fraction against the test organisms is indication that the bioactive principles were absent in the fraction.

The differential MIC and MBC values observed for leaf and stem-bark extracts are indication that they differ in the composition and quantity of bioactive compounds.

The presence of all (but amino acids) phytochemicals tested in the leaf extract; and all (but saponins, cardiac glycosides, amino acids) in the stem-bark extract may be indication of their possible role in the observed antibacterial activities as observed before (ur Rahman *et al.*, 2014). These constituents have being reported to be associated with different pharmacological, including antimicrobial, activities of *Acacia nilotica* by Banso, (2009) who reported that ethanol extract exhibited antimicrobial activity against *Streptococcus viridans, Staphylococcus aureus, Escherichia coli, Bacillus subtilis* and *Shigella sonnei*.

The antimicrobial activity of the plant might be due to the polyphenolic compounds. The studies of Cheesbrough and Kolattukudy, (1984), also indicated that polyphenolic compounds and/or volatile oils cause inhibition of wide range of microorganisms. Phenol is a well-known chemical antiseptic and the presence of tannin may accelerate wound healing probably due to their astringent effect.

This study has shown that the ethanolic leaf and stem-bark extract of *Acacia nilotica* contains bioactive constituents that may be beneficial as an antimicrobial agent and also in accelerating wound healing.

CONCLUSION

The study has shown that both the ethanolic leaf and stem-bark extracts of *Acacia nilotica* possesses antibacterial activity against common bacteria associated with wound infections, with leaf extract being more active; the extracts contain phytochemicals which are thought to have possible role in the activities. This study showed that *Acacia nilotica* has useful antimicrobial properties and therefore, has justified its use in traditional medicine for wound healing.

Recommendations

Further studies should be carried out on the leaf extract fractions to isolate and purify the compound(s) responsible for the antibacterial activity against the wound pathogens. Acute toxicity studies on the plant should be investigated to determine its safety.

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