WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 5, 8-18. Research Article ISSN 2277– 7105

PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL PROPERTIES OF CRUDE LEAVES EXTRACT AND FRACTIONS OF *ACACIA NILOTICA* (LINN.)

A. Abdulhamid*[1], Y. U. Dabai[2] and Amar Mohamed Ismail[3]

[1]Department of Biochemistry, Faculty of Science, Kebbi State University of Science and Technology, PMB 1144, Aliero, Kebbi State, Nigeria.

[2]Department of Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto, Nigeria.

[3]Department of Biochemistry and Molecular Biology, Faculty of Science and Technology, University of AL-Neelain, Kartoum, Sudan.

Article Received on
03 Jan. 2018,

Revised on 23 Jan. 2018,
Accepted on 13 Feb. 2018

DOI: 10.20959/wjpr20185-11187

***Corresponding Author**
A. Abdulhamid
Department of Biochemistry, Faculty of Science, Kebbi State University of Science and Technology, PMB 1144, Aliero, Kebbi State, Nigeria.

ABSTRACT

Infectious diseases caused by bacteria, fungi, parasites and viruses are still a major threat to public health. Their impact is particularly large in developing countries due to the relative unavailability of medicines and the emergence of widespread drug resistance. This has lead to the search of new antimicrobial agents mainly among plant extracts. As part of our ongoing research to purify, isolate and characterized antibacterial compounds from the extracts of some Nigerian medicinal plants, *Acacia nilotica* (Linn.) was screened for its preliminary phytochemical and antibacterial activity. The preliminary phytochemical screening of the extracts was carried out using standard methods while the antibacterial activity was done using disc diffusion method. The results for the phytochemical screening showed the presence of most of the phytochemicals tested. The results for the antibacterial activity of the crude methanolic extract and fractions of the leaf of *Acacia nilotica* showed varying degree of antibacterial activity against the bacterial isolates. However, crude methanolic extract, ethyl acetate and butanol fractions showed relatively high zone of inhibition (mm), minimum inhibitory concentrarion (MIC) and minimum bactericidal concentration (MBC). They were found to inhibit the growth of most of the test bacterial isolates comprising of both Gram-positive and Gram-negative organisms. On the other hand, hexane and aqueous fractions

shows little or no activity against tested isolate. These findings support previous reports on the antimicrobial activity of this plants. The result of the present study signifies the potential of *Acacia nilotica* leaf as a source of therapeutic agents, which may provide leads in the ongoing search for antimicrobial agents from plants.

KEYWORDS: *Acacia nilotica,* antibacterial activity, phytochemicals and zone of inhibition.

INTRODUCTION

Nigeria is well known for its rich ethno botanical wealth, particularly regarding medicinal plants which are traditionally used in the treatment of ailments and could be a good source for discovery of new, safe and biodegradable drugs. High population growth rate (2.8% per annum) and poverty coupled with dwindling economic reserves in the country make Nigerians resort to more affordable sources for their immediate health needs. As the population increases, demand for traditional medicine will increase.[1,2]

Plants are rich in various active compounds including antimicrobial agents.[3] The recent discovery of novel drugs such as artemisinin, atropine, digitoxin, digoxin, emetine, pilocarpine, quabain, quinidine, quinine, reserpine, vinblastine, vincristine, etc., from medicinal plants implies that vast potential still exist for the production of numerous more novel drugs. Consequently, the area of ethno pharmacology of medicinal plants has attracted increasing attention in new drugs research and development.[4,5] It is estimated that two-thirds of the world population depend on traditional medications due to the limited availability, the high prices of most pharmaceutical products and the various side effects that they cause.[6] This further justifies the search for alternative products from plants used in traditional medicine.

Acacia nilotica (L.) Willd. ex Del. also known as Gum Arabic tree, Babul, Egyptian thorn, or prickly Acacia is multipurpose nitrogen fixing tree legume. It is a pioneer species, relatively high in bioactive secondary compound and are important for a variety of functions. It is economically used as a source of tannins, gums, timber, fuel and fodder. In Nigeria, the plant is traditionally used to treat infections such as diarrhoea, dysentery, oxidative stress, intestinal pains, ulcer, cold, haemorrhages, tuberculosis, congestion, coughs and fever.[7,8]

As part of our ongoing research to purify, isolate and characterized antibacterial compounds from the extracts of some Nigerian medicinal plants, the extract and fractions of *Acacia*

nilotica (Linn.) leaf was screened for its antibacterial activity.

MATERIALS AND METHODS

Materials

Solvents for extraction

The solvents used were: Butanol (BDH), Distilled water, Ethyl Acetate (BDH), Methanol (BDH) and N-Hexane (BDH).

Materials for antimicrobial test

Microbiological media (nutrient broth): Muller Hinton agar.

Test Organisms: *Staphylococcus aureus, Streptococcus pneumoniae, Salmonella typhi, Klebsiella pneumoniae, Escherichia coli, Psedomonas aeruginosa and Proteus spp.*

Petri Dishes, Sterile Pipette, 6 mm cork borer, Incubator, Autoclave, Dimethylsulphoxide (DMSO) 10%.

Methods

Plant Sample Collection and Identification

Fresh disease-free leaves of the plant used was separately collected from Bodinga, Sokoto State, Nigeria and was identified and authenticated by a Botanist at the Biological Sciences Department, Usmanu Danfodiyo University, Sokoto, Nigeria. The plant was identified as *Acacia nilotica* (Linn.) with voucher number UDUH/ANS/0247. The sample was shed-dried, ground and kept in air-tight containers till further use.

Preparation of Plant Extracts

The methanolic crude extract was prepared by soaking a sample (1000g) of powdered plant material in 90% methanol (6.0 litres) for 72 h. At the end of the extraction, the extract was filtered using Whatman filter paper. The filtrate was concentrated in vacuum at 30^{o}C and stored in sterile sample containers at 4^{o}C until further use.

Phytochemical Screening

The extracts were screened for the presence of major phytochemicals using standard qualitative methods as described previously.[9,10,11] The plant extracts were screened for the presence of saponins, tannins, alkaloids, flavonoids, terpenoids, steroids, phenols, cardiac glycosides and anthraquinones.

Solvent Partitioning of the Crude Methanolic Extract

About 100 grams of crude methanolic leave extract of *Acacia nilotica* was resolved in sterile distilled water (500mL) in a separatory funnel and extracted with n-hexane. The resulting n-hexane phase was concentrated to dryness and the resulting powder was kept in a freezer in an air-tight container. The resulting aqueous phase was further extracted with ethyl acetate. The ethyl acetate fraction obtained was concentrated to dryness and the recovered powder was kept in freezer for further use. The n-butanol fraction was obtained using the above procedure. The remaining aqueous fraction was dried to powder.[12] This was also kept in freezer in an air-tight container till further use. The procedure is presented in the flowchart shown below:

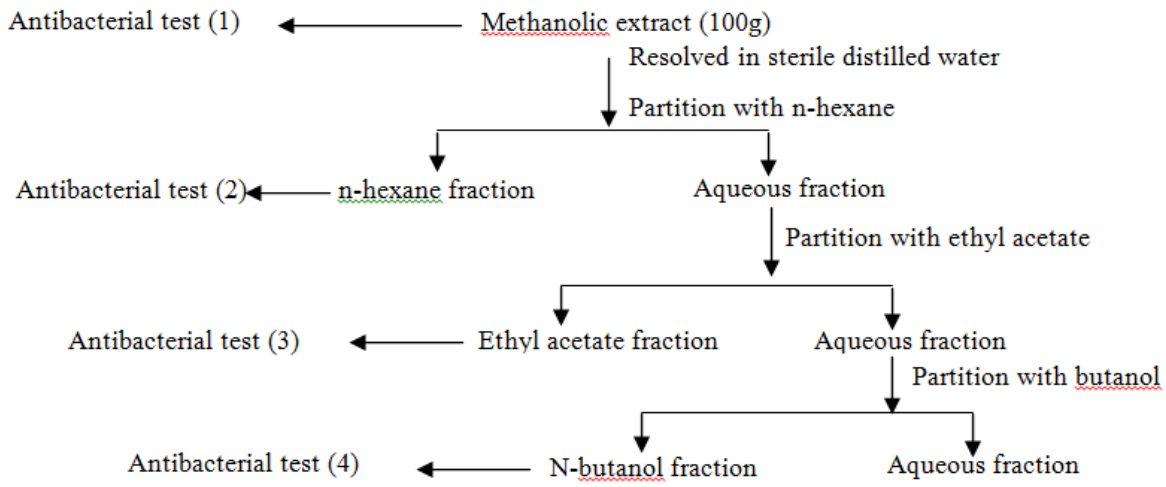

Figure 1: Extraction and fractionation scheme of the methanolic crude extract of *A. nilotica*.

Antibacterial Screening

Preparation of inoculums of test organisms

0.5 McFarland turbidity standard was used to standardise the organisms. The scale was prepared by adding 0.05 mL of 1% barium chloride ($BaCl_2$) to 9.95 mL of 1% H_2SO_4. Suspensions of the organisms were made in normal saline and compared with 0.5 McFarland turbidity standard by holding the suspension and McFarland turbidity standard in front of a light against a white background with contrasting black lines. The bacterial suspension was diluted with normal saline when the density is higher and additional bacteria were added to the suspension when the density is lower. This continues until the density of the bacterial suspension matched with that of 0.5 McFarland turbidity standard which corresponds to 1.5 x 10^8 CFU/mL.[13]

Sensitivity test of the crude extracts

Agar well diffusion method was employed to assay for the antibacterial activity.[14] The antibacterial activity of the crude methanolic extract and fractions of *A. nilotica* were determined using stock concentration of 100 mg/mL. The standardised inocula of the isolates were uniformly streaked unto freshly prepared Mueller Hinton agar plates with the aid of a sterile swab stick. Using a sterile cork borer (6 mm in diameter), three appropriately labelled wells were bored into each agar plate. A 0.2 mL of the appropriate extract concentrate was placed in each well and then allowed to diffuse into the agar. The plates were later incubated at 37°C for 24 h after which zone of inhibition (diameter) formed was determined as an indication of antibacterial activity. These effects were compared with that of the standard antibiotic amoxicillin at a concentration of 1 mg/ml.

Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration of the extract was carried out on the microorganisms that were sensitive to the extract and was done using broth dilution method.[15] Different concentrations of the extract that exhibited antimicrobial activity against the test organisms were prepared in the test tube containing Mueller Hinton Broth (MHB). The organisms were inoculated into each tube containing the diluted extracts. The plates were incubated at 37^{o}C for 24 hours. The lowest concentrations of the extract which shows no turbidity was recorded as the minimum inhibitory concentrations.

Minimum Bactericidal Concentration (MBC)

Minimum Bactericidal Concentrations of the extracts were carried out to check whether the test microbes were killed or only their growth was inhibited. Mueller Hilton agars were prepared according to the manufacturer's instruction, boiled to dissolve and were sterilized at 121^{o}C for 15 minutes, the media were cooled to 45^{o}C and the medium (20 ml) was poured in to sterile Petri dishes, the plates were covered and allowed to cool and solidify. The contents of the MIC in the serial dilution was inoculated on to the media, the plates were incubated at 37^{o}C for 24 hrs, after which the plate were observed for colonies growth. The MBC was the plate with lowest concentrations of the extract without colony growth.[16]

RESULT AND DISCUSSION

Table 1: Phytochemical constituents present in the crude leaves extract of *A. nilotica.*

Phytochemical Constituent	Result
Flavonoid	+
Tannins	+
Saponins	+
Glycosides	+
Alkaloids	+
Cardiac glycosides	+
Steroids	+
Balsams	N.D
Anthraquinones	+
Terpenoids	+

Keys: + = present – = not detected.

Table 2: Weight (g) recovered and percentage (%) yield of the crude methanolic leaves extract and fractions of *Acacia nilotica*.

Extract	Weight (g)	Percentage yield (%)
Crude methanolic extract	242.81	24.28
N – Hexane	0.41	0.041
Ethyl acetate	31.67	3.17
Butanol	13.32	1.33
Aqueous	7.44	0.74

Table 3: Antibacterial activity of crude methanolic leaves extract and fractions of *Acacia nilotica.*

Bacterial isolates	Zone of Inhibition (mm)*					
	Methanol	N-Hexane	Ethylacetate	Butanol	Aqueous	Amox.
E. coli	25.00±1.00	0 ±0.00	24.33±1.53	21.33±1.53	7.67±0.58	28.67±1.15
K. pneumoniae	23.33±2.08	3.33±1.15	24.33±0.58	20.00±1.00	0 ±0.00	26.67±0.58
Proteus spp.	17.00±1.00	0 ±0.00	21.00±1.00	18.00±1.00	0 ±0.00	30.67±1.15
P. aeruginosa	18.67±0.58	0 ±0.00	25.00±1.00	20.33±0.58	0 ±0.00	25.37±1.53
S. aureus	30.33±0.58	0 ±0.00	27.67±1.15	30.33±0.58	4.33±0.58	23.00±1.00
S. typhi	21.33±2.08	0 ±0.00	20.00±1.00	19.33±0.58	0 ±0.00	28.00±2.00
S. pneumonia	23.67±1.15	0 ±0.00	25.00±1.00	23.00±1.00	0 ±0.00	21.67±1.53

**values are mean and standard deviation of three (3) replicates, 0 ±0.00 = No activity*

Amox. = Amoxicillin as positive control

Minimum Inhibitory Concentration (MIC)

Table 4: The minimum inhibitory concentrations (mg/ml) of the methanolic crude extract, N-butanol and ethyl acetate fractions against bacterial isolates.

Bacterial isolates	Methanol	Ethyl acetate	Butanol	Amoxicillin
E. coli	3.12	3.12	3.12	0.25
K. pneumoniae	1.56	3.12	3.12	0.50
Proteus spp.	3.12	6.25	6.25	0.13
P. aeruginosa	3.12	6.25	3.12	0.13
S. aureus	1.56	3.12	3.12	0.25
S. typhi	3.12	6.25	3.12	0.50
S. pneumonia	1.56	3.12	3.12	0.50

Minimum Bactericidal Concentration (MBC)

Table 5: The minimum bactericidal concentration (mg/ml) of the methanolic crude extract, N- butanol and ethyl acetate fractions against tested bacterial isolates.

Bacterial isolates	Methanol	Ethyl acetate	Butanol	Amoxicillin
E. coli	N.D	N.D	N.D	0.50
K. pneumoniae	100	50	50	1.00
Proteus spp.	N.D	100	100	1.00
P. aeruginosa	100	50	50	0.25
S. aureus	N.D	50	100	0.50
S. typhi	50	100	N.D	1.00
S. pneumonia	N.D	100	N.D	0.50

The result of the phytochemical analysis of the crude leaves extract of *A. nilotica* is presented in Table 1. The result reveals the presence of flavonoids, tannins, saponins, glycosides, alkaloids, cardiac glycosides, anthraquinones and terpenoids in the leave extract of *A. nilotica*. Only steroids were not detected. Several other studies have reported similar phytochemicals from this plant[20,22]; these support the data reported in this research. These compounds are known to be biologically active[24,25] and thus may contribute to the observed antibacterial activities in these plants.

Sequential extraction involving solvent of varying polarity (n-hexane, ethyl acetate, butanol and water) was used to extract varied compounds from the leaf of *A. nilotica*. A sequential extraction procedure was chosen mainly because the nature and polarity and hence the solubility of the bioactive compound in the leaves of the *A. nilotica* were unknown.[21] In general n-hexane was used to extract hydrophobic or non-polar compounds such as fatty acids, waxes fatty acids some alkaloids and terpenoids.[22] Ethyl acetate is known to extract both medium polarities and some polar compounds such as phenols, flavonoid, tannin and

some terpenoid.[23,24] On the other hand butanol and water are known to extract hydrophilic or polar compounds such as carbohydrate, amino acids and their derivatives.[24]

The amount (weight in grams) and the percentage (%) yield of the four fractions (and the crude methanolic extract) are presented on table 2. The weight and percentage yield of crude methanolic of *A. nilotica* were 242.81 grams and 24.28% respectively. Of all the four solvent fractions, ethyl acetate fraction has the highest percentage yield (3.17%), followed by butanol fraction (1.33%), aqueous fraction (0.74%) and lastly N-hexane fraction (0.041%).

The antibacterial activities of the partitioned fractions against test isolates show different degrees of activity. Out of the four fractions derived from the crude methanolic extract of *Acacia nilotica*, only ethyl acetate and butanol fractions show strong activity, while the N-hexane and aqueous fractions showed little or no activity against the test isolates used (Table 3). This suggests that ethyl acetate and butanol will be good solvents for the isolation and purification of the active principles present in the leaf of *Acacia nilotica*.

The minimum inhibitory concentration (MIC) was determined for the crude methanolic extract of *A. nilotica*, ethyl acetate and butanol fractions. The MIC results is presented in Table 4 reflect a trend that tends to show different interactions among bioactive components of the leaf extract of *A. nilotica*. The lowest MIC exhibited by the crude methanolic leaf extract against *K. pneumoniae, S. aureus* and *S. pneumoniae* was 1.56 mg/mL; while the highest MIC of 3.12 mg/mL was exhibited against *E. coli, Proteus spp., P. aeruginosa* and *S. typhi*. The lowest MIC observed for the ethyl acetate fraction was 3.12 mg/mL against *E. coli, K. Pneumoniae, S. aureus* and *S. pneumoniae*; while the highest MIC observed was 6.25 mg/mL against *Proteus spp., P. aeruginosa* and *S. typhi*. With regards to butanol fraction, the lowest MIC exhibited against *E. coli, K. Pneumoniae, P. aeruginosa, S. aureus, S. typhi* and *S. pneumoniae*; while the lowest MIC of 6.25 mg/mL was exhibited against *Proteus spp.*

Furthermore, simultaneous comparison of the MIC values exhibited by crude methanolic extract, ethyl acetate fraction and butanol fraction against each test bacterium showed that MIC values of crude methanolic leaf extract against test bacteria were smaller than they were for ethyl acetate and butanol fractions. An exception was observed for *E. coli* which has the same MIC values for crude extract, n-hexane fraction and aqueous fraction (Table 4). This shows that there might be synergistic antibacterial-enhancing interactions between different bioactive components of the leaf extract. Antibacterial-enhancing interactions among

bioactive components of plant extracts have been reported.[25] An example of the possibility of a synergistic interaction between the bioactive components of the leaf extract of *A. nilotica* can be observed by comparing the MIC values exhibited by the crude extract, ethyl acetate fraction and butanol fraction against *K. pneumoniae*. The MIC value of 1.5 mg/mL exhibited by crude leaf extract against *K. pneumoniae* was half than the 3.12 mg/mL MIC value exhibited by the ethyl acetate fraction and butanol fraction against this same test organism (Table 4).

Table 5 shows the minimum bactericidal concentrations (MBC) exhibited by the crude extract, ethyl acetate and butanol fractions against the susceptible test isolates. The MBC exhibited by the crude extract against the test isolates ranged between 50 mg/mL and 100 mg/mL. Also, the ethyl acetate and butanol fractions showed a MBC ranging between 50 mg/mL and 100 mg/mL. Thus, the MBC exhibited by both extract and the two fractions followed the same pattern.

CONCLUSION

The result of the present study signifies the potential of *Acacia nilotica* leaf as a source of therapeutic agents, which may provide leads in the ongoing search for antibacterial agents from plants. Further, the activity exhibited by the extracts against tested bacteria species that are associated with various infectious diseases, may provide scientific justification for the ethnomedicinal uses of the plant.

REFERENCE

1. Ekeanyanwu CR. Traditional medicine in Nigeria: Current status and the future. Research Journal of Pharmacology, 2011; 5(6): 90-94.
2. Monier MAE. Traditional medicinal plants of Nigeria: an overview. Agriculture and Biology Journal of North America, 2016; 7(5): 220-247.
3. Sati SC, Joshi S. Aspects of antifungal potential of ethnobotanically known medicinal plants. Research Journal of Medicinal Plants, 2011; 5: 377-391.
4. Geyid A, Abebe D, Debella A, Makonnen Z, Aberra F, Teka F, Kebede T, Urga K, Yersaw K, Biza T, Mariam BH, Guta M. Screening of some medicinal plants of Ethiopia for their antimicrobial properties and chemical profiles. Journal of Ethnopharmacology, 2005; 97: 421–427.

5. Jazari SK, Bhouri W, Skandrani I, Limem I, Ghedira LC, Ghedira K. Phytochemical, antimicrobial, antioxidant and antigenotoxic potential of *Cyperus rotundus* extracts. South African Journal of Botany, 2011; 77: 767-776.
6. Tagboto S, Townson S. Antiparasitic properties of medicinal plants and other natural occurring products. Advances in Parasitology, 2001; 50: 199–295.
7. Aliyu BS. Common Ethinomedicinal Plants of the Semiarid Regions of West Africa; Triumph Publishing Company Limited; Nigeria, 2006; 8 – 26.
8. Saini ML. Comparative Pharmacognostical and antimicrobial studies of Acacia species (Mimosaceae). Journal of Medicinal Plants Research, 2008; 2(12): 378-386.
9. Harborne JB. Phytochemistry. London, Academic press, 1998; 92-135.
10. Sofowora EA. Medicinal plants and traditional medicine in Africa. 3rd ed. Spectrum book Ltd. Ibadan Nigeria, 1993; 23-25.
11. Trease GE, Evans WO. Trease and Evans Pharmacognosy. 16th ed. New York: Sauders Elsevier Limited, 1989; 104-262.
12. Abioye EO, Akinpelu DA, Aiyegoro OA, Adegboye MF, Oni MO, Okoh AI. Preliminary Phytochemical Screening and Antibacterial Properties of Crude Stem Bark Extracts and Fractions of *Parkia biglobosa* (Jacq.), Molecules, 2013; 18: 8485-8499.
13. National Committee for Clinical Laboratory Standard NCCLS. Performance Standard Stand for Antimicrobial Susceptibility Test approved Standard M2–A5 NCCLS, Villanorapa, 1990.
14. Njoku OU, Boniface JAE, Obitte NC, Odimegwu DC, Ogbu HI. Int. J. Appl. Res. Nat. Prod., 2010; 2(4): 11-19.
15. Vollekova A, Kostalova S. Sochorova R. Isoquinoline Alkaloids from *Mahonia aquifolium* stem bark is active against Malassezia Sp. Folia Microbiology, 2001; 46: 107-111.
16. Mann A, Banso A, Clifford LC. An antifungal property of crude plant extracts from *Anogeissus leiocarpus* and *Terminalia Avicennioides*, Tanzania J. Health Res., 2008; 10(1): 34-38.
17. Banso A. Phytochemical and antibacterial investigation of bark extracts of *Acacia nilotica*. J. Med. Plants Res., 2009; 3(2): 082-085.
18. Okoro SO, Kawo AH, Arzai AH. Phytochemical screening, antibacterial and toxicological activities of *Acacia nilotica* extracts. Bayero Journal of Pure and Applied Sciences, 2014; 7(1): 105-115.

19. Bruce B, Spiller GA, Klevay LM, Gallagher SK. A diet high in whole and unrefined foods favourably alters lipids, antioxidant defences and colon function. Journal of the American College of Nutrition, 2000; 19(1): 61–67.
20. Ververidis F, Trantas E, Douglas C, Vollmer G, Kretzschmar G, Panopoulos N. Biotechnology of flavonoids and other phenylpropanoid-derived natural products. Part 1: Chemical diversity, impacts on plant biology and human health. Biotechnology Journal. 2007; 2: 10-12.
21. Satyajit D, Sarker Z, Latif A, Gray I. Natural product isolation. Second edition, Humana Press Inc., 2006.
22. Cowan MM. Plant products as antimicrobial agents. Clinical Microbiology Review., 1999; 12: 564 – 582.
23. Eloff JN. Antibacterial activity of Murula (*Sclerocarya birrea* (A. rich) Hochst. Subsp. Caffra (Sond) Kokwaro) (Anacardiaceae) bark and leaves. J. Ethnopharmacol., 2001; 76: 305–308.
24. Scalbert A, Johnson IT, Saltmarsh M. Polyphenols: antioxidants and beyond. Am. J. Clin. Nutr., 2005; 81: 215S - 217S.
25. El-Mahmood AM, Doughari JH, Chanji FJ. (2008) *In vitro* antibacterial activities of extracts of *Nauclea latifolia* and *Daniella oliveri*. Sci. Res. Essay, 2008; 3: 102–105.