ANTIBACTERIAL ACTIVITIES OF *Acacia nilotica* SEED EXTRACTS

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ABSTRACT

Antibacterial activities of the crude extract of *Acacia nilotica* seeds using methanol, chloroform, ethyl acetate and water as solvents were tested on *Klebsiella pneumoniae, Shegella dysenteriae* and *Staphylococcus aureus.* These strains were clinical isolates and confirmatory biochemical test were conducted. The phytochemical analyses of the seed extracts indicated the presence of alkaloids, flavonoids, glycosides and steroids. Disc diffusion method was used to examine the bacterial susceptibility of the extracts. There were various zones of inhibition by the different extracts. Methanolic extract inhibited the growth of *Klebsiella pneumoniae* at 90 and 120 mg/ml (2.0 and 4.3mm respectively), *Shegella dysenteriae* was inhibited at 60 to 120 mg/ml (1.0, 3.5 and 4.1 mm respectively).*Staphylococcus aureus* was inhibited at all the concentrations. The trend of extract inhibition was similar in the chloroform and ethyl acetate while the water extract inhibited the three microorganisms at all the four levels of concentrations. There is an indication that the plant could be used to advanced medical practice.

Key words: *Acacia nilotica*, Phytochemical, Antibacterial activity, Seed extracts

INTRODUCTION

Plants have chemical substances that may have effects on the animals' system, but some with therapeutic properties, can be and have been utilized in the treatment of human diseases (Sofowora, 1979). However, in the literature of folklore medicine, many plant remedies have been reported to cure infectious diseases. A large number of these plant species have been known not to contain any active constituents after various extraction and purification processes (Adesaramu, 1982). Inspite of these failures, yet many of these plants still provide an avenue of effective antimicrobial agents (Waterman, 1990). In view of the unfortunate drug resistance of pathogens to antimicrobial agents and their limited spectrum activity, however, new modified compounds are actively being used (Mitcheri and Rao, 1984). Consequently, the search for new effective antimicrobial agents is a continuous process.

Bacterial infections have been and still remained significant causes of human morbidity and mortality in the tropical countries; hence, due to indiscriminate used of antibiotics make the bacteria developed resistance. Some species of bacteria are considerably known as normal flora of the body. On the other hand, some conditions such as chilling, smoking, anesthesia and malnutrition can make microorganisms to become pathogenic (Olanipekun and Montitiove, 1978; Sofowora, 1985 and Rotimi *et al.,* 1994). Extracts of different parts of crushed *Acacia nilotica* seed dissolved in some organic solvents have been studied and were

found to posses antibacterial effects (Lutere *et al.*1994; Abrevetal, 1999; Hussain *et al.* 1999 and Tona *et al*. 1999). *Klebsiella* sp is a facultative anaerobe, it causes chest infection; intestinal and occasionally severe bronchopneumonia with lung abscesses. The infections occur in persons with chest disease or diabetes mellitus or in malnourished persons. *Klebsiella* sp often produce beta lactomas and are resistant to ampicillin and cephalosporin (Monica, 2002).

Shigella dysenterae is primarily human pathogen, Gram negative, non-motile, non sporing, non capsulated and non lactose fermenting rods. It is a facultative anaerobe, but grows best aerobically. It produces convex, circular translucent colonies with entire edge measuring 2mm in diameter within 24 hours; it causes gastroenteritis (Bacillary dysentery). The organism invades the mucosal epithelia and subsequently causes superficial ulceration and bleeding with an incubation period of 1-4 days. *Staphylococcus aureus* is a non fastidious pathogen; it can grow in the presence of high concentration of salt with optimum temperature of 37^{O}C and pH 7.4-7.6. It produces golden yellow colour colonies and has a number of features on patients such as, pustule, boils, carbuncles and impetigo (Merchant and Parker, 1967). The antimicrobial activities of crushed *Acacia nilotica* seeds on some commonly encountered bacteria pathogens were investigated.

MATERIALS AND METHODS

Plant Materials and Extraction

The seeds from the pods of the plants were obtained within the savanna region of northern Nigeria. The seeds were sun dried for five days, ground and sieved to fine powder. Distilled water, methanol, ethyl acetate and chloroform were used as solvents for extraction of the active constituents. A soxhlet extractor was used with continuous reflux until enough was collected. This was decanted and the solvents evaporated to concentrate (Lyamabo, 1991).

Enumeration of bacteria and biochemical analysis

Bacteria isolates were obtained from clinical stock cultures at Usmanu Danfodiyo University Teaching Hospital, Sokoto. Five milliliters of each stock culture was transferred into 9 ml of 0.85% normal saline as a stock solution, serial dilution up 10^{-9} were made. Following the methods of Fawole and Oso (1998), 0.l ml each dilution was plated on nutrient agar, blood agar and MacConkey agar. Sterilized glass spreader was used to spread the suspension on the surfaces of the agar media. The plates were then incubated at 37 ^{O}C for 24 hours. Distinct colonies were selected to subculture into nutrient agar slants for further use. 10^{-6} and 10^{-7} dilution of each extract was streaked on nutrient agar plate. This was prepared as described by Smith (1977). This was incubated at 37^{O}C for 24 hours, the growths observed on the plate were subcultured again into plates of nutrient agar after the isolation of the pure culture and the biochemical tests were as well carried out. These were as follows: Litmus milk reaction, Gram stain, Lactose, Dextrose, Sucrose, Indole test, MRVP test, Citrate test, Urease test, Catalase test, Oxidase test, Gelatine liquefaction, Starch hydrolysis and Lipid hydrolysis as described by Jewetz *et al.,* (1988) and Cowan and Steel (1995).

Antibacterial activity

This was carried out according to Cheesbrough (1984). The method was for extract evaluation of the presence of alkaloid, saponin, glycoside, steroid and flavonoids according to (Harbone, 1991; El-Olemy *et al*., 1994; Okeretu and Ani, 2000).

RESULTS

The extracts of *Acacia nilotica* seeds indicated the presence of alkaloids, flavonoids, glycoside and steroids (Table 1). The antimicrobial activities of methanol extracts showed that *Klebsiella pneumonia*e at 30 and 60 mg/ml concentration had no inhibition while 90 mg/ml concentration had a zone of inhibition of 2mm and 120 mg/ml had a zone of inhibition of 4.30mm. 30 mg/ml could not inhibit *Shegella dysenteriae* but was inhibited at 60, 90 and 120 mg/ml concentration by 1.00, 3.50 and 4. 10 mm respectively. The various concentrations of the methanolic extracts inhibited the growth of *Staphylococcus aureus* by 0.5, 1.50, 4.50 and 4.80 mm respectively (Table 2). The chloroform extract at 30 mg/ml concentration could not inhibit the growth of *Klebsiella pneumoniae* but could inhibit its growth effectively at 60 to 120 mg/ml from 2.00, 2.50 and 2.70mm respectively. *Staphylococcus aureus* resisted the concentration at 30 mg/ml but not at 60 to 120 mg/ml. The Ethyl acetate extract was more affective on *Klebsiella pneumoniae* at higher concentration of 60 to 120 mg/ml where inhibition was 1.70, 2.10 and 2.67 mm respectively. *Shegella dysenteriae* and *Staphylococcus aureus* were more resistant to the ethyl acetate extracts at 30 to 90 mg/ml but could not resist it at 120 mg/ml. Water extract was very effective on the inhibition of the growth of the microorganism in all the concentration levels (Table 2).

Table 1. Phytochemical constituents of *Acacia nilotica* seeds.

Solvents/extracts	alkaloids	flavonoids	Glycosides	saponnins	steroids
Methanol	+	+	+	-	+
Chloroform	+	-	+	-	+
Ethyl acetate	+	-	-	-	+
Water	+	+	+	-	+

Table 2.Antimicrobial activity of aqueous and phytochemical extracts of *Acacia nilotica* seeds.

Conc. Extracts(mg/ml)	Zone of inhibition (mm)		
	K. pneumoniae	*S. dysenteriae*	*S. aureus*
Methanol 30	-	-	0.50
60	-	1.00	1.50
90	2.00	3.50	4.50
120	4.30	4.10	4.50
Chloroform 30	-	-	-
60	2.00	-	2.00
90	2.50	1.50	2.50
120	2.70	1.50	2.50

Ethyl acetate				
	30	-	-	-
	60	1.70	-	-
	90	2.10	-	-
	120	2.67	1.50	1.50
Water	30	1.20	1.10	2.04
	60	2.20	0.50	3.04
	90	4.60	2.50	5.10
	120	6.00	4.00	7.10

DISCUSSION

The antimicrobial activities of the extracts of *Acacia nilotica* seeds studied using different solvents is clearly demonstrated by inhibitory effect on the tested organisms; i.e. *K.pneumoniae, S. dysenteriae* and *Staphylococcus aureus*. However, the difference in the rate at which the test organisms were killed by the extract varies, this was due to differences in susceptibility by the bacterial species (Cheesbrough, 1994). For any compound to be effective it must penetrate the cell enveloped and attained sufficiently high concentration at the target site to exert effectiveness. The antimicrobial activity against gram negative rod organisms as well as the gram positive cocci, is of particular interest owing to the increasing resistance of the organisms to the conventional antibiotics. (Oyekan and Okafor, 1989 and Paulo *et al*., 1994). These results are in agreement with those of Ijeh *et al* (2005), Okoli and Iroegbu (2005) and Nair *et al.*(2008).

The tested organisms showed higher susceptibility from the aqueous extract than the other solvents (table 2). This could be explained by the higher level of merceration of water. Hot water extracts are reported to contain higher amounts of plant constituents (Okeke *et al*. 2001; Okoli *et al*., 2002 and Nkere and Iruegbu, 2005). The high level of toxicity of the plant extracts to the microorganisms had been confirmed by similar results of Ajayi (2008) in a study of the medicinal plants in Nigeria. Tannins, were found to be more in methanol and ethyl acetate extracts than any other extract, and steroids were found to be generally absent, alkaloids are well known for their wide pharmacological activities ranging from antibacterial and antifungal to stimulation of almost all organisms in the body including the nervous system (Traese and Evans, 1983). The variation in phytochemical contentment may be attributed to the various amounts of precursors present in the plant. Although much investigation work still need to be carried out. Ours was to determine the exact nature and identity of particular constituents implicated in the antimicrobial activity observed in *Acacia nilotica* seeds. The plant alkaloids and tannins were responsible for the antimicrobial activities (Table 2).

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