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Antibacterial Activity of *Acacia nilotica* Stem-Bark Fractions against *Staphylococcus aureus* and *Escherichia coli*

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Authors' contributions

This work was carried out in collaboration between all authors. Authors RDJ and SBM designed the study, wrote the protocol and the first draft of the manuscript. Author ADD managed the literature searches and author UDN managed the analyses of the study and performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This research was carried out to determine the antibacterial activity of *Acacia nilotica* stem bark extract and bioactive fractions against the test bacteria (*Staphylococcus aureus* and *Escherichia coli*).
Place and Duration of Study: *Acacia nilotica* was collected within Aliero town, Kebbi State, Nigeria between April and September, 2017.
Methodology: The crude and bioactive fractions were obtained using soxhlet extraction and column chromatographic method respectively. The qualitative phytochemical screening was conducted to detect the presence of some phytochemical constituents in the crude extract and fractions. The antibacterial activity was determined at various concentrations (10, 50, 100, 150 and 200 mg/ml) using disc diffusion method.

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Results: The crude antibacterial activity indicated that ethanol extract showed higher activity than the n-hexane extract with 14.0±0.00 and 12.0±0.00 mm zones of inhibition compared with the control drug (10 µg Ciprofloxacin drug), which showed 14.0±0.00 and 13.0±0.00 mm zone of inhibition against the test bacteria. The MIC and MBC values determined for ethanol extracts against the test bacteria was 12.5 mg/ml and 25 mg/ml, while the MIC and MBC values obtained for n-hexane extracts were 25 and 50 mg/ml against the test bacteria. The bioactive fractions (Yellow, Purple and Blue Black Fractions) tested against the test bacteria showed higher activity compared with the crude extract. The phytochemical properties of the plant crude extract and the bioactive fractions indicated the presence of phenol, tannins, alkaloids, saponins, flavonoids, terpenoids, steroids and glycosides and this attributed to the high antibacterial activities of 17.0±0.00 and 16.0±0.00 mm showed by the fractions against *Staphylococcus aureus* and 15.67± and 14.0±0.00 mm against *Escherichia coli* respectively.
Conclusion: *Acacia nilotica* crude extract and fractions exhibited antibacterial activity which was comparable to the standard drug ciprofloxacin. This validates the folkloric medicinal use of this plant by the indigenous people of Aliero, Kebbi State.

Keywords: Antibacterial activity; Acacia nilotica; minimum inhibitory concentration; minimum bactericidal concentration Staphylococcus aureus and Escherichia coli.

1. INTRODUCTION

Antibacterial agent is any chemical substance that destroys or suppresses bacterial growth or their ability to reproduce. Many chemical and physical agents like antibiotics, heat and radiation can have antimicrobial properties. Antimicrobial agents also include naturally occurring antibiotics, synthetic derivatives of naturally occurring antibiotics (semi-synthetic antibiotics) and chemical antimicrobial compounds (chemotherapeutic agents). However, antibiotics are used to describe antimicrobial agents (antibacterial) that can be used to treat microbial infections [1].

Staphylococcus aureus are Gram- positive bacteria; naturally associated with the skin, skin glands and mucous membranes of humans and many other animals. They are sometimes found in the intestinal, genitourinary and upper respiratory tracts of the hosts and are known to cause a range of illnesses from minor skin infections such as pimples, impetigo, boils, cellulitis, folliculitis, carbuncles, scalded skin syndrome and abscesses to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteremia and sepsis [2].

Escherichia coli are Gram-negative, non-spore-forming, straight rods usually arranged in pairs or singly; are motile and may have capsules or microcapsules; *E. coli* is a normal inhabitant of the human gastrointestinal tract[3][4]. Virulent strains of *E. coli* can cause gastroenteritis, urinary tract infections and neonatal meningitis. In rare cases, virulent strains are also responsible for haemolytic-uremic syndrome (HUS), peritonitis, mastitis, septicemia and Gram-negative pneumonia [5]. Most *E. coli* infections seen in Northern Nigeria are urinary tract infection, bacteremia and gastroenteritis (diarrhoea) [6].

Acacia nilotica (Wild) is a genus of shrubs and trees belonging to the subfamily Mimosoideae [7], of the family Fabaceae or Leguminosae [8]. *A. nilotica* (Wild) has been used traditionally for decades in the treatment of many diseases such as diarrhea, dysentery, leprosy, cancers, ulcer, burns, boils, wound ulcer and diabetes [9]. Parts of this plant are also used against inflammation, ophthalmia, hemorrhoid, bleeding piles, and leucoderma problems [10]. Due to the increase in bacterial resistance against the common antibiotics, attention has been focused on finding new or alternative substances that will have a broad- spectrum activity and that will also be readily available and affordable to the common rural inhabitants who are mostly victims of microbial infections [11]. Therefore, this study involved the use of *in vitro* experiment in investigating the efficacy of *A. nilotica* stem bark crude extract and bioactive extract against *S. aureus* and *E. coli,* which may help in the synthesis of new plant, based antibiotics with regulation for potency.

2. MATERIALS AND METHODS

2.1 Sample Collection

Fresh stem bark of *Acacia nilotica* (wild) (L.) Delile was collected in the month of April, 2017 at

Aliero, Kebbi State, Nigeria in a clean polythene bags and was transported to the herbarium, Botany unit of Department of Biological Science, Kebbi State University of Science and Technology, Aliero, Kebbi State. The plant was identified and a voucher specimen number (V. No. 284) was deposited.

2.2 Preparation of Plant Extract

The freshly collected stem bark of *A. nilotica* was neatly air-dried at ambient temperature, which was pounded into powder [12]. A Soxhlet system was assembled, where 300 g of *A. nilotica* stem bark powder was loaded and 250 ml of ethanol was filled into a distillation flask, placed on a heating mantle. When the liquid reached the overflow level, a siphon aspirated the solution of the thimble-holder and loads it back into the distillation flask, carrying extracted solutes into the bulk liquid. In the solvent flask, solutes were separated from the solvent using distillation. Solutes were left in the flask and fresh solvent passes back into the plant solid bed. The operation was repeated until complete extraction was achieved [12]. Likewise, the same process was applied in n-hexane solvent. The solvents was later separated from the extract with the aid of a rotary evaporator at 40°C leaving a small yield of extract of the plant material (about 3 ml) in the round bottom flask [14]. Each extract was subsequently weighed and the percentage yield calculated as follows:

Percentage (%) yield =

$$\frac{Amount\ of\ extract\ obtained}{Amount\ of\ initial\ sample} \times 100\%$$

2.3 Standard Drug

Ciprofloxacin was purchased from the Hero's Land Pharmaceutical Limited, Birnin Kebbi, Kebbi State, Nigeria. And all other chemicals, media and reagents were of analytical grade.

2.4 Quantitative Phytochemical Screening of *Acacia nilotica*

Five grams (5 g) of *Acacia nilotica* dried stem bark crude extract obtained by the use of n-hexane and ethanol solvent was dissolved in 40 ml of distilled water to qualitatively detect the presence of alkaloids, resins, saponins, tannins, phenol, flavonoids, terpenoids, anthraquinones, steroids and phlobatannins using standard analytical methods described by Harborne [13], Sofowora [14] as well as Trease and Evans [15].

2.5 Test Bacteria

Two (2) bacterial species, *Staphylococcus aureus* and *Escherichia coli,* were used in this research work. The isolates were collected from the Microbiology Laboratory of Federal Medical Centre Birnin Kebbi, Kebbi State. Nigeria. The isolates were aseptically transported to Microbiology Laboratory, Kebbi State University of Science and Technology, Aliero, and were sub-cultured into Manitol Salt Agar (MSA) and Eosin Methylene Blue Agar (EMB) for *Staphylococcus aureus* and *Escherichia coli* respectively and were incubated at 37°C for 24 hrs in order to obtain pure culture of the bacterial. The bacteria were examined microscopically after gram staining techniques and were further subjected to biochemical test such as catalase, coagulase, oxidase, indole, motility and urease test to confirm the isolates to the species level [1].

2.6 Preparation of Varying Concentrations of Crude Extract

Exactly 0.01, 0.05, 0.1, 0.15 and 0.2 grams of *A. nilotica* stem bark extract was weighed and dissolved in 1 ml each of distilled water to obtain the following concentrations: 10, 50, 100, 150 and 200 mg/ml respectively [16].

2.7 Preparation of Sterile Disc

Paper discs from Whatman's No.1 filter paper was prepared by cutting 6mm disc form using a 6 mm puncher and were sterilized. Twenty (20) pieces were dipped into the varying concentrations of each of the prepared extracts [16].

2.8 Preparation of Mcfarland Turbidity Standard

McFarland turbidity standards were prepared by mixing various volumes of 1% sulfuric acid and 1% barium chloride to obtain solutions with specific optical densities. 0.5 McFarland turbidity standard was used to provide an optical density comparable to the density of the bacterial suspension 1.5 x 10^8 colony forming units (CFU/ml) [17].

2.9 Antibacterial Sensitivity Test of the Crude Extracts of *Acacia nilotica*

The antibacterial testing of the crude extracts was carried out according to the method

proposed by Mohan et al. [18]. The test organisms (*Staphylococcus aureus* and *Escherichia coli*) were inoculated into the sterile Mueller Hinton agar using a sterile swab. The sensitivity discs were applied by placing on the agar surface in each of the extracts. The plates were incubated at 37°C for 24 hrs and the zone of inhibition was measured and was recorded millimeters [19].

2.10 Determination of Minimum Inhibitory Concentration (MIC) of *Acacia nilotica* Stem- Bark Extract

The MIC was determined according to the method proposed by Mohan et al. [19] and Samie et al. [20]. Twelve sterile test tubes were used and 1 ml of sterile nutrient broth was dispensed from test tube 2 to test tube 12, a stock solution of *A. nilotica* stem-bark extracts was prepared i.e 400 mg of the crude extract was dissolved into 2 ml of distilled water, 1 ml of the stalk solution was dispensed aseptically into tube 1 and 1 ml into tube 2 and from the contents of test tube 2 a doubling dilution was performed using 1 ml transfer to tube 10, leaving tube 11 and 12 and 1 ml was taken out of tube 10 and discarded, the concentration in each tube from tube 1 to 10 is 200, 100, 50.25, 12.5, 6.25, 3.125, 1.562, 0.78125 and 0.390625 mg/ml respectively. 1:100 (10^{-2}), broth culture of the organisms (*Staphylococcus aureus* and *E. coli)* were prepared separately and the dilution of the broth culture was compared with 0.5 McFarland turbidity standards (as in section 2.9) and 1 ml of the prepared broth culture was dispensed into each test tube with the exception of the test tube 11 and 1 ml of sterile nutrient broth was added to test tube 11, and were then incubated at 37°C for 24 hrs. After 24 hrs, the test tubes were examined for turbidity in order to determine the MIC and MBC. The MIC was the concentration in the tube that fails to show evidence of growth (turbidity), just immediately after the last one that showed growth [21].

2.11 Minimum Bactericidal Concentration (MBC) of the Crude Extracts of *Acacia nilotica*

Samples from the MIC tubes that showed no visible growth after 24 hrs of incubation was sub-culture into freshly prepared sterile nutrient agar. The least concentration that did not produce growth after 24 hrs was regarded as the MBC [21].

2.12 Chromatographic Separation of Bioactive Fractions

The crude extract was separated using column and thin layer chromatographic techniques on silica gel. The extract was dissolved in methanol and then placed on top of the silica gel (60-120) column (85 cm × 18 cm). The column was packed with n-hexane and was eluted with n-hexane: chloroform (100:0, 70:30, 50:50, 30:70 v/v, 1L×5 fractions, each), chloroform: methanol (100:0, 99:1, 98:2, 97:3, 96:4, 95:5, 94:6, 93:7, 92:8, 91:9, 90:10, 89:11, 88:12, 87:13, 86:14, 85:15, 84:16, 83:17, 82:18, 81:19, 80:20, 79:21): gel G in water was degassed and poured on TLC plates (20 cm × 10 cm × 1 cm). The plates were activated at 110°C for 2 hrs. Spot(s) were developed in iodine chamber. And R_f value(s) was calculated for compounds identification. The chemical property of the active component was studied using the phytochemical screening (in section 2.5 above) and the information was used to identify the appropriate method to be used in purifying the compound. Where more than one compound is involved, each of them were isolated and purified [22].

2.13 Antimicrobial Activity of the Bioactive Compounds from the Column Chromatographic Fractions against the Test Bacteria

The activity of each of the reconstituted fractions was tested against *E. coli* and *S. aureus*. Here, 0.1 and 0.2 g of the dried fractions was dissolved in 1 ml of sterile distilled water to give a concentration of 100 mg/ml and 200 mg/ml respectively. Sterile disc were soaked into each of the concentration and was placed on the plate containing the inoculated test organisms, the plates were incubated for 24 hours at 37°C. The zones of inhibition was measured and expressed in milliliter and the result was recorded in triplicate [23]. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration of the active component were determined using the MIC and MBC method stated (in section 2.10 and 2.11 above).

2.14 Data Analysis

The data collected was subjected to statistical analysis as the zones of inhibition were expressed as Mean± Standard Deviation (SD) using Instat Graphpad software version 21.

3. RESULTS

3.1 Percentage Yield of *A. nilotica* Ethanol (Wild) Extract

The ethanol extraction of 500 g of *A. nilotica* stem bark obtained, yielded 12%. And the extract was powdery and brownish.

3.2 Phytochemical Composition of *A. nilotica* (Wild) Ethanol Extract

The result of the phytochemical analysis of *A. nilotica* extract revealed the presence of some secondary metabolites as shown in the table below Table 1.

Table 1. Qualitative phytochemical properties of *Acacia nilotica* (wild) ethanol crude extracts

Phytochemicals	Solvent	Stem bark
Tannins	Ethanol	+
	n-Hexane	+
Alkaloids	Ethanol	+
	n-Hexane	+
Saponins	Ethanol	-
	n-Hexane	-
Flavonoids	Ethanol	+
	n-Hexane	+
Terpenoids	Ethanol	+
	n-Hexane	+
Glycosides	Ethanol	+
	n-Hexane	+
Steroids	Ethanol	+
	n-Hexane	+
Phenols	Ethanol	+
	n-Hexane	+
Anthraquinones	Ethanol	-
	n-Hexane	-

Key: (+) present, (-) not detected

Table 2. Antibacterial activity of *Acacia nilotica* (wild) ethanol stem-bark extracts against the test bacteria

Extracts	Conc. (mg/ml)	Zone of inhibition in mm/bacterial isolate	
		Staphylococcus aureus	***Escherichia coli***
Stem bark	10	7.33±0,58	3.67±0.58
	50	11.0±0.00	10.0±0.00
	100	12.33±0.58	10.0±0.00
	150	13.0±0.00	11.0±0.00
	200	14.0±0.00	12.0±0.00
Control (Ciprofloxacin)	10 µg/ml	14.0±0.00	13.0±0.00

Table 3. Antibacterial activity of *Acacia nilotica* (Wild) N-Hexane stem-bark crude extracts against the test bacterial

Extracts	Conc. (mg/ml)	Zone of inhibition in mm/bacterial isolate	
		Staphylococcus aureus	***Escherichia coli***
Stem bark	10	8.0±0.00	6.33±0.58
	50	10.33±0.58	9.0±0.00
	100	10.67±0.58	7.33±0.58
	150	11.33±0.58	8.0±0.00
	200	12.0±0.00	11.0±0.00
Control (Ciprofloxacin)	10 µg/ml	14.0±0.00	13.0±0.00

Table 4. Minimum inhibitory concentration (MIC) and MBC of *Acacia nilotica* (Wild) ethanol and N-Hexane crude extracts

Extract	Org.	200 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	3.125 mg/ml	1.5625 mg/ml	0.78125 mg/ml	0.390625 mg/ml	MIC	MBC
Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Acacia nilotica* (Wild) Ethanol crude extract													
Stem Bark	SA	-	-	-	-	+	+	+	+	+	+	12.5	25
	EC	-	-	-	+	+	+	+	+	+	+	25	50
Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of N-Hexane crude extracts of *Acacia nilotica* (Wild)													
Stem Bark	SA	-	-	-	+	+	+	+	+	+	+	25	50
	EC	-	-	-	+	+	+	+	+	+	+	25	50

Key: Staphylococcus aureus (SA), Escherichia coli (EC) and organism (Org.)

Table 5. Qualitative phytochemical properties of bioactive fractions of *Acacia nilotica* (wild) stem bark

Phytochemicals	Fraction Y	Fraction P	Fraction BB
Alkaloids	+	+	+
Saponins	+	+	-
Tannins	-	+	+
Phenols	+	+	+
Glycoside	+	+	+
Flavonoids	-	+	-
Terpenoids	-	+	+
Anthraquinones	+	+	+
Steroids	+	+	+

Key: (+) Present, (-) not detected, fraction Y (yellow), fraction P (purple) and fraction BB (blue black)

Table 6. Antibacterial activities of fractions of *A. nilotica* (wild) stem bark

Fractions/conc (mg/ml)		Zone of inhibition in mm	
		Staphylococcus aureus	***Escherichia coli***
Fraction Y	100	13.67±0.58	12.0±0.00
	200	17.0±0.00	15.67±0.58
Fraction P	100	15.67±0.58	14.0±0.00
	200	16.67±0.58	15.0±0.00
Fraction BB	100	16.0±0.00	11.67±0.58
	200	16.0±0.00	14.0±0.00
Ciprofloxacin drug	10 µg/ml	14.0±0.00	13.0±0.00

Key: Fraction Y (yellow), fraction P (purple) and fraction BB (blue black)

Table 7. MIC and MBC of bioactive fractions of *Acacia nilotica* (wild) stem bark

Concentration (µg/ml)	2000	1000	500	250	125	62.5	31.25	15.625	7.8125	3.90625	MIC	MBC	Bacteria
Fraction Y	-	-	-	-	-	+	+	+	+	+	62.5	125	*S. aureus*
	-	-	-	-	-	+	+	+	+	+	62.5	125	*E. coli*
Fraction P	-	-	-	-	-	-	-	+	+	+	15.625	31.25	*S. aureus*
	-	-	-	-	-	-	-	+	+	+	15.625	31.25	*E. coli*
Fraction BB	-	-	-	-	-	-	+	+	+	+	31.25	62.5	*S. aureus*
	-	-	-	-	-	-	-	+	+	+	15.625	31.25	*E. coli*

Key: Fraction Y (yellow), fraction P (purple), fraction BB (blue black), Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

4. DISCUSSION AND CONCLUSION

4.1 Discussion

The increase in bacterial resistance against the common antibiotics, necessitate the reason of giving attention/focused on finding an alternative substance that will have a broad-spectrum activity and that will also be readily available and affordable to the common rural inhabitants. This research revealed that *A. nilotica* (stem bark) is highly active against the test bacteria used in this study; hence it could serve as a remedy to this outstanding problem.

The stem-bark ethanol crude extracts of *A. nilotica* has shown dose dependent activity against *Staphylococcus aureus* and *Escherichia coli,* increase in concentration of the extract resulted at a higher antibacterial activity of the plant extract tested in all concentrations (10, 50, 100, 150 and 200 mg/ml) used, however, the ethanol extract showed a higher activity than the N-Hexane extract based on the zones of inhibition. The Mean value of the zone of inhibition of N-Hexane stem-bark was 43.4 mm and 34.2 mm against *Staphylococcus aureus* and *Escherichia coli*. On the other hand, the zones of inhibition of the control drug (10 µg/ml ciprofloxacin) were 14 and 13 mm.

The presence of high phytochemical components in the stem-bark extract of *A. nilotica* (Wild) has attributed to the higher antimicrobial activity observed. This result also recorded at the lowest zone of inhibition of 6 mm. This is in line with the findings of Bauer et al. [24] who stated that the microbicidal activity is classified into resistance or inactive if the zone of inhibition in millimeter is less than 7, 7-9 mm intermediate and it is regarded active or sensitive if it is 10 mm and above.

The ethanol extract yielded a better activity than the N-Hexane extract, this agrees with the findings of Adaramola et al. [25] who carried out the antibacterial activity of *A. nilotica* (Wild) against some strains of organism, it was stated that ethanol extract yielded a better activity than the n-hexane extracts which might be due to the differences in the polarity of the solvent as ethanol is a polar solvent which enable a better extraction of the most active ingredient of the plant parts than the n-hexane.

Phenol compound present in both extracts (ethanol and hexane) may be responsible for their antibacterial activities as a report showed that phenol compound exhibits antimicrobial activity against pathogens [26,27,28]. Tannins have also been reported to be utilized traditionally in the treatment of diarrhea and dysentery while saponins were reported to have the natural tendency to ward off microbes [25]. The result also agrees with the findings of Deshpande [29], who conducted a study on ethanol and petroleum ether extract of stem-bark of *Acacia nilotica*; it was discovered that there was a high antibacterial activity against *Staphylococcus aureus, Escherichia coli, Proteus vulgaris, Proteus mirabilis, Salmonella paratyphi, Klebsiella pneumoniae.* The results showed that both the extracts exhibited inhibitory action on the pathogens above mentioned. But, never the less, ethanol extract showed greater activity as Compared to corresponding petroleum ether extract.

This research also revealed that *S. aureus* is highly susceptible to both crude extract and the column chromatographic compounds than *E. coli.* This result is in line with the findings of [30] who worked on the antimicrobial activity in leaf; seed extract and seed oil of *Jatropha curcas* against *Bacillus thuringiensis*, *Bacillus subtilis, Agrobacterium tumefacciens, E. coli, Pseudomonas flourescens, Acinetobacter junii, Rhizopus oryzae, Mucor indicus and Tilletia indica,* it was stated that the reason for higher sensitivity of gram positive bacteria than gram negative could be ascribed to the differences between their cell wall compositions. The gram positive bacteria contain an outer peptidoglycan layer which is an ineffective permeability barrier [31].

The MIC and MBC of ethanol and n-hexane stem-bark extract of *A. nilotica* revealed potent activity against *Staphylococcus aureus* and *Escherichia coli* respectively. It was also observed that the activity is dose dependent, the increase in concentrations of the extracts resulted in the increase in antibacterial activities. This result is in line with the findings of [32] who reported that higher concentrations of antimicrobial substances of the same extract could show appreciable inhibition.

Phytochemical screening of stem-bark crude extracts of *Acacia nilotica* showed that tannins, alkaloids, flavonoids, terpenoids, glycosides, steroids and phenols were found positive while saponins and anthraquinones were negative. The presence of these phytochemical compounds in *A. nilotica* (Wild) ethanol and N-Hexane extracts could be responsible for the

observed effect of this plant on *E. coli* and *S. aureus.* Therefore, the medicinal values of this plant may be related to their constituent Phytochemicals. Secondary metabolites (phyto-chemicals) and other chemical constituents of medicinal plants account for their medicinal value. Phenols were found to be present in *Acacia nilotica,* according to Osakabe et al. [33] phenol exhibit antiulcer activity.

The most active compounds of the chromatography (fraction Yellow, Purple and Blue Black) revealed the presence of phenol, saponins and tannins in their phytochemicals as well as saponins and anthraquinone even though these were absent in the crude extract of *A. nilotica* (stem bark). This might be attributed to the fact that other compounds present in the crude extract were hindering or interfering with the activity of the pure compound. This is in line with the findings of Ibrahim [34], who stated that other compounds present in the crude extract were hindering or interfering with the activity of the pure compound.

The antibacterial activity of fractions showed higher activity than the crude extract. Similarly, the MIC and MBC of bioactive fraction, was observed to be effective with a higher potency activity when compared with the crude extract. This implies that the fractions are very effective against the test bacteria because it is a pure active component of the plant extract. This is in line with the findings of Ibrahim [34], who carried out a research on antibacterial property of the hexane extract from the pods of *A. nilotica*. It was stated that the n-hexane crude extract of *A. nilotica* pods have less activity against *S. aureus* and *S. dysenterae* compared to the bioactive compound CY2 which had a better antibacterial and antifungal activity.

4.2 Conclusion

The antibacterial activity of *A. nilotica* stem-bark crude extract revealed that the plant crude extract was very effective; The phytochemical properties of the plant crude extract and the bioactive components indicated the presence of phenol, tannins, alkaloids, saponins, flavonoids, terpenoids, steroids and glycosides and this attributed to the high antibacterial activities showed by the fractions of *A. nilotica* against *S. aureus* and *E. coli respectively*. The column chromatography active component of *A. nilotica* showed an MIC and MBC value of 15.625 and 31.25 µg/ml against *Staphylococcus aureus* and *E. coli* respectively.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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