

ANTIBACTERIAL ACTIVITY OF CRUDE N-BUTANOL FRACTION OF *TERMINALIA AVICENOIDES*

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ABSTRACT

Eight gram of the primary extracts was fractionated using N-butanol. Preliminary phytochemical screening and antibacterial activity was determined. Qualitative analysis of the extracts revealed the presence of glycosides, saponins, tannins, flavonoids, alkaloids, steroids and phenol. Different concentrations ranging from 10mg/ml to 50mg/ml were used to test the antibacterial activity. Result of the study showed that the fraction is active against Methicillin Resistant *Staphylococcus aureus* with the means zone of inhibition ranging from 14.50±0.02^e to 22.00±0.06^a. Zones of inhibition were measured in millimeter to the nearest whole number using meter rule.

Keywords: Phytoscreening, MRSA, antibacterial activity, *Terminalia avicenoides*

INTRODUCTION

It is believed that nature has provided the cure for every disease in one way or the other. Plants have been known to relieve various diseases worldwide. Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Ncube *et al.*, 2008). The knowledge of phytochemicals presence in extracts is a credit for area of specialization. The correct identification of the herbal material and the active materials is crucial to quality control, safety, efficacy, acceptability and possible integration into the national healthcare system of herbal remedies. Herbal preparations are increasingly being used in both human and animal healthcare systems. Diarrhea is one of the common clinical signs of gastrointestinal disorders caused by both infectious and non-infectious agents leading to human and livestock debilitating condition. Herbs are used by humans for the treatment of bacterial and fungal infections (Mann, *et al.*, 2009). Many people in developing countries still rely on medicinal plant for their daily healthcare needs, in spite of the advancement in modern medicine (Passalacqua *et al.*, 2006). Most of these are either used as therapeutic or prophylactic in human or animal diseases. In Nigeria, farmers are known to treat animal diseases with herbs before the advent of standard medicine. Traditional medical and veterinary practices remain relevant and vital in many areas in the country (Ojewole, 2004). Therefore, this study was carried out to determine the antibacterial activity of crude extract of N-butanol of *Terminalia avicenoides* extracts against one of the most implicated bacterial species in human infections - MRSA.

MATERIALS AND METHOD

Collection and identification of the plant

Fresh root, stem and leaf of *Terminalia avicenoides* were collected from the open field at Doka village in Doka District, Kachia Local Government Area of Kaduna State, Nigeria along Kaduna-Abuja express way. It was identified at the Department of Botany, Faculty of Science, Ahmadu Bello University Zaria, with the herbarium number 900239.

Preparation of the plant material

The root, stem and leaf *Terminalia avicenoides* were air-dried and later pulverized with the aid of mortar and pestle into powder form. The powder was packed into a clean plastic container with screw cap for subsequent bench work.

Fractionation

Eight grams of the parent extracts was fractionated using N-butanol. The residue was dried in hot air oven at low temperature. The fraction obtained was stored in screw cap bottle.

Collection of wound samples

A total of 100 wound samples were collected from Yusuf Dantsoho Memorial Hospital Tudun Wada, and Gwamna Awan General Hospital Kaduna. The samples were analysed in the Medical laboratory, Department of Microbiology, Faculty of Science Kaduna State University.

Test Organisms The methicillin resistant *Staphylococcus aureus* used in study was isolated from wound sample collected Yusuf Dantsoho Memorial Hospital Tudun Wada and Gwamna Awan General Hospital Kaduna, Nigeria. The isolate was checked for purity and maintained in slants of Nutrient agar.

Antibiogram sensitivity Agar well diffusion method was used (Clinical Laboratory Standard Institute, 2017) Mueller Hinton agar was prepared according to the manufacturer's instruction: dissolved and sterilized at 121°C for 15 minutes. The medium were allowed to cool to 45°C and 20mL of the medium was dispensed into sterile plates and then allowed to cool and solidify. The media was seeded with 1mL standard inoculums of the bacterium and streak evenly over the surface of the medium with the use of sterile swabs. The plates were dried at 37°C for 25 minutes. By the use of a standard sterile cork borer (6mm) in diameter, holes were made at the centre of each inoculated plate and 0.1mL of the solution of the extract was introduced into each well. The plates were then incubated at 37°C for 24 h. After the incubation, the zones of inhibition were observed. The zones of inhibition were measured with a transparent ruler and the result

was record in millimeters (mm).

RESULTS

One hundred wound samples were collected from the studies centres. Fifty one samples were positive with *Staphylococcus* species. Out of the fifty one *Staphylococcus* species isolated from the infected wound, five were Methicillin Resistant *Staphylococcus aureus* (Table 1, 2).

Table 1. Distribution of *Staphylococcus aureus* obtained from the clinical specimens

Study Centre	Number Examined	Number Positive (%)
G A G H	50	25(49.0)
Y D M H	50	26(50.9)
TOTAL	100	51(100)

Key: GAGH = Gwamna Awan General Hospital, YDMH = Yusuf Dantsoho Memorial Hospital

Table 2. Oxacilin Characterization of Methicillin resistant *Staphylococcus* Species

Hospital	Number Examined	OS (%)	OR (%)
G A G H	25	23(45.1)	2(3.9)
Y D M H	26	23(45.1)	3(5.8)
TOTAL	51	46(90.1)	5(9.7)

Key: GAGH = Gwamna Awan general Hospital, YDMH = Yusuf Dantsoho Memorial Hospital, OS = Oxacillin sensitive, OR = Oxacillin resistant

Table 3. Bioconstituents of N-butanol fraction of *Terminalia avicenoids*

Biocompounds	Root Bark	Bark	Leaf
Alkaloids	-	-	+
Tanins	+	+	+
Saponins	+	+	+
Glycoside	+	-	+
Flavonoids	+	+	-
Phenol	-	+	-
Steroids	+	-	+

Key: (+) = Detected, (-) = Not detected

Leaf fraction had high zone of inhibition ranging from 14.50±0.02^e to 22.00±0.06^a mm. However the activities of the root and bark fractions were low (16.00±0.08^c to 18.40±0.09^a) (16.45±0.02^d to 20.00±0.05^a) for the root and bark respectively (Table 4).

Table 4. Antibacterial susceptibility pattern of normal-butanol fraction of *Terminalia avicenoids*

Conc (mg/ml)	Zone of inhibition (mm)			
	Root bark	Stem bark	Leaf	CT
10	17.25±0.02 ^b	16.45±0.02 ^d	15.04±0.05 ^d	36.00±0.11 ^a
20	17.35±0.03 ^b	19.40±0.02 ^a	14.50±0.02 ^e	
30	18.00±0.09 ^a	18.50±0.03 ^b	20.10±0.07 ^c	
40	18.40±0.09 ^a	20.00±0.05 ^a	21.00±0.09 ^b	
50	16.00±0.08 ^c	17.59±0.08 ^c	22.00±0.06 ^a	
P. Value	0.0002	0.0004	0.0001	

Key: ZI = zone of inhibition (mm), Conc = concentration, CT = control, abcde = Significant difference between the group

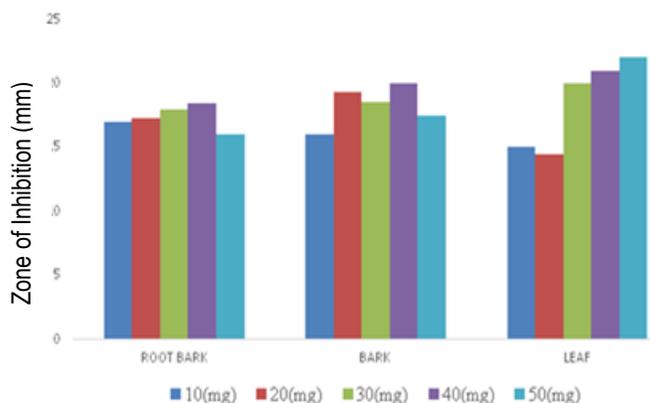


Figure 1: Antibacterial Susceptibility Patterns of N-butanol Fraction against Methicillin Resistant *Staphylococcus Aureus*

DISCUSSION

The high prevalence of *Staphylococcus* species may be attributed to the improper dressing of the wound or lesion. The prevalence of the *Staphylococcus* species agree with finding of Abdullahi *et al.* (2011), who reported that *Staphylococcus* were predominant in wound infection. Screening for phytochemicals revealed the presence alkaloids, tannins, saponin, flavonoids, phenol, steroids and glycosides in all the three parts of the plants (table 3). The presence of these secondary metabolites may be due to the type of solvent used for the extraction. This is attributed to the antibacterial. These results agree with the finding of Abdullahi *et al.* (2009). Different concentrations ranging from 10mg/ml to 50mg/ml of the fraction were used to evaluate the activity of the extracts. The root, bark and leaf extract all showed activity against Methicillin Resistant *Staphylococcus aureus*. The presence of the secondary metabolites detected from the extract contributes

to the antibacterial activity. Plants that are rich with biocompounds such as tannins, saponin, flavonoids and alkaloids have been confirmed to exhibit activity on both Gram positive and Gram negative bacteria because they tend to inhibit protein synthesis (Idris *et al.*, 2009). The low activities of the root and bark extracts may be attributed to the fact that fewer compounds were detected. There was no significant difference between the concentrations because all the P-Values (0.0002, 0.0004, 0.0001) were less than 0.5, but the mean zones of inhibition showed significant difference within the concentrations. Generally, the activity of the plant was low. This may be as a result of fewer biochemical compounds was revealed.

Conclusion

Fifty one samples were positive with *Staphylococcus aureus*. Out 51(90.1%) samples 9.7% (5) isolates were Methicillin Resistant *Staphylococcus aureus* (MRSA). The phytochemical analysis revealed the presence of Alkanoids, saponins, flavonoids, tannins, phenol, glycosides, and steroids. All the plant parts exerted zone of inhibition against the test organism but the activity was low ranging from 14.50 ± 0.02^a to 22.00 ± 0.06^a . This research showed that *Terminalia avicenoides* has potential antibacterial activity which could be useful in the Pharmaceutical industries to develop therapy against Methicillin Resistant *Staphylococcus aureus*.

Recommendation

The activity of the extracts against Methicillin - resistant strains (MRSA) of pathogenic microbes should be further studied to discover the full spectrum of the extract so as to gain the full knowledge of the potential of this plant as an antibacterial agent.

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