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Research Article

ANTIBACTERIAL PROPERTIES OF CRUDE POD EXTRACT OF ACACIA NILOTICA (FABACEAE)

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ABSTRACT

Antibacterial activity of different concentrations of *Acacia nilotica* pod crude extract (200, 400, 600, 800 and 1000 mg/ml of water) *on Staphylococcus aureus, Streptococcus pyogenes, Bacillus subtilis, Corynebacterium pyogenes, Klebsiella pneumoniae, Salmonella typhi and Candida albicans* was determined. Minimum inhibitory concentration of *A. nilotica* pod crude extract was 12.5 mg/ml for *Bacillus subtilis, C. pyogenes* and *K. pneumoniae,* while it was 25 mg/ml for *S. pyogenes* and *C. albicans* and 200 mg/ml for *S. aureus* Minimum bactericidal concentration of *Acacia nilotica* pod crude extract was 25 mg/ml for *B. subtilis* and *K. pneumoniae*, 100 mg/ml for *S. pyogenes* and 200 mg/ ml for *S. aureus* and *C. pyogenes*. This study revealed the importance of *A. nilotica* pod as an antibacterial agent and its applications in the treatment of bacterial diseases.

Key words: *Acacia nilotica*, antibacterial properties, crude extract, pods

There are roughly 1,300 species of Acacia worldwide with about 950 species being native to Australia and remaining species spread around the dry tropical to warm temperate regions of both hemispheres, including India, Africa, Southern Asia, and the Americas. Flowers of *Acacia nilotica* are globulous, 1.2 to 1.5 cm in diameter with bright golden-yellow color. Pods are strongly constricted, white-grey, hairy and thick (Baravker *et al*., 2008). The extract from various parts of *A. nilotica* is found to stimulate the synthesis and release of prolactin in rats and may stimulate lactation in women (Lompo *et al*., 2004). *A. nilotica* pods have anti-hypertensive, antispasmodic, anti-diarrhoeal, astringent, anti-fertility and anti-oxidant activities (Gilani *et al*., 1999; Singh *et al*., 2009).

Traditionally the pods, leaves, bark and flowers have been reported to be used in the management of different disease conditions (Meena *et al*., 2006). *A. nilotica* pods have been reported to inhibit the growth of some bacterial and fungal organisms that cause diarrhoea (Umaru *et al*., 2011). Hence, this study was conducted to ascertain the antibacterial activity of *A. nilotica* pods on most common bacterial organisms incriminated in the cause of deaths in the tropics.

MATERIALS AND METHODS

Sample Collection, Identification and Extract Preparation: The pods of *A. nilotica* were obtained from Lassa, Askira/Uba Local Government Area, Borno state, Nigeria and were authenticated at the Department of Biological Sciences, University of Maiduguri, Nigeria. The pods were air-dried, ground and powdered. Two hundred and fifty grams of the powder was mixed with 5.0 L of distilled water (25^{0}C) and the mixture was shaken manually. Shaking was repeated after every 30 min for 6 h before it was allowed to stand for 18 h, thereafter it was shaken vigorously and filtered using Whatman filter paper No. 1. The filtrate was dried in an oven at 50^{0}C and the crude powder was stored at 4^{0}C.

Phytochemical Screening of *A. nilotica:* The aqueous extract of *A. nilotica* was prepared by cool water extraction and was subjected to qualitative chemical screening for identification of the various classes of active chemical constituents (Trease and Evans 1989; Sofowora, 1993; Edeoga *et al*., 2005).

Culture Media: Nutrient agar and nutrient broth (Oxoid, England) of pH 7.3 were used for antibacterial investigation.

Preparation of Microbial Culture: *Staphylococcus aureus, Streptococcus pyogenes, Bacillus subtilis, Corynebacterium pyogenes, Klebsiella pneumoniae, Salmonella typhi and Candida albicans* cultures were obtained from the Department of Veterinary Medicine, University of Maiduguri, Nigeria. The isolates were cultured separately on nutrient agar plates and incubated for 24 h. A colony of each test organism was sub-cultured on 10 ml nutrient broth and incubated at 37^{0}C for 8 h. One milliliter of the broth culture was used to flood the agar plate.

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Preparation of Inocula: The inoculum size of all bacterial isolates tested was standardized using of overnight broth cultures prepared by inoculating 3 loopful of well-isolated colonies of test bacteria in 10 ml of nutrient broth which was incubated at 35^0C for 24 h. A loopful of the overnight broth culture was diluted in 4ml of sterile normal saline in such a way that its turbidity matched with that of 0.5 MacFarland standard (Cheesbrough, 2002).

Preparation of Antimicrobial Discs: Graded concentrations of 200, 400, 600, 800 and 1000 mg/ml of the extract were reconstituted using distilled water. One ml of sterile distilled water was added to each plate containing the extract and stirred. Filter paper discs were then placed in each plate and stirred so as to ensure the impregnation of the disc by the extract. Tetracycline (250 mg/ml) as the control drug was prepared the same way as the extract and was placed at the centre of each inoculated plate and the plates were incubated at 37^0C for 18-24 h.

Minimum Inhibitory Concentration (MIC): The MIC of the crude pod extract of *A. nilotica* was determined using standard protocol. Serial dilutions of the extract were prepared so as to obtain the concentrations of 200, 100, 50, 25 and 12.5 mg/ml respectively and used to determine the MIC. The MIC was recorded as the least concentration of the extract that completely inhibited the growth of the organisms (Greenwood, 1989; Geidam *et al.*, 2007).

Minimum Bactericidal Concentration (MBC): The MBC of the extract was determined using standard protocol. Samples were taken from test tubes used in the MIC assay and sub-cultured unto freshly prepared nutrient agar medium and later incubated at 37^0C for 24 h. The MBC was taken as the lowest concentration of the extract that inhibited bacterial growth on the agar plates (Olorundare *et al*., 1992).

RESULTS AND DISCUSSION

Phytochemical Contents of Crude Pod Extract of *A. nilotica*: Phytochemical analysis of the crude pod extract of *A. nilotica* revealed the presence of carbohydrates, tannins, saponins, cardiac glycosides and flavonoids (Table 1).

Antibacterial Activity of *A. nilotica* Pod Crude Extract: The crude extract possessed antimicrobial activities against *S. aureus, S. pyogenes, B. subtilis, C. pyogenes, K. pneumoniae,* S. *typhi and C. albicans* in graded concentrations of 200, 400, 600, 800, and 1000 mg/ml, while *E. coli, P. aeruginosa* and *Proteus mirabilis* were resistant to the extract. Tetracycline (250 mg/ml), used as a control drug, inhibited the growth of all the micro-organisms including those resistant to the aqueous extract of *A. nilotica* (Table 2).

MIC of *A. nilotica* Pods Extract on Some Bacterial Isolates: The MIC test showed that *B. subtilis* and *K. pneumoniae* were inhibited by 12.5 mg/ml, *S. pyogenes*, *C. pyogenes* and *C. albicans* by 25 mg/ml while *S. aureus* by 100 mg/ml of crude pod extract, respectively (Table 3).

MBC of *A. nilotica* Pods Extract on Some Bacterial Isolates: The MBC showed that *B. subtilis* was inhibited at 25 mg/ml, *K. pneumonia* and *C. albicans* at 50 mg/ml, *S. pyogenes* at 100 mg/ml, while *S. aureus* and *C. pyogenes* were inhibited at the concentration of 200 mg/ml (Table 3).

The aqueous extract of *A. nilotica* antibacterial activity was compared against a standard drug, tetracycline. Tetracycline showed a better antibacterial property with highest zone of inhibition of 48 mm for *S. pyogenes* at 250 mg/ml and least zone of inhibition of 16mm for *S. aureus* and *S. typhi* as compared to the highest zone of inhibition of 25 mm at the 1000 mg/ml for *B. subtilis* and *K. pneumoniae* and least zone of inhibition of 12 mm for *S. aureus* and *S. typhi*, respectively for *A. nilotica*.

Table 1
Qualitative phytochemistry of aqueous extract of *Acacia nilotica* pod

Phytochemical constituents	Type of test	Inference
Carbohydrate	Molisch's	+
	Barfoed's	-
	Free reducing sugar	+
	Combined reducing sugar	-
	Ketones	+
	Pentoses	+
Tannins	Ferric chloride	+
	Formaldehyde	+
	Chlorogenic	-
Anthraquinones	Free anthraquinones	-
	Combined anthraquinones	-
Saponins	Frothing	+
Glycosides	General test	+
Terpenes and steroids	Lieberman- Buchard's	-
	Salkowski's	-
Flavonoids	Lead acetate	+
	Sodium hydroxide	-
	Ferric chloride	+
	Pew	+
Alkaloids	Dragendorff's	-
	Mayer's	-

-=Not detected; +=Present

Table 2
Antibacterial activity of *Acacia nilotica* (Fabaceae) pod crude extract on some bacteria isolates

Extract	Extract conc (mg/ml)	Zones of inhibition (mm) for									
		S. aureus	*S. pyogenes*	*B. subtilis*	*C. pyogenes*	*K. pneumoniae*	*S. typhi*	*E. coli*	*P. aeruginosa*	*P. mirabilis*	*C. albicans*
*A*cacia	1000	16	17	25	15	25	12	R	R	R	18
nilotica	800	13	15	23	13	20	10	R	R	R	16
pod extract	600	11	13	20	11	19	9	R	R	R	14
	400	7	11	17	9	16	7	R	R	R	12
	200	7	9	14	7	13	R	R	R	R	10
Tetracycline	250	16	48	32	24	32	16	28	16	18	20

R = Resistance

Table 3
Determinations of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Acacia nilotica* aqueous pods extract on some bacterial isolates

Organism	Concentration of *Acacia nilotica* aqueous pods extract (mg/ml)									
	MIC					MBC				
	200	100	50	25	12.5	200	100	50	25	12.5
S. aureus	-	+	+	+	+	-	+	+	+	+
S. pyogenes	-	-	-	+	+	-	-	-	+	+
B. subtilis	-	-	-	-	+	-	-	-	-	+
C. pyogenes	-	-	-	+	+	-	-	-	+	+
K. pneumoniae	-	-	-	-	+	-	-	-	-	+
C. albicans	-	-	-	+	+	-	-	-	+	+

The phytochemical screening and qualitative analysis of *A. nilotica* crude pods extract studied showed that the extract is rich in carbohydrates, tannin, saponins, cardiac glycosides and flavonoids. These phytochemicals have been known to show medicinal activity as well as physiological activities (Burger, 1990; Sofowora, 1993; Nwze *et al.*, 2004).

Hydrolyzable tannins tested against *Helicobacter pylori* have been reported to cause dose dependent membrane damaging effect hence indicating the potential in tannins to be used as new and safe therapeutic regimen for treatment of bacterial diseases (Keiji *et al*., 2004).

Saponins have been reported to disturb the permeability of bacterial outer membrane by increasing the permeability of bacterial cell wall to fluids and (Arabski *et al*., 2009). Preparations that contain flavonoids as principal physiological active constituents have been used by physicians and traditional healers in attempts to treat human diseases (Havsteen, 1983). The activity of quercetin, for example, has been at least partially attributed to inhibition of DNA gyrase, hence giving flavonoids its antibacterial activity (Tim-Cushnie and Lamb, 2005).

The study of phytochemical properties and antibacterial activity of aqueous pods extract of *A. nilotica* revealed the presence of three principal phytochemicals (tannins, saponins and flavonoids) that have been reported to possess antibacterial activity (Giovana *et al*., 2013). This could be the reason for antibacterial activity of aqueous pods extract of A. *nilotica.* This study therefore reveal the potentials of *A. nilotica* pod extract as antibacterial agent especially in the management of ailments caused by organisms such as *S. pyogenes*, *B. subtilis*, *C. pyogenes*, *K. pneumoniae* and *C. albicans*. The pods of *Acacia nilotica* can be used in future to develop antibiotics that can be of benefit to humans and animals.

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