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Antibacterial Activity and Phytochemical Screening of Acacia Nilotic a Leaf Extracts Against Clinical Isolates of Some Bacteria

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Abstract

Several hundred genera of plants were used traditionally for medicinal purposes. The study was aimed to screen for phytochemicals and to determine the antibacterial susceptibility of Acacia nilotica leaf extracts against clinical isolates of Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa isolated from patients with gastrointestinal infection. The qualitative phytochemical screening of the plant leaf was conducted using conventional method while the quantitative phytochemical analysis was conducted using analytical method. Agar well diffusion method was used for determination of antibacterial activity of the extracts while dilution method was used for determination of minimum inhibitory concentration (MIC) of the extract. The result of phytochemical screening showed that the plant's leaf extracts contain alkaloid (7.2%), followed by flavonoid (3.85%), terpenoid (2.6%), tannin (2.3%) and saponin (1.25%). Least content was recorded by glycoside and reducing sugar with 1% and 0.9% respectively. The antibacterial activity of the leaf extract showed that ethanolic extract has the highest activity (15.04 mm) than aqueous extracts (14.19 mm). The average zone of inhibition recorded by E. coli was 16.15 mm while those recorded by S. aureus and P. aeruginosa was 14.39 and 13.32 mm respectively. It is concluded that the leaf extract of nilotica leaf possesses antibacterial activity.

Keywords: Acacia Nilotica, Antibacterial Activity, Phytochemicals, Staphylococcus Aureus

Introduction

Currently, there has been a lot of attention focused on producing medicines and products that are natural. Several leaves and leaf extracts have been found to have antimicrobial activity against microorganisms [1]. The plants with antimicrobial action may be a source of compounds that can be used to inhibit the growth of pathogens [2]. There is no plant that does not have medicinal value [3]. The active components are normally extracted from all plant structures, but the Concentration of these compounds varies from structure to structure. However, plant parts known to contain the highest concentration of these phytochemicals constituents for therapeutic purpose can be leaves, stem barks, root, bulks, corms, rhizomes, wood, flowers, fruits or the seeds [4]. The presence of phytochemical constituents in medicinal plants made them useful for healing as well as for curing of human diseases [5]. Phytochemicals are naturally occurring compounds in the medicinal plants [6]. Large populations of the world, especially in developing countries depend on the traditional system of medicine to treat variety of diseases [7]. Several hundred genera of plants are used traditionally for medicinal purposes. The World Health Organization reported that 80% of the world population relies chiefly on traditional medicine and a major part of the traditional therapies involving the use of plant extracts and their constituents [8].

Acacia nilotica (L) is multipurpose plant [9]. A nilotica is a plant 5 to 20 m high with a thick spherical crown, stems and branches usually sinister to black colored, grey-pinkish slash, fissured bark, exuding a reddish low-quality gum. The plant has straight, light, thin, grey spines in axillary pairs, usually in 3 to 12 pairs, 5 to 7.5 cm long in young trees, mature trees commonly without thorns. The leaves are bipinnate, with 3 to 6 pairs of pinnulae and 10 to 30 pairs of leaflets each, rachis with a gland at the bottom of the last pair of pinnulae. Flowers in globulous heads 1.2 to 1.5 cm in diameter of a bright golden-yellow color set up either axillary or whorly

on peduncles 2 to 3 cm long located at the end of the branches. Pods are strongly constricted, white-grey, hairy and thick [10]. A. nilotica is a pantropical and subtropical genus with species abundant throughout Asia, Australia, Africa and America. A. nilotica occurs naturally and is imperative in traditional rural and agro-pastoral systems [11]. A. nilotica is an imperative multipurpose plant used broadly for the treatment of various diseases [12].

Natural medicinal plants promote self-healing, good health and durability in ayurvedic medicine practices and have acknowledged that A. nilotica can provide the nutrients and therapeutic ingredients to prevent, mitigate or treat many diseases or conditions). The phytochemicals contribute chemically to a number of groups among which are alkaloids, volatile essential oils, phenols and phenolic glycosides, resins, oleosins, steroids, tannins and terpenes [13]. It has been reported that different parts of the plant are prosperous in tannins (ellagic acid, gallic acid and tannic acid), stearic acid, vitamin-C (ascorbic acid), carotene, crude protein, crude fiber, arabin, calcium, magnesium and selenium [14]. A number of medicinal properties have been ascribed to various parts of this highly esteemed plant. Traditionally the bark, leaves, pods and flowers were used against cancer, cold, congestion, cough, diarrhea, dysentery, fever, gall bladder, hemorrhoid, ophthalmia, sclerosis, tuberculosis and small pox, leprosy, bleeding piles, leucoderma and menstrual problems. The study was aimed to screen for phytochemicals and to determine the antibacterial susceptibility of A. nilotica leaf extracts against clinical isolates of S. aureus, E. coli and P. aeruginosa isolated from patients with gastrointestinal infection [15].

Materials and Methods

Study Area

The study was conducted at Sheikh Muhammad Jiddah Hospital and School of Technology, Kano State Polytechnics both in Kano Metropolis. Kano State is one of the states located in Northern Nigeria. It is geographically coordinated at 110 3'N and 80 3'E latitude and longitude respectively. It shares borders with Kaduna State to the West, Bauchi State to the South, Jigawa State to the East, Katsina State to the North. It has a total area of 20,131 km2 (7,777 sqm) and population of 13,405,300 [16].

Collection and Identification of Plant Materials

The leaves of A. nilotica were collected at Ketawa town of Gezawa Local Government in Kano state, Nigeria at about 6:00 am. The identification and authentication of the leaves was done at Herbarium in the Department of Plant Science Bayero University Kano. Voucher specimens were deposited there for future reference. The leaves were washed thoroughly with distilled water and air-dried in a shade for two weeks, then cut into pieces and grinded into powder using a sterile pestle and mortar under laboratory condition. The powder was then kept in air tight container for future use.

Test Isolates

Clinical isolates of Staphylococcus aureus, E. coli, and P. aeruginosa isolated from Microbiology laboratory of Sheikh Muhammad Jiddah Hospital Kano were used in the study. The isolates were transported to Laboratory of Science Laboratory Technology for identification and further processing. Distinctive morphological properties of the pure culture such as colony form, elevation of colony and colony margin were observed. Further microbial identification was based on the methods of Holt et al. [16].

Extraction of A. Nilotica leaves

Aqueous (water) and ethanol solvents were used for extraction of the active components of the leaves of A. nilotica. For aqueous extraction, water extraction method as described by Ahmed and Beg was used 50 g of each of the fruits powder were extracted by successive soaking for 3 days using 500 ml of distilled water and ethanol in a sterile conical flask respectively [17]. The extracts were filtered using Whatman filter paper and the filtrates concentrated in water bath at 40°C. The solid concentrated filtrate, now the extracts were then stored in universal bottle for further use. The powdered plant part was extracted in 500 ml of ethanol for 3 days mixture was filtered al bottles in the refrigerator at 4°C before use. For ethanol and chloroform extraction, 50 g of using Whatman No.1 filter paper and the extracts were evaporated to dryness using rotary evaporator at 40°C. The solid residues obtained were reconstituted in 10% DMSO at stock concentration, stored in the refrigerator at 4oC until used.

Preliminary Phytochemical Screening

Phytochemical screening of the plant materials was conducted using the method adopted by Sofowora and Tiwari et al. [18, 19]. Wagner's test for alkaloid, Ferric chloride test for phenol, gelatin test foe tannin, lead acetate test for flavonoid, foam test for saponin, acetic acid test for steroid, Salkowski test for terpenoid detection, Fehling's test for glycoside

Quantitative Phytochemical Analysis

Various methods will be employed in determining the amount of bioactive components (phytochemicals) present in the plant materials. Terpenoids, steroids, and tannins will be determined using Spectrophotometric method while phenol will be determined using Folin Ciocalteu procedure. The alkaloids, flavonoids, and the content of saponins will be evaluated using analytical method [20].

Determination of Antibacterial Susceptibility of the Extracts

The agar well method was used to determine the antibacterial activity of the extracts. 0.1ml of the different standardized organism were inoculated on the surface of Mueller Hinton Agar in a sterile Petri dish and allowed to set and then labeled. A sterile cork borer 6mm was used to punch holes (i.e. 5 wells) in the inoculated agar and the agar was then removed. Four wells that were formed were filled with different concentrations of the extract which were labeled accordingly; 50, 100, 150 and 200 mg/ml while the 5th well contained the solution used for the research to serve as control, Ciprofloxacin (Micro lab limited) 100 mg/ml, was used as control in this research. These were then left on the bench for 1hour for adequate diffusion of the extracts and incubated at 37°c for 24 hours. After incubation, the diameter of the zones of inhibition around each well, were measured to the nearest millimeters. The experiment was conducted in triplicate and average value was calculated [21].

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of the extracts was determined using broth dilution technique. Two-fold serial dilutions of the extracts were prepared by adding 2ml of 100mg/ml of the extract into a test tube containing 2ml of Nutrient broth, thus producing solution containing 50mg/

ml of the extract. The process continues serially up to test tube No. 5, hence producing the following concentrations; 50, 25, 12.5, 6.25 3.125 mg/ml. Test tube No. 6 do not contain extracts and serve as negative control. Exactly 0.5 ml of 0.5 McFarland equivalent standards of test organisms were introduced into the test tubes and incubated at 370 C for 24 hours. After incubation the test tubes were observed for growth by checking for turbidity [21].

Determination of Minimum Bactericidal Concentration (MBC)

From each tube that did not show visible growth in the MIC, 0.1ml was aseptically transferred into extract free Mueller Hilton agar plates. The plates were incubated at 37°C for 24 hours. The MBC was recorded as the lowest concentration of the extract that had less than 99% growth on the agar plates [21].

Statistical Analysis

The data of average zone of inhibition produced by the isolates against the antibiotics used was analyzed using One-Way ANOVAs and the statistical program SPSS 21.0 (Statistical Package for the Social Sciences). The results were presented as the means ± standard deviation. Significance level for the differences was set at $p<0.05$.

Results

Qualitative Phytochemical Screening

The qualitative phytochemical screening of A. nilotica aqueous and ethanolic leaf extract is presented in Table 1. The result indicated the presence of alkaloid, saponin, phenol, flavonoid, and glycoside, tannin, reducing sugar and terpenoid in both the extracts.

Table 1: Qualitative phytochemical screening of Acacia nilotica leaf extract

S/N	Phytochemicals	Test	Aqueous extract	Ethanol extract
1	Alkaloid	Wagner's test	+	+
2	Saponin	Foam test	+	+
3	Phenol	Ferric chloride test	+	+
4	Flavonoid	Lead acetate test	+	+
5	Glycoside	Fehling test	+	+
6	Tannin	Gelatin test	+	+
7	Reducing sugar	Fehling test	+	+
8	Steroid	Acetic test	-	-
9	Terpenoid	Salkowski test	+	+

Key: + = Presence of phytochemical, - = Absence of phytochemical.

Quantitative phytochemical Screening

The quantitative phytochemical screening of *A. nilotica* leaf extract is presented in Table 2. The result showed that the extract has highest percentage of alkaloid (7.2%), followed by flavonoid (3.85%), terpenoid (2.6%), tannin (2.3%) and saponin (1.25%). Least content was recorded by glycoside and reducing sugar with 1% and 0.9% respectively.

Table 2: Quantitative phytochemical screening of Acacia nilotica leaf extract

S/N	Phytochemicals	Quantity (%)
1	Alkaloid	7.20±0.05
2	Flavonoid	3.85±0.02
3	Phenol	1.15±0.01
4	Saponin	1.25±0.01
5	Tannin	2.30±0.02
6	Glycoside	1.00±0.01
7	Reducing sugar	0.90±0.01
8	Terpenoid	2.60±0.01

Antibacterial Activity of Aqueous Extract

The antibacterial activity of aqueous extract of A. nilotica leaf is presented in Table 3. The results showed that zones of inhibition recorded by the isolates depend on the type of bacterial isolates and concentration of the extracts. Highest zone of inhibition was demonstrated by E. coli (19.2± mm) at 200 mg /ml. The zone of inhibition of the control (Ciprofloxacin 100 mg/ml) ranges from to 21-24 mm.

Table 3: Antibacterial activity of aqueous extract of A. nilotica leaf

	Concentration (mg /ml)/zone of inhibition (mm)				
Isolates	**25**	**50**	**75**	**100**	**Control**
Pseudomonas aeruginosa	10.20±0.10a	12.00±0.20a	13.50±0.13^{a}	15.60±0.20^{b}	21
Escherichia coli	12.30±0.14a	13.60±0.14a	17.80±0.15^{b}	19.20±0.32^{c}	24
Staphylococcus aureus	11.30±0.17a	12.50±0.18a	14.80±0.20^{b}	17.50±0.18^{c}	23

Key: Values having different superscript on the same row are considered significantly different at $p<0.05$

Antibacterial activity of Ethanol Extract

The antibacterial activity of ethanol extract of A. nilotica leaf is presented in Table 4. The results showed that zones of inhibition recorded by the isolates depend on the type of bacterial isolates and concentration of the extracts. Highest zone of inhibition was demonstrated by E. coli (20.6 mm) at 200 mg /ml. The zone of inhibition of the control (Ciprofloxacin 100 mg/ml) ranges from to 21-24 mm

Table 4: Antibacterial activity of ethanol extract of *A. nilotica* leaf

	Concentration (mg /ml)/zone of inhibition (mm)				
Isolates	**25**	**50**	**75**	**100**	**Control**
Pseudomonas aeruginosa	10.80±0.12a	13.50±0.18a	14.30±0.15^{b}	16.70±0.23^{b}	21
Escherichia coli	12.80±0.13a	14.70±0.11b	18.20±0.19^{c}	20.60±0.12^{c}	24
Staphylococcus aureus	11.20±0.17a	12.80±0.15a	16.30±0.13^{b}	18.60±0.15^{c}	23

Key: Values having different superscript on the same row are considered significantly different at $p<0.05$

MIC and MBC of the Leaf Extract

MIC and MBC of aqueous and ethanol extract of A. nilotica leaf is represented in Table 5. The result showed dilutions of various concentrations of aqueous and ethanol extracts can inhibit and/or kill the isolates. Lower MIC (3.125 mg/ml) was shown by ethanol extract. MBC of ethanol extract ranges between 12.5 - 50mg/ml while the MBC of aqueous extract ranges from 25 – 50 mg/ml.

Table 5: Minimum inhibitory concentration (MIC) and MBC of the extracts

Isolates	**Aqueous extract**		**Ethanol extract**	
	MIC (mg/ml)	**MBC (mg/ml)**	**MIC (mg/ml)**	**MBC (mg/ml)**
S. aureus	12.5	50	6.25	25
Escherichia coli	6.25	25	3.125	12.5
P. aeruginosa	12.5	50	12.5	25

Discussion

The plant product or natural products show an important role in diseases prevention and treatment through the enhancement of antioxidant activity, inhibition of bacterial growth, and modulation of genetic pathways. In the present study, phytochemical screening and antibacterial activity of aqueous and ethanol extracts of Acacia nilotica leaves against clinical isolates of S. aureus, P. aeruginosa and E. coli were determined. From the present study, the result of phytochemicals shows the presence of alkaloid, saponin, phenol, flavonoid, and glycoside, tannin, reducing sugar and terpenoid in both the extracts. The quantitative phytochemical constituents of Acacia nilotica leaves extract showed that the extract has highest percentage of alkaloid (7.2%), followed by flavonoid (3.85%), terpenoid (2.6%), tannin (2.3%) and saponin (1.25%). Least content was recorded by glycoside and reducing sugar with 1% and 0.9% respectively. The result of phytochemical analysis of this work is inconformity with several researchers who worked on antibacterial activity of the plant. Several studies were conducted to determine the phytochemical present in A. nilotica plant [13,22,23]. This result was in conformity with that of Kalaivani et al. who confirmed that all the tested extracts of A. nilotica contain phenolic compounds, flavonoid and saponins [22]. The finding also correlates with that of Jigam et al and Banso et al who both found terpenoids, alkaloids, saponins and glycosides in A. nilotica plant [13, 23].

The result of the antimicrobial activities of the extracts by agar well diffusion showed that the extracts produce zones of inhibition all the test organisms even at the lowest concentration. Ethanolic extract is the first in term of activity with average zone of inhibition of 15.04 mm while average zone of inhibition recorded by aqueous extracts was 14.19 mm, highest zone of inhibition of ethanol extracts is due to better solubility of the phytochemical constituents of the plant parts. The findings of antibacterial activity of this study correlate with the findings of several researchers [13,23].

The finding of this study was in conformity with that of Banso who confirms the antimicrobial activity of Acacia against Streptococcus viridans, Staphylococcus aureus, Escherichia coli, Bacillus subtilis and Shigella sonnei using the agar diffusion method [13]. This finding also justifies the study conducted by Kalaivani and Methew who found the extract of A. nilotica demonstrated highest activity against three bacterial (E. coli, S. aureus and Salmonella typhi) and two fungal strain (Candida albicans and Aspergillus niger) [23]. On the other hand, the results of antibacterial activity of the extracts show that the extracts were more active against E. coli than S. aureus and P. aeruginosa. The average zone of inhibition recorded by E. coli was 16.15 mm while those recorded by S. aureus and P. aeruginosa was 14.39 and 13.32 mm respectively. The antibacterial activity of the extracts is due to the present of phytochemicals they contained. The presence of phytochemical constituents in medicinal plants made them useful for healing as well as for curing of human diseases [5]. Phytochemicals are naturally occurring compounds in the medicinal plants. The MIC and MBC of aqueous and ethanol extract of A. nilotica leaf showed dilutions of various concentrations of aqueous and ethanol extracts can inhibit and/or kill the isolates. Lower MIC (3.125 mg/ml) was shown by ethanol extract. MBC of ethanol extract ranges between 12.5 - 50mg/ml while the MBC of aqueous extract ranges from 25 – 50 mg/ml.

Conclusion

The study screened for phytochemical and determination of antibacterial activity of aqueous and ethanol extracts of A. nilotica leaf against clinical isolates of S. aureus, P. aeruginosa and E. coli. From the results of the study, it is concluded that the aqueous and ethanol extracts of A. nilotica contain the following bioactive components; alkaloid (7.2%), followed by flavonoid (3.85%), terpenoid (2.6%), tannin (2.3%) and saponin (1.25%). Least content was recorded by glycoside and reducing sugar with 1% and 0.9%. The antibacterial activity of aqueous and ethanol extracts of Acacia nilotica leaf against the isolates showed that the ethanol extract demonstrated higher activity than aqueous extract. It is recommended that the extract from A. nilotica leaf is a good candidate for production of antibiotics.

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