



COMPARATIVE ANTIBACTERIAL ACTIVITY OF *ACACIA NILOTICA* WILD. LEAVES EXTRACTS AGAINST MULTI DRUG RESISTANT BACTERIA

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Abstract

Antibiotic resistance among bacteria is becoming a major problem in the treatment of many infections. Hence, there is need to find a potential alternative way of curing disease, this includes the use of medicinal plants in overcoming the antibiotic resistance. The present study was aimed at investigating the antibacterial activity of *Acacia nilotica* Wild. leaves extracts against some selected multi drug resistant bacteria (*Staphylococcus aureus*) and (*Pseudomonas aeruginosa*). Extraction of *Acacia nilotica* were performed using water and chloroform, on the basis of their increasing polarity with varying concentrations and were screened for the antibacterial activity using disc diffusion assay. Phytochemical screening reveals presence of alkaloids, tannins, saponins, phenols, terpenoids and steroids. Aqueous extract was found to be more potent against all the selected bacterial pathogens with zone of inhibition ranges from (10 mm-13 mm) and (6 mm-10 mm) while that of chloroform extract were (3 mm-5 mm) and (2 mm-4 mm) against *S. aureus* and *P. aeruginosa* respectively. The minimum inhibitory concentration (MIC) were (7 mg/ml) and (15 mg/ml) for aqueous extract while (15 mg/ml) and (25 mg/ml) for the chloroform extract on the test bacteria. Therefore this study determined the value of *Acacia nilotica* plant as alternative treatment for bacterial infections that can be used to completely overcome or minimize the resistance of bacteria observed in synthetic antimicrobial agents.

Keywords: *Acacia nilotica*, chloroform, extract, pathogens, multidrug resistant

INTRODUCTION

Natural sources, such as plants and their products, have been used in the indigenous system of medicine from time immemorial for curing diseases. Being plant a rich source of secondary metabolites such as phenolic acids, flavonoids, tannins, alkaloids, and other small compounds (Ncube *et al.*, 2008). Various plant extracts and phytochemicals offer considerable potential for the development of new agents effective against infections and could help curb the problem of multidrug-resistant organisms (Ncube *et al.*, 2008). The genus *Acacia* belongs to the family Mimosaceae. It is a cosmopolitan genus containing more than 1350 species, distributed throughout tropical and warm temperate areas of the world (Maslin *et al.*, 2003).

Out of these species, *Acacia nilotica* (also known as *Bagaruwa* in Hausa language, Gum Arabic tree, Babul, Egyptian thorn, or Prickly Acacia) is widely distributed in subtropical Africa from Egypt to Mauritania to South Africa, and in Asia eastwards to Pakistan and India (Bennison and Paterson, 1994). Traditionally, the plant is used widely for the treatment of

various ailments, but scientifically few of them were screened out (Malviya *et al.*, 2011).

Several species of *Acacia* have been proven as an effective medicine in the treatment of a cough, toothache, diarrhea, dysentery, jaundice, and skin disorders (Sahu *et al.*, 2011). In addition to the afore mentioned, its various parts possess significant antibacterial and antifungal properties (Mahmood and Qureshi, 2012). *A. nilotica* is also reported to be effective against multidrug-resistant strains of bacteria and fungus causing nosocomial and community-acquired infections (Khan *et al.*, 2009).

Phytochemical screening of different parts of *A. nilotica* showed distinct classes of secondary metabolites having therapeutic potential (Sawant *et al.*, 2014). A number of secondary metabolites have been reported from various *Acacia* species including amines and alkaloids, cyanogenic glycosides, cyclitols, fatty acids seed oils, fluoroacetate, gums, non-protein amino acids, terpenes, hydrolyzable tannins, flavonoids and condensed tannins (Seigler, 2003).

The development of resistance in microorganisms to presently available antibiotics has necessitated the search for new antimicrobial agents. Antibiotics provide the main basis for the therapy of microbial infections. Since the discovery of these antibiotics and their uses as chemotherapeutic agents there was a belief in the medical fraternity that this would lead to the eventual eradication of infectious diseases. However, overuse of antibiotics has become the major factor for the emergence and dissemination of multi drug resistant strains of several groups of microorganisms (Harbottle *et al.*, 2006).

The Leaves and Bark extracts of *Acacia nilotica* have been used for the determination of antimicrobial activity, but little information was documented on effect of *Acacia nilotica* leaves extract against multi drugs resistant bacteria. Therefore, this study aims to report on the antibacterial potential of *A. nilotica* crude extract and its bioactive compounds against multi drug resistant (MDR) bacteria.

MATERIALS AND METHODS

Study Area

This study was carried out within Kebbi State University of Science and Technology, Aliero headquarter of Aliero local government area of Kebbi state North-Western Nigeria. It is located in the South-Eastern part of Kebbi State on the Al higher way 12° C'16° C'42° C'N4'27'066E. It was created 1991 with a total land mass of 412Km², the local government is bounded in the North-East by Gwandu local government area, in the south by Jega local government, in the East by Tambuwal local government area of Sokoto State, in the North-West by Birnin Kebbi local government area. Kebbi State share boundary with Sokoto State in the North-Eastern axis, Zamfara State on the Eastern part, Niger State in Southern part and Republic of Niger on Western part, Kebbi State has the total population of 3,238,628 (NPC, 2006).

Plant Materials

Fresh leaves of *Acacia nilotica* Wild. were collected from Aliero Local Government Area in Kebbi state. *A. nilotica* was authenticated and voucher specimen numbered (284) was deposited in the Herbarium, Botany unit, Department of Biological Sciences, Kebbi State University of Science and Technology, Aliero.

Test Bacteria

Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Pseudomonas aeruginosa*) with multi drug resistance property were obtained from Usmanu Danfodio University Teaching Hospital Sokoto which were

isolated from clinical specimens. Thereafter, antibiotic susceptibility test was conducted at microbiology laboratory Kebbi State University of Science and Technology, Aliero for confirmation.

Preparation of *Acacia nilotica* Leaves Extracts

The basic procedure include steps, such as pre-washing, drying of plant materials, grinding to obtain a homogenous sample and often improving the kinetics of analytic extraction and also increasing the contact of sample surface with the solvent system. The leaves were dried under the shade to assure that potential active constituents are not lost distorted or destroyed during the preparation of the extract from plant samples.

The aqueous and chloroform extracts of the plant were obtained according to the method described by Leonard *et al.*, (2013). 100g of the dried powder of *A. nilotica* leaves sample was suspended in distilled water, and 75% chloroform each separately. The mixture was gently stirred, tightly covered with cotton wools and foiled, then allowed to stand for 3 days at room temperature. Each extract was decanted and filtered through muslin cloth and subsequently with Whatman's filter paper 02. The filtrates obtained were concentrated to dryness on a water bath at 65°C. The extracts were stored at 4°C for screening of antibacterial activity.

Media Preparation

The media used in this study were nutrient agar, nutrient broth, and Mueller Hinton agar (MHA). The media were prepared according to Manufacturer's instructions using standard aseptic technique as adopted from (Cheesbrough, 2000).

Phytochemical Screening of the Plant Extracts

A preliminary phytochemical screening of 2g of each of the plant extracts aqueous and chloroform was mixed with 10ml of distilled water to detect the presence of saponins, tannins, phenols, flavonoid, terpenoids, alkaloids, and steroids using standard analytical protocol reported by Trease and Evans, (1989).

Screening for Antibacterial Activity

Antibacterial activity of *Acacia nilotica* leaves extracts were tested using disc diffusion technique on Muller Hinton agar according to Agarry *et al.* (2005). The presence of zone of inhibition is an evidence of antibacterial activity. Each extract (aqueous and chloroform) of *Acacia nilotica* leaves was tested against the test bacteria in triplicates.

The sterilized medium was seeded with 0.1ml of the standard inoculum of the test bacteria and spread evenly over the surface of the media with a sterile swab. Discs incorporated with the extracts were aseptically placed on the surface of the medium. The plates were allowed for the pre-diffusion time of 15 minutes after which they were incubated for 24 hours at 37°C. Diameter zone of inhibition was measured using millimeter ruler.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Minimum inhibitory concentration and minimum bactericidal concentration were determined according to a method described by Kubo *et al.* (2004). The extracts were prepared to obtain the concentrations of 250, 125, 62.5, 31.25, 15.27, 7.81, 3.91, and 1.95mg/ml. Each inoculum was adjusted to 0.5 McFarland standards and then diluted to 1:100 for broth dilution method. The standardized inoculum was introduced in each concentration of extract. The test tubes containing nutrient broth along with standardize inoculum were used as growth control. The test tubes containing broth without inoculum and extract were used as negative control. The test tubes were further incubated for 24 hours at 37°C and the lowest concentration that had no visible

growth after 24 hours incubation was considered as MIC.

MBC was determined by sub-culturing all concentrations that had no detectable growth. Zero (0.1ml) from each dilution was inoculated on the surface of freshly prepared nutrient agar plates and incubated for 24 hours at 37°C. The minimum concentration that had no visible growth on agar plates after 24 hours incubation was considered as MBC.

Statistical analysis

All experiments were carried out in triplicates and results are expressed as mean values with standard deviation (\pm SD) of three replicates. One-way analysis of variance (ANOVA) and Duncan's multiple range tests were carried out to determine significant differences using SPSS statistical software package (SPSS, version 20.0).

RESULTS

Table 1 show the results of phytochemical screening of aqueous and chloroform extract of the leaf of *Acacia nilotica* Wild. All the extracts contain alkaloids, tannins, phenols, saponins terpenoid, and steroids which are likely responsible for the antimicrobial activity while flavonoid was not detected in all the extracts. Terpenoid was not observed in chloroform extract.

Table 1: Preliminary phytochemical screening of *Acacia nilotica* leaves extracts

S/No	Phytochemical	Aqueous extract	Chloroform extract
1.	Alkaloids	++	++
2.	Saponins	+	+
3.	Tannins	++	+
4.	Phenols	+	+
5.	Flavonoids	ND	ND
6.	Terpenoids	+	ND
7.	Steroids	+	+

Key

+ = slightly present, ++ = moderately present

+++ = significantly present

ND= not detected

The extracts prepared from *Acacia nilotica* Wild. leaves using different solvents showed varying degree of antibacterial activity against both Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Pseudomonas aeruginosa*) multi drug resistant bacteria used for this study. Both the test bacteria were found to be sensitive to all the extracts prepared and the control antibiotic as well.

The antibacterial activity of aqueous extract against *Staphylococcus aureus* and *Pseudomonas aeruginosa* ranges from (10.3 mm - 12.7 mm) and (6.7 mm - 10.3 mm) respectively. Standard drug used as control recorded zone of inhibition (13.3 mm) and (11.7 mm) on the test bacteria respectively as shown in Table 2.

Table 2: Antibacterial activity of *A. nilotica* leaves aqueous extract on *S. aureus* and *P. aeruginosa*

Extract	Conc. mg/ml	Diameter zone of inhibition (mm)	
		<i>S. aureus</i>	<i>P. aeruginosa</i>
Aqueous	90	10.3±0.57 ^a	6.7±1.15 ^a
	120	11.7 ±1.52 ^{ab}	9.0±1.00 ^b
	150	13.7 ±1.15 ^b	10.3±0.57 ^{bc}
Control (Cipro.)	250	13.3 ±1.15 ^b	11.7±1.52 ^c

The results were expressed as mean ± SD of triplicates. Values with different superscripts are significantly different when compared to control within a column. P <0.05

Chloroform extract was found to be the least effective as compared to aqueous extract with diameter zone of inhibition ranging from (3.3 mm - 5.7 mm) and (2.3 mm -4.7 mm) against *S. aureus* and *P. aeruginosa* respectively. The

activity of the standard control was more effective with zones of inhibition (13.3 mm) and (11.7 mm) respectively. The result is depicted in Table 3.

Table 3: Antibacterial activity of *A. nilotica* leaves chloroform extract on *S. aureus* and *P. aeruginosa*

Extract	Conc. (mg/ml)	Diameter zone of inhibition (mm)	
		<i>S. aureus</i>	<i>P. aeruginosa</i>
Chloroform	90	3.3±0.57 ^a	2.3±0.57 ^a
	120	5.0±1.00 ^{ab}	3.3±0.57 ^{ab}
	150	5.7±1.52 ^b	4.7±1.15 ^b
Control (Cipro.)	250	13.3±1.15 ^c	11.7±1.52 ^c

The results were expressed as mean ± SD of triplicates. Values with different superscripts are significantly different when compared to control within a column. P <0.05

Table 4 shows the minimum inhibitory concentration and minimum bactericidal concentration of the crude extracts of *Acacia nilotica* Wild. leaves against *Streptococcus aureus* and *Pseudomonas aeruginosa*. The MIC of aqueous extract was low (7.81)/ml indicating its effectiveness as compared to chloroform

extract of 15.6 mg/ml on *S. aureus*, whereas on *P. aeruginosa* the MIC of the crude extracts was 15.6 mg/ml and 25.25 mg/ml respectively. Minimum bactericidal concentration ranges from (15 mg/ml-25 mg/ml) and (15 mg/ml-31 mg/ml) on *S. aureus* and *P. aeruginosa* respectively.

Table 4: Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of *A. nilotica* crude extracts

Test Bacteria	Minimum Inhibitory Conc.		Minimum Bactericidal Conc.	
	Aqueous (mg/ml)	Chloroform (mg/ml)	Aqueous (mg/ml)	Chloroform (mg/ml)
<i>S. aureus</i>	7.81	15.61	15.00	25.00
<i>P. aeruginosa</i>	15.63	25.25	15.50	31.00

DISCUSSION

Plants are important source of potentially useful substances for the development of new antimicrobial agents. The first step towards the achievement of the target objective is the *in vitro* antibacterial activity assay. Many reports are available on the antibacterial properties of these plants. Some of these observations would help in identifying the bioactive compounds responsible for such activity and the development of newer drugs for the therapeutic purposes. However, not many reports are available on the plants for developing commercial drug for applications to overcome and curtail the spread of antibiotic resistant among microbial populations (Mattana *et al.*, 2012). Thus, in this study crude extract of *A. nilotica* leaves were evaluated for exploration of their antibacterial activity against *S. aureus* and *P. aeruginosa* which were regarded as human pathogenic bacteria that are resistant to many antibiotic drugs (MDR).

In this research, secondary metabolites including alkaloids, tannins, phenols, saponins, terpenoid, and steroids were detected. The presence of these bioactive compounds in the leaves extracts of *A. nilotica* is thought to be responsible for the antibacterial activity. Numerous investigations have proved that *A. nilotica* contains diverse classes of bioactive compounds such as tannins, alkaloids, terpenoids and flavonoids, which exhibit various pharmacological properties (Emam, 2010).

The phytochemical screening of *A. nilotica* leaves extracts revealed the presence of alkaloids, tannins, phenols, flavonoids, terpenoids and steroids. This result is consistent with the findings by Muhammad *et al.* (2015), who reported same compounds as in this study. But the result of phytochemical screening in this study contradicted the result reported by Angelo, (2015), who reported that, tannins and flavonoids were not detected in aqueous extract of *A. nilotica*; this might be attributed to different reagents and procedure employed in both studies. Several plants which contain alkaloids, tannins, glycosides have been shown to possess antimicrobial activity against number of microorganisms (Seigler, 2003).

The antibacterial activity of Acacia extracts were investigated by disc diffusion assay at different concentrations (90 120 and 150

mg/ml). Positive control used in this study (Ciprofloxacin) shows significantly sized inhibition zones (13.3 mm) and (11.7 mm) against *S. aureus* and *P. aeruginosa* respectively. However, there were no significant differences between the extracts aqueous and the standard control used at certain concentrations ($p < 0.05$).

The lowest concentration used (90 mg/ml) of Acacia inhibited the growth of both clinical isolates. This is in accordance with the findings by Rosina *et al.* (2009) that reported the efficacy of *A. nilotica* extracts against bacteria isolated from clinical samples. Similarly, Kavitha *et al.* (2013), reported the effectiveness of *A. nilotica* against various multi drug resistant bacteria isolated from clinical samples. The leaves aqueous extract at concentration of 150 mg/ml shows the maximum zone of inhibition (10.3 ± 0.57 mm) and (12.7 ± 1.15 mm) against *P. aeruginosa* and *S. aureus* respectively. These results are in line with the result previously reported by Chelon *et al.* (2014) on antibacterial activity of aqueous extracts of the plant.

Chloroform extract had the lowest zone of inhibition (4.7 ± 1.15 mm) and (5.7 ± 1.52 mm) against *P. aeruginosa* and *S. aureus* respectively. This result correlate with the result obtained by Rahman, *et al.* (2014) who reported that, the chloroform extract showed least zone of inhibition as compared to other extracts used in their study.

The MIC of aqueous extract was low (7.8 mg/ml and 15.6 mg/ml) on *S. aureus* as compared to the chloroform extract (15.6 mg/ml and 25.2 mg/ml) on *P. aeruginosa*. The lower MIC is an indication of high effectiveness of extract. This result corroborates with the findings by Rosina *et al.*, 2009 on effectiveness of different Acacia extracts on multi drug resistant bacteria.

Conclusion

This research concluded that, the plant have very promising antimicrobial activities and thus can be used traditionally to cure various infectious diseases cause by these resistant bacteria, and could serve as useful source of new antibacterial agents. It is recommended that, further studies should be carried out for the isolation and characterization of the bioactive compounds. Investigations on the toxicity of the plant resulting from over dosage cannot be over emphasized.

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