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Research Article

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Antibacterial Activity of Column Fractions of *Acacia nilotica* Leaves Extract

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Abstract *Acacia nilotica* is used in traditional formulation in Nigeria for the treatment of infectious diseases. The susceptibility of bacteria strains against *Acacia nilotica* leaves extracts was determined using the disk diffusion method. The result showed *A. nilotica* leaves to possess antibacterial activity against *S. typhi*, *P. aerugonisa*, *E. coli* and *S. aureus*. At the highest extract concentration (120 mg/ml), the most susceptible microorganism was E. coli, while the least susceptible was S. typhi. Antibacterial activity of column fractions of *A. nilotica* revealed fraction 4 to have the highest activity of 9, 10, 14 and 1mm zones of inhibition against the test organisms respectively. Phytochemical analysis revealed the presence of saponins, terpenoids, flavonoids, phenols, carbohydrates, tannins and steroids. This study validates and supports the use this plant in the treatment of infectious diseases.

Keywords Zones of inhibition, leaves extract, Test organism, Antibacterial activity.

Introduction

Infectious diseases represent an important cause of morbidity and mortality among the general population, particularly in developing countries [1]. In recent years, due to the constant emergence of microorganisms resistant to conventional antimicrobials, pharmaceutical companies have been motivated to develop new antimicrobial drugs. Medicinal plants have been proved to be effective in the treatment of infectious diseases with little or no side effects as experienced with synthetic drugs [2]. This has lead to an increase in search for plant derived antibacterial agents.
Herbs have been used as sources of food and medicinal purposes for centuries and this knowledge have been passed on from generation to generation [3]. In Nigeria, traditional healers treat different kinds of diseases including many microbial infections using herbal preparations. *Acacia nilotica* belongs to Mimosaceae family is grown in virtually every part of Nigeria and other countries like India, Asia, Australia, America [4]. Various preparations of this plant have been advocated in folk medicine for the treatment of diarrhea, to reduce fever, stop excessive bleeding, diabetes and widely employed against all types of infection [5]. The present study was therefore carried out to evaluate the antibacterial activity of *Acacia nilotica* leaves as well as its phytochemical constituents.

Materials and Methods

Collection, Preparation and Extraction of Plant Material

Fresh leaves of *Acacia nilotica* were collected from wilds of Kangiwa Local Government Area in Kebbi state, Northern-west Nigeria. The plant was identified by a botanist with a voucher specimen number 286 deposited at the Herbarium unit, Department of Biological Sciences, Kebbi State University of Science and Technology, Aliero. The leaves were then dried at room temperature and then ground to fine powder using clean mortar and pistol. Four hundred grams (400 g) of the dried plant powder was extracted using 70 % methanol for 72 hrs. The extract was filtered and concentrated.

Phytochemical Analysis

Standard screening test were used to detect the presence of phytochemicals such as saponnins, tannins, glycosides, phenols, flavonoids, anthraquinones, phlobatannins, carbohydrates, steroids and terpeniods [6-9].

Test Microorganisms

The four bacterial strains which were clinical isolates obtained from Specialist Hospital, Sokoto were used for this studies. The bacteria used were *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Salmonella typhi.*

Antibacterial Activity

The antibacterial activity was carried out using agar well diffusion technique [10]. The Muller Hinton agar medium was prepared, allowed to solidify and left on the bench untouched for 18–24 hours to check for sterility of the medium. Standard drug (Ampicillin 250 mg) was used as the positive control while extracts and column fractions of *A. nilotica* leaves were incorporated into four wells based on the concentrations of 30, 60, 90 and 120 mg/ml respectively on the inoculated plates. The wells were sufficiently spaced out to prevent overlapping of the zones. The plates were then allowed for the pre-diffusion time of 15 minute after which they were incubated for 24 hours at 37 ^{o}C. Diameters of zones of inhibition were measured using millimeter ruler and the results were expressed in millimeter.

Fractionation of the Crude Extract of *A. nilotica* leaves

Column Chromatography

Two grams (2 g) of the hydromethanolic leaves extract of *A. nilotica* was subjected to column chromatography to separate the extract into its component fractions. Silica gel 100 - 200 mesh was used as the stationary phase, and the solvent system chloroform: methanol as mobile phase. In the setting up of the column chromatography, the lower part of the glass column was stocked with glass wool with the aid of glass rod. The slurry prepared by mixing 25g of silica gel and 250 ml of the solvent system chloroform: methanol was poured down carefully into the column , the tap of the glass column was left open to allow flow of the solvent system into a conical flask below, the set-up was seen to be in order when the solvent system drain freely without carrying the silica gel or the glass wool along, at the end of the packing the tap was locked, the column was allowed to settle, after which the clear solvent was allowed to drain down to the silica gel meniscus. The column was set up using wet packing method. The sample was prepared by adsorbing 2 g of the extract to 10 g of silica gel in methanol then allowed to dry. The dried powder was gently layered on top of the column then a glass wool was put on top so as to avoid splashing of the solvent system when pouring it into the column. The column tap was opened to allow the eluent to flow. The elution of the extract was done with solvent system chloroform: methanol 9:1 (1.3 L), the solvent system was measured and poured into the column each time the solvent system run down to prevent solvent droplets from falling directly and disturbing the topmost layer of the column, the eluted fractions were collected in bottles. The eluted fractions collected were 60 bottles [11].

Analytical thin layer chromatography (TLC)

The content of each bottle was concentrated then spotted on the pre-coated (silica gel) aluminum plates and placed in a chromatographic tank to separate the different fractions based on the relative mobility and color reactions. Using a capillary tube a spot of the sample was applied on the plate about 1.0 cm from the edge of the plate, it was allowed to dry and lowered into the chromatographic tank that contains the solvent system chloroform: methanol (9:1). The tank was tightly covered to prevent escape of the solvent. After the solvent have traveled a distance from the origin and the plant components separated, the plate was removed, allowed to dry then it was put in a tank that contains iodine crystals for visibility of bands [12]. After the thin layer chromatography was done, the fractions that have the same mobility were joined together, so out of the 60 bottles (column fractions) that were used to run the thin layer chromatography, 8 fractions (F1 –F8) were pooled together and then tested for antibacterial activity.

Results and Discussion

Plant compounds are of interest as a source of safer or more effective substitutes than synthetically produced antimicrobial agents. Pathogenic bacteria have developed resistance to antibiotics or they produce antibiotics resistance strain against broad spectrum drugs. Research has shown that the potential use of plant extract for treatment was due to the phytochemicals present in the extract [13]. Phytochemical screening of *Acacia nilotica* leaves extract revealed that the plant contain terpenoids, saponins, tannins, flavoniods, carbohydrate, steroids and phenols (Table 1).

Table 1: Phytochemical analysis of hydromethanolic leaves extract of *Acacia nilotica*.

Phytochemical Compounds	Result
Carbohydrate	++
Phenols	++
Saponins	++
Tannins	++
Flavonoids	+
Steroids	++
Terpenoids	++
Glycosides	_
Phlobatannins	_
Antraquinones	_

Key: += slightly present, ++ = moderately present, -- = not detected.

Active compounds produced during secondary vegetal metabolism are usually responsible for the biological properties of some plant species used throughout the globe for various purposes, including treatment of infectious diseases [1]. The presence of these phyto-constituents in the leaves extract of this plant is thought to be responsible for the antibacterial activity. Numerous investigations have proved that medicinal plants contain diverse classes of bioactive compounds such as tannins, alkaloids and flavonoids, which exhibit various pharmacological properties [14].

The agar well diffusion method for antimicrobial susceptibility test was performed to determine the antibacterial activity of *A. nilotica* leaves extract against *Salmonella typhi*, *Pseudomonas aeruginosa*, *Escherichia coli* and Staphylococcus *aureus*. The results showed increasing inhibition of bacterial growth with increasing extract concentration (Table 2). Antibacterial susceptibility of the *A. nilotica* leaves extracts at the highest concentration (120 mg/ml) against the test organisms showed the highest activity against *E. coli* with a 13 mm zone of inhibition while lowest activity was observed in *S. typhi* having 7 mm zone of inhibition. The inhibitory effect of growth of the test organisms was higher in the standard drug (Ampicillin) compared to the extract. Disc diffusion assay showed that *E. coli* was the most inhibited bacterial.

Table 2: Antibacterial activity of *Acacia nilotica* leaves extract.

Test Organism	**Test Organism/ zone of inhibition (mm)**				
	***Acacia nilotica* Leaves Extract**				**Ampicillin**
	30 (mg/ml)	60 (mg/ml)	90 (mg/ml)	120 (mg/ml)	250 (mg/ml)
Salmonella typhi	2	4	6	7	10
Pseudomonas aeruginosa	3	5	8	9	15
Escherichia coli	6	8	9	13	16
Staphylococcus aureus.	4	6	8	10	12

Extraction is the crucial first step in the analysis of medicinal plants, because it is necessary to extract the desired chemical components from the plant materials for further separation and characterization [15]. Plant extracts usually occur as a combination of various type of bioactive compounds. It is a common practice in isolation of these bioactive compounds that a number of different separation techniques such as TLC, column chromatography, flash chromatography, Sephadex chromatography and HPLC, are used to obtain pure compounds [15]. The extraction procedure in this study resulted in obtaining concentrated preparations of bioactive substances. To achieve comprehensive knowledge of their properties, it was subjected to column and thin layer chromatographic techniques. The antibacterial activity of column chromatographic fractions of *A. nilotica* leaves demonstrated antimicrobial susceptibility only in fractions 4, 5 and 6 (Table 3).

Table 3: Antibacterial activity of column fractions of *Acacia nilotica* leaves.

S/No	**Column fraction**	**Organism/ zone of inhibition (mm)**			
		S. aureus	***E. coli***	***P. aeruginosa***	***S. typhi***
1	F1(1-2)	-	-	-	-
2	F2(3-6)	-	-	-	-
3	F3(7-11)	-	-	-	-
4	F4(12-20)	9	10	14	1
5	F5(21-24)	5	3	-	5
6	F6(25-32)	3	5	4	-
7	F7(33-45)	-	-	-	-
8	F8(46-60)	-	-	-	-

Once an extract is separated into several fractions and the parent extract and fractions are tested in an assay several outcomes are possible. One outcome is that all activity may be lost in the daughter fractions, in which case the separation method is deemed unsuitable. Loss of biological activity may be due to irreversible binding to the separation media, or to instability of the active compound. A second outcome would be for all or most daughter fractions to have some low amount of activity. This too is undesirable and simply indicates that the separation mode is not suitable. The third and desired outcome is that one or several daughter fractions contain substantial bioactivity, and that the mass of active fractions has been reduced from the parent with a corresponding increase in potency [16]. In the present study, the third outcome is clearly established in fractions 4, 5 and 6.

Most plants contain several compounds with antimicrobial properties for protection against aggressor agents, especially microorganisms. The mechanisms of action of natural compounds are related to disintegration of cytoplasmic membrane, destabilization of the proton motive force, electron flow, active transport and coagulation of the cell content [17]. Not all action mechanisms work on specific targets, and some sites may be affected due to other mechanisms. The degree of concordance between traditional use of *Acacia nilotica* leaves and the observed antibacterial properties of this study suggest that there may be some truth to this remedy.

Conclusion

The results from the study suggest that the leaves of *Acacia nilotica* showed antibacterial activity against different bacterial species. They could be used as alternatives to common antimicrobial agents for treatment of bacterial infections. Further research is needed toward further isolation and identification of active principles present in the column fractions which could possibly be exploited for pharmaceutical use.

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