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Antibacterial Evaluation of Ethnomedicinal Plants Used Against Diarrhea in Niger, Western Africa

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Abstract

Background: In Niger as in other developing countries, the diarrheal diseases constitute a serious problem of public health. To treat diarrhea, most Niger people living in the rural areas do rely on medicinal plants. To evaluate the traditional use of these plants, scientific validation is needed.

Objectives: The plant species studied here are traditionally used in Niger to treat diarrhea. The aim of this study was to investigate the antibacterial activities of these plants against enteropathogenic bacteria isolated from clinical stool samples.

Materials and Methods: The collected plant materials were dried, pulverized, and methanol extracts were prepared. The total and serially diluted fractions of the extracts were assayed for antibacterial activities against selected enteropathogens by agar well diffusion and deep-well microdilution methods.

Results: The extracts of *Acacia nilotica*, *Sclerocarya birrea* and *Combretum nigricans* exhibited the highest antibacterial activities against *Escherichia coli* (21.9±0.6 mm zone of inhibition and minimum inhibitory concentration [MIC] 0.9 mg/mL), *Salmonella typhi* (19.6±0.2 mm zone of inhibition and MIC 0.9 mg/mL) and *Shigella flexneri* (18±0.6 mm zone of inhibition and MIC 3.7 mg/mL), respectively.

Conclusion: The results of this study provided some insight into the antibacterial activities of the plants traditionally used by the people of Niger republic to treat diarrhea.

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Background

Gastrointestinal disorders are significant health concerns in Niger and other least developed countries which substantially affect worldwide morbidity and mortality rates. Diarrhea in particular, is a leading killer of children, accounting for 9% of all deaths among children under age 5 worldwide in 2015, with over 1400 young children dying each day, or about 526 000 children per year.[1,2] It is a common cause of death in developing countries including Niger republic and the second most common cause of infant deaths worldwide.[3] In modern medicine, a number of successful synthetic drugs do exist across the world including Africa for the treatment of diarrhea. However, the poverty hitting majority of the African countries has significantly encouraged most communities to reconsider phytomedicine as an alternative to survive the disease. Though, these realities have encouraged and attracted many investigators in conducting interesting studies to know more about the medicinal plants across the African countries.[4-8]

In Niger, numerous published and/or unpublished works in the field of ethnomedicine have advised various medicinal plant preparations and their usages locally against diverse diseases. Most of the indigenous medicinal plants cited from Niger have a significant traditional medicinal role in the treatment of diarrhea.[9-19] Our previous study reviewing the ethnobotanical use of medicinal plants for the treatment of gastrointestinal disorders including diarrhea (manuscript under review) forms a back-bone to further research on evaluating their biological activity. In this review, a total of 20 plant species belonging to 12 families were documented as anti-diarrheal treatments. Eight out of these 20 plant species used in this study were selected based on their best respective scores as the most used and cited ones as ethnomedicinal plants to treat diarrhea. However, no information regarding the antimicrobial activities of these plants from Niger were reported elsewhere. One of the goals of our laboratory (Key Laboratory of Natural Substances) in the Department of Applied Science and

Technology is to document and establish knowledge bases for natural substances derived from plants used in Niger's traditional medicine. Thus, the interest in screening these selected medicinal plants for anti-diarrheal activity is justified.

Objectives

The aim of the present study was to evaluate the antibacterial activity of methanol extracts of *Lannea acida, Acacia nilotica, Bauhinia rufescens, Boswellia dalzielii, Combretum micranthum, Sclerocarya birrea, Prosopis africana,* and *Combretum nigricans* against *Shigella flexneri, Salmonella typhi,* and *Escherichia coli* by agar-well diffusion and deep-well microdilution methods.

Materials and Methods

Plant Material

Plant parts were collected from Niamey city (Niger republic), from the Botanical Garden of the Abdou Moumouni University (UAM) and from the markets during June-August 2016 and January-February 2017. All the plants were identified and verified by a competent botanist, a researcher at the Faculty of Science, UAM, Niger. The plant materials were rinsed, air dried under shade at room temperature, and pulverized by the use of metallic mortar and pestle. The obtained powder was then stored in plastic bags.

Preparation of the Methanol Extracts

Thirty grams of ground air-dried plant material were shaken (120 cycles/min) in 150 mL 96% methanol (MeOH) (Blulux Laboratories Ltd-121001) at room temperature for 48 hours. The insoluble material was filtered by filter paper (Whatman Grade 4) (Whatman/GE healthcare, Cat No. 1004-150) and evaporated to almost dryness in a water bath at 50°C (Isotemp 210, Fisher Scientific). The extract was weighed and dissolved in 2.5% dimethyl sulfoxide (DMSO) (Merck KGaA, Germany) at a concentration of 200 mg/mL and then serial two-fold dilution was made in concentration range of 0.2~200 mg/mL.

Antibacterial Assays

Bacterial Test Strains and Culture Media

Clinical strains of *S. flexneri, S. typhi* and *E. coli* isolated from stool samples collected from admitted patients with diarrheal episodes were obtained from the Bacteriological Laboratory, Niamey National Hospital (HNN), Niger. Conventional microbiological methods (Clinical and Laboratory Standards Institute, CLSI, USA) were used for isolate identification and characterization. Nutrient agar (NA) (Deben Diagnostics Ltd., UK) slant was used for the maintenance of bacterial cultures. Bacterial strains were activated by sub-culturing into fresh nutrient agar slants and then placed in an incubator (Incubator IN160, Memmert, Germany) overnight at 37°C prior to the test. Mueller-Hinton Agar (MHA) (Deben Diagnostics Ltd., UK) was used for minimum inhibitory concentration (MIC).

Agar Well Diffusion Method

The agar well diffusion method was used to evaluate the antibacterial activity of the plant extracts.[20-22] An inoculum size of 10^8 CFU/mL of test bacterium was used. MHA (Deben Diagnostics Ltd, UK) plates (90 mm diameter) were then inoculated with the already prepared inoculum using sterile swab stick. About 80 uL of methanol plant extract (stock 200 mg/mL) prepared in DMSO (Merck KGaA, Germany) was introduced in a well of 6 mm diameter in an MHA (Deben Diagnostics Ltd, UK) petri dish. The wells were aseptically made using a sterile borer. The seeded petri dishes were then turned upside down and the respective wells were labeled with a maker (Signierstift Nr.1181, Germany). Similar experiments were set up for comparison with 100% DMSO (Merck KGaA, Germany) used as negative control and standard antibiotics (Oxoid Ltd., England), gentamicin and ciprofloxacin, used as positive controls. The zone of inhibition was measured excluding the well diameter.

Minimum Inhibitory Concentration

Two-fold serial dilutions of the methanol extracts were prepared by reconstituting with DMSO (Merck KGaA, Germany). Each test bacterium inoculum adjusted with an electronic Densimat (Marcy-l'Etoile, Biomerieux SA, France) to 0.5 McFarland standard (10^8 CFU/mL) was seeded in a 96-well microplate (Sterilin Ltd, Parkway, Newport, NP11 3EF, UK) and treated with various concentrations of the methanol extract, ranging from 0.23 to 100 mg/mL. The microplates (Sterilin Ltd, Parkway, Newport, NP11 3EF, UK) were then incubated at 37°C and the MIC was recorded after 18-24 hours. All determinations were carried out in duplicate. The MIC is the least concentration of the methanol extract at which the test bacteria does not show visible growth.[23]

Results and Discussion

Test results for antibacterial activity of plant extracts used in the present study are shown in Table 1 and Figure 1. Five out of 8 plant species (62%) used by the Nigerien healers were found to be effective in suppressing the growth of at least one of the test bacteria, as exhibited by an agar well diffusion assay (Table 1). For this method of diffusion, a plant extract is considered active when it induces an inhibition zone superior or equal to 10 mm. Thus, the plant extract of *A. nilotica, Combretum micranthum* and *S. birrea* were the most potent active extracts against *Salmonella typhimurium* and *E. coli*. The extracts of *Prosopis africana, C. micranthum* and *C. nigricans* exhibited profound activities against *S. flexneri* (Figure 1.B,C). *S. flexneri*, *S. typhi*, and *E. coli* were inhibited by 3 (60%), 4 (80%) and 3 (60%) plant species, respectively. However, the diameters of the inhibition zones induced by all these extracts remained inferior to those of the reference antibiotics, gentamicin and ciprofloxacin, for all

Table 1. Mean (±S.D, n = 3) Zone of Inhibition (mm)

Family	Scientific name	Local Name (Hausa)	PPU	Test Bacteria		
				Sf	St	Ec
Anacardiaceae	*Lannea acida* A. Rich.	Faru	Bk	0	1.7±0.8	1.7±0.6
Mimosaceae	*Acacia nilotica* Linn.	Bagaruwa	Se	11±0.7	16.7±0.4	21.9±0.6
Burseraceae	*Boswellia dalzielii* Hutch.	Hano	Bk	6,4±0.4	1.8±0.9	5.8±0.7
Combretaceae	*Combretum micranthum* G. Don.	Geza	Lf	17±0.8	17.5±0.5	14.8±0.4
Anacardiaceae	*Sclerocarya birrea* (A. Rich.) Hochst	Dania	Bk	3.9±1.9	19.6±0.2	16.7±0.5
Olacaceae	*Ximenia Africana* Linn.	Tsada	Bk	0	8.9±0.3	2.9±0.6
Mimosaceae	*Prosopis Africana* (R. Br.) Guill & Perr.	Kiriya	Bk	15.9±0.6	14.7±0.3	7.5±1.1
Combretaceae	*Combretum nigricans* var. elliotii (Engl. &Diels) Aubrév.	Tsiriri	Ap	18±0.6	2.5±1	1.5±0.4
Gentamicin				24	24	24
Ciprofloxacin				26	25	18
DMSO				0	0	0

Abbreviation: PPU, plant part utilized; Bk, bark; Se, seed; Lf, leaf; AP, aerial part; Sf, *Shigella flexneri*; St, *Salmonella typhi*; Ec, *Escherichia coli*; DMSO, dimethyl sulfoxide.

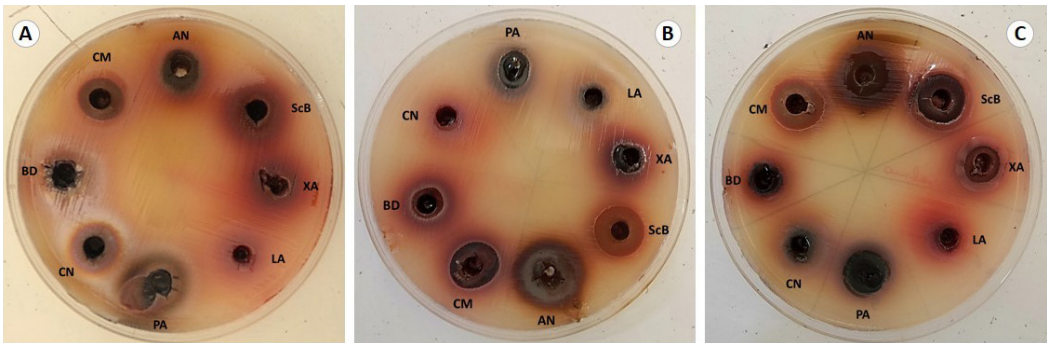

Figure 1. Antibacterial Activity of Plant Extracts Against: (A) *Shigella flexneri*; (B) *Salmonella typhi*; (C) *Escherichia coli*.
Abbreviations: AN, *Acacia nilotica*; CM, Combretum micranthum; BD, *Boswellia dalzielii*; CN, *Combretum nigricans*; PA, *Prosopis africana*; LA, *Lannea acida*; XA, *Ximenia africana*; ScB, *Sclerocarya birrea*.

the tested bacteria.

Table 2 shows the results obtained by the dilution method in liquid medium for the determination of the MIC. The MIC values obtained, in general, significantly matched with those of the diameters of the inhibition zones. Plant extracts that induced an important zone of inhibition presented the smallest MIC value with respect to the correspondent test bacteria. It is the case of *A. nilotica* on *E. coli*, or *C. micranthum* and *S. birrea* on *S. typhi*. *A. nilotica* and *S. birrea* both were found to present the smallest MICs on *E. coli* and *S.* respectively. *C. nigricans* extract displayed MIC value of 3.7 mg/mL for *S. flexneri*

Table 2. Minimum Inhibitory Concentration Results of Effective Plant Extracts

Plant species	PPU	Test Bacteria		
		Sf	St	Ec
Acacia nilotica	Lf	7.5	3.7	0.9
Combretum micranthum	Lf	7.5	1.8	7.5
Sclerocarya birrea	Bk	NT	0.9	3.7
Prosopis africana	Bk	7.5	3.7	NT
Combretum nigricans	Ap	3.7	NT	NT

Abbreviation: PPU, plant part utilized; Bk, bark; Se, seed; Lf, leaf; AP, aerial part; Sf, *Shigella flexneri*; St, *Salmonella typhi*; Ec, *Escherichia coli*; NT, not tested.

whereas *A. nilotica*, *C. micranthum* and *Prosopis africana* showed MIC value of 7.5 mg/mL. The potent activity of *A. nilotica* against diarrhea causing microorganisms demonstrated in this study is similarly reported elsewhere by Mathabe et al[24] Garba et al,[25] and Mohamed et al.[26] *S. birrea* displayed significant inhibitory efficacy against *S. typhi* (MIC of 0.9 mg/mL) and *Escherichia coli* (MIC of 3.7 mg/mL). In previous studies, Galvez et al[27] and Eloff[28] reported the antidiarrheal and antibacterial activity of the bark of *S. birrea* respectively. Kutama et al reported the antibacterial activity of the methanol extract of *S. birrea* against *Escherichia coli* using the agar-well diffusion method.[29] Bark extract of *P. africana* displayed significant inhibitory efficacy against *S. flexneri* (>15 mm zone of inhibition) and *S. typhi* (>14 mm zone of inhibition). Results obtained from a study conducted by Ajiboye et al highlighted the antimicrobial activity of the extracts of *P. africana* against most of the tested bacterial species.[30] High susceptibility to the leaf extract of *C. micranthum* was recorded for *S. flexneri* (> 16 mm zone of inhibition), *S. typhi* (> 17 mm zone of inhibition) and for *E. coli* (> 14 mm zone of inhibition) and to the extract of *C. nigricans* for *S. flexneri* (> 17 mm zone of inhibition). Toua et al reported the sensitivity of *E. coli* to the extract of *C.*

micranthum, a plant used in the treatment of diarrheal diseases in the Far-North region of Cameroon.[31]

Conclusion

The present study justified the importance of the ethnomedicinal use of these plants by the traditional healers in Niger to treat diarrheal diseases with bacterial origin and demonstrated their significant antibacterial activity against the tested enteropathogens, commonly associated with diarrhea; however, it should be noted that other pathogens not tested herein may also contribute to the development of diarrheal diseases and as such, further studies involving these plants should be considered in recruiting other enteric pathogens. For the meantime, there is an urgent need to investigate the toxicity profile or index of the purified extracts of these plants and further to plan the optimization of an improved traditional medicine.

Authors' Contributions

LMM, IM and KI: designed the study; LMM: designed and performed the laboratory experiments; LMM and IM: analyzed the data; LMM: drafted the manuscript; LMM, IM and KI: revised and approved the manuscript.

Conflict of Interest Disclosures

None.

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