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Phytochemical screening of selected medicinal plants used against diarrhea in Niger, West Africa

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Abstract
Medicinal plants including *Lannea acida, Acacia nilotica, Bauhinia rufescens, Boswellia dalzielli, Combretum micranthum, Sclerocarya birrea, Prosopis americana* and *Combretum nigricans* are the main herbal agents traditionally used by most Niger people to treat diarrhea.
To value the traditional use of these plants, different organs were collected, dried, powdered and separately extracted using water, methanol and ethanol. The alkaloid, flavonoid, saponin, tannin, steroid, triterpenoid and phenol contents in all these plants were estimated using standard methods. It was observed that all eight plants contained flavonoids, tannins, steroids, terpenoids, saponins and phenols. The alkaloids were present in all plants except *Sclerocarya birrea*.
Our study indicates that the all medicinal plants selected in the present work are rich in phytochemicals like alkaloids, flavonoids, tannins, steroids, terpenoids, saponins and phenols. The presence of these important chemicals groups could permit to justify their traditional usage against diarrhea.

Keywords: Phytochemicals, ethnomedicinal plants, diarrhea, Niger

1. Introduction
Medicinal plants remain the principal therapeutic arsenal accessible to most African populations. According to the World Health Organization (WHO), about 80 % of Africans use traditional medicine to treat themselves [1–3]. This situation is observed in both rural and urban areas. Most of these traditional therapists, in general, know the expected therapeutic effect of selected plants against a given pathology. The use of medicinal plants to treat different diseases is well documented in most tropical zones from West Africa [4–6].
In sub-saharan African countries including Niger, diarrheal disease constitutes a serious public health concern affecting all age groups. The development of the disease can be due to bacteria, viruses, parasites, and fungus. Dehydration and systematic use of standard antibiotics to treat diarrhea were mostly recommended. Antibiotics function at killing or suppressing the growth of microorganisms responsible for the diarrheal infection. However, the abuse in prescribing broad-spectrum antibiotics to treat diarrhea has significantly contributed to the development of microbial resistance [7–9]. This situation has encourage most investigators to search for alternatives. Over the last decades, considerable effort has been made to discover chemically bioactive antimicrobial drugs of plant origin [10–13].
Various studies published elsewhere have reported the antimicrobial activity of medicinal plants mostly recommended by traditional healers to treat gastrointestinal disorders [14–25]. Many researchers from different horizons have reported that many phytochemicals synthetized by plants to be beneficial as they possess various medicinal activities in humans [26]. These phytochemicals, generally grouped as primary or secondary metabolites are the main active principles present in plant [27, 28]. Secondary metabolites like tannins, saponins, flavonoids, alkaloids, terpenoids, glycosides, etc are important therapeutic agents in humans, now receiving even greater attention by most drugs' development companies [29–32].
In Niger, the numerous number of published and or non-published research works in the field of ethnomedicine did mentioned various medicinal plants' preparations and their usages locally against diverse diseases. Most of the indigenous medicinal plants from Niger cited have a significant traditional medicinal role in the treatment of diarrhea [33–42].
Our previous study reviewing the ethnobotanical use of medicinal plants for the treatment of gastrointestinal disorders including diarrhea (manuscript accepted) forms a back-bone to further research on evaluating the phytochemical constituents of these plants. In this review, a total of 20 plant species belonging to 12 families were documented as anti-diarrheal treatments. 8 out of these 20 plant species used in this study were selected based on their best respective scores as the most the most cited ones as ethnomedicinal plants to best treat diarrhea. One of the goals of our laboratory (Key laboratory of Natural Substances) through

the department of Chemistry, faculty of Applied Science and Technology is to document and establish knowledge bases for natural substances derived from plant use in Niger's traditional medicine. Owing to the significance in the above context, the interest in screening these selected medicinal plants based on phytochemical tests is therefore justifiable.

2. Materials and Methods

2.1 Plant materials

Plant parts were collected in Niamey city (Niger republic) in the Botanical Garden of the Abdou Moumouni University (UAM) and in the markets during June-August 2016 and January-February 2017. All the plants (Table 1) were verified and identified by a competent botanist, a researcher at the Faculty of Science, Abdou Moumouni University, Niger. The plant materials were rinsed, air dried under shade at room temperature and powdered by the use of metallic mortar and pestle. The obtained powders were then stored in plastic bags.

2.2 Solvents

Ethanol, methanol, and distilled water were used as solvents for the extraction of the plant materials.

2.3 Reagents

Divers chemical reagents were used for phytochemical screening: hydrochloric acid, sulfuric acid, ammoniac (30%), sodium hydroxide, ferric chloride, chloroform, sodium nitrite, acetic anhydride magnesium. Specific chemical reagents available on the market were purchased or kindly provided by our collaborators for the purpose of the experiments: Dragendorff and Mayer reagents.

2.4 Preparation of plant extracts

Three types of extracts were prepared for each studied plant: two organic extracts (methanol and ethanol) and one aqueous extract.

2.4.1 Extraction with organic solvents

Firstly, 30 grams of grounded air-dried plant material were shaken (120 cycles/min) in 150 ml of each solvent (methanol, ethanol), at room temperature for 48 hours. The insoluble material was filtered using filter paper (Whatman No.4) and evaporated to almost dryness in a water bath at 50ºC. The crude extracts were weighed and placed in a refrigerator at -4 °C in sealed glass bottles until use.

2.4.2 Aqueous maceration

20 grams of grounded air-dried plant material were macerated in 200 ml of distilled water, at room temperature, under shaken, during 48 hours. The macerate is then filtered using filter paper (Whatman No.4) and the filtrate were concentrated to almost dryness in a water bath at 50ºC. The crude extracts were weighed and placed in a refrigerator at -4 °C in sealed glass bottles until use.

2.5 Phytochemical tests

Standard methods were used for the screening of the 8 selected medicinal plants for various phytochemical constituents [11, 34]. Phytochemical constituents tested include: tannins, saponins, flavonoids, alkaloids, terpenoids, steroids, phenols and quinones. The reading of the results is done by direct visual observation of the coloration profile of the reactions and or the formation of precipitates.

2.5.1 Test for alkaloids

For the test of alkaloids, the Dragendorff and Mayer reagents were used. For the Dragendorff test: in each tube containing 0.2 ml of crude extract is added 1.5 ml hydrochloric acid (2%), then two to three drops of the Dragendorff or the Mayer reagent. The presence of a red or orange precipitate indicates the presence of alkaloids for the Dragendorff test, while for the Mayer test the precipitate characterizing the presence of alkaloids appears whitish.

2.5.2 Test for tannins

To characterize the presence of tannins, Braymer's test is used. In each tube containing 2 ml of crude extract is 2 ml of distilled water, then two to three drops of 5% Ferric chloride. The formation of brownish green or a dark-blue color indicated the presence of tannins.

2.5.3 Test for steroids and terpenoids

For the test of steroids and terpenoids, Liebermann Burchard's test is performed. To 0.5 ml of crude extract is added 2 ml of acetic anhydride, then 2 ml of sulfuric acid. The formation of a purple or violet to blue ring at the interface and the green-blue or violet coloration of the upper layer solution indicated the presence of steroids and terpenoids in the extract respectively.

2.5.4 Test for flavonoids

For the test of flavonoids, Shinoda test is performed. To 1 ml of the crude extract is added eight to ten drops of hydrochloric acid and a pinch of magnesium powder. The mixture is then boiled for ten to 15 minutes. A red coloration indicates the presence of flavonoids.

2.5.5 Test for saponins

To characterize the presence of saponins, Foam test is performed. To 5 ml of the crude extract is added 5 ml of distilled water. The tube containing the mixture is then boiled. The formation of a froth indicates the presence of saponins.

2.5.6 Test for phenols

For the test of phenols, Liebermann's test is performed. To 1 ml of the crude extract is added 1 ml of sodium nitrite, few drops of diluted sulfuric acid and then 2 ml of diluted sodium hydroxide. A deep red or green or blue color indicates the presence of phenols.

3. Results and discussion

3.1 Phytochemical analysis

The results of phytochemical screening of the plant extracts were presented in table 2. The sign (+) indicates a positive reaction while the sign (-) indicates a negative reaction.

3.1.1 *Lannea acida A. Rich.* (Anacardiaceae)

The bark contained alkaloids, tannins, flavonoids, saponins, terpenoids and phenols. Any of these phytochemical constituents was observed in at least one extract. The presence of flavonoids and phenols was observed in methanol, ethanol and aqueous extracts. Early studies on *Lannea acida* have reported the few phytochemical investigations [43, 44] and pharmacological properties [44, 45]. Etuk EU *et al*., (2009) [44] has reported the presence of tannins and alkaloids and the absence of flavonoids from the aqueous extract of the bark of *Lannea acida.* While in a study conducted in Burkina Faso, Ouattara L *et al*., (2011) [43] reported the presence of flavonoids and phenols from the ethanolic and aqueous extracts of the bark of *Lannea acida*.

3.1.2 *Acacia nilotica* Linn. (Mimosaceae)
The pods of *Acacia nilotica* is rich in tannins, flavonoid, saponins, steroids, terpenoids, alkaloids and phenols. These results are comparable to those published by Garba S *et al*., (2015) [19]. *Acacia nilotica* has beenreported to be very useful in treating diarrhea [46, 47]. Numerous number of studies have reported the antibacterial activity of the extracts of *Acacia nilotica* against most enteropathogenic bacteria [48–52].

3.1.3 *Boswellia dalzielli* Hutch (Burseraceae)
The phytochemical screening of the bark showed the presence of alkaloids, tannins, flavonoids, saponins, steroids, terpenoids and phenols. Any of these chemical constituents was observed in at least one extract. The presence of these constituents gives an indication of the medicinal values of the bark of *Boswellia dalzielli* and suggest that the plant is pharmacologically active. The antibacterial activity of different extracts of *Boswellia dalzielli* against most enteropathogenic bacteria is thoroughly reported in many valuable research works [53–55]. Nwinyi FC *et al*., [55] investigated the aqueous extract of *Boswellia dalzielli* for therapeutic properties using aspirin-induced ulceration in rats, gastrointestinal motility in mice and castor oil-induced diarrhea in rats. It was found that, the aqueous extract of *Bowellia dalzielii* had anti-ulcer activity and reduced gastrointestinal motility. This suggest that, it contains active ingredients that could be developed for such gastrointestinal problems as have been claimed by traditional medical practitioners.

3.1.4 *Combretum micranthum* G. Don. (Combretaceae)
The phytochemical screening of the leaves of *Combretum micranthum* showed the presence of alkaloids, tannins, flavonoids, saponins, steroids, terpenoids and phenols. Any of these chemical constituents was observed in at least one extract. Nounagnon S *et al*., [56] investigated the phytochemical constituents of the extracts of the leaves of *Combretum micranthum*. The results obtained were comparable to those obtained in the present study. Abdullahi MH *et al*., [57] investigated the phytochemical constituents and the effect of aqueous leaf extract of *Combretum micranthum* on isolated rabbit jejunum, guinea pig ileum and rat uterus. The result showed that, the aqueous leaf extract contains pharmacologically active principle (s), which may account for the beneficial effect of the plant in the management of diarrhea.

3.1.5 *Sclerocarya birrea* (A. Rich.) Hochst (Anacardiaceae)
The bark of the *Sclerocarya birrea* is rich in tannins, flavonoids, saponins, steroids, terpenoids and phenols. Alkaloid is not detected in any of the three different extracts of the plant. Mohammed MM *et al*., [58] in a study conducted in Nigeria detected and evaluated the secondary metabolites' constituents from the extracts of *Sclerocarya birrea*. Phytochemical screening of the bark extracts revealed the presence of the same secondary metabolites that were reported in the present study even though alkaloids were not investigated. Watt and Breyer-Brandwijk [59] reported that the bark of the *Sclerocarya birrea* yields 3.5-20.5 per cent tannin and traces of alkaloids. Kutama AS *et al*., [23] reported the presence of tannins, flavonoid in both methanolic and aqueous extracts of the bark of *Sclerocarya birrea;* while alkaloid was found present in the aqueous extract and absent in the methanolic extract. Many pharmacological studies have been performed on the basis of the chemical constituents of *Sclerocarya birrea* and traditional uses as antidiarrheal.

3.1.6 *Ximenia americana* Linn. (Olacaceae)
The results of phytochemical analysis of the barks' extraxts of *Ximenia americana* indicated the presence of tannins, flavonoids, saponins, terpenoids and phenols. However, alkaloids were absent in the extract of water. Maika VA *et al*., [60] and Zeinab MMA *et al*., [61] reported very close similar results with the methanolic and the aqueous extracts of the bark of *Ximenia americana*. Shagal MH *et al*., [62] and Abdalfatah A *et al*., [63] also both detected the same phytochemical constituents in the extract of ethanol. Investigations conducted in the past 15 years showed that the chemical constituents of *Ximenia americana* have shown several biological activities. Several other studies to evaluate the antimicrobial activity of the crude extract of *Ximenia americana* were performed [60–64].

3.1.7 *Prosopis africana* (R. Br.) Guill & Perr (Mimosaceae)
The results of phytochemical analysis of the barks' extracts of *Prosopis africana* indicated the presence of alkaloids, tannins, flavonoids, saponins, steroids, terpenoids and phenols. All the extracts tested positive for any of the detected chemical constituent. Most studies investigated the phytochemical constituents present in the leaves, roots and or fruits of *Prosopice Africana* and its antibacterial activity [24, 65–67]. Frequently detected chemical constituents include tannins, alkaloids, flavonoids, saponins, steroids and terpenoids.

3.1.8 *Combretum nigricans* var. elliotii (Engl. & Diels) Aubrév. (Combretaceae)
The aerial part of *Combretum nigricans* is rich in alkaloids, flavonoids, saponins, tannins, and terpenoids. Several studies describing the bioactivities of extracts and isolated compounds from the species of the genus *Combretum* were performed [68]. Although many species of *Combretum* including *Combretum nigricans* have not been extensively investigated for their chemical constituents, various classes of secondary metabolites. Existing phytochemical investigation that indicated the presence of triterpenoids from *Combretum nigricans* was reported by Jossang A *et al*., [69]. Baba-Moussa F *et al*., conducted [70] a chemical survey of extracts from *Combretum nigricans* and revealed the presence of large quantities of saponins and tannins, which agrees with the study of Hodouto *et al*., [71].
Several plants of the genus *Combretum* have been reported for their biological activities. Antibacterial activity of different extracts (ethanol, chloroform, methanol or water) of *Combretum micranthum* was noted against various bacteria strains [56, 57, 72, 73].

4. Conclusion
The presence of different secondary metabolites (mainly phenols, tannins, alkaloids, flavonoids, saponins, steroids and terpenoids) in the different medicinal plant extracts may justify the therapeutic properties of these herbal agents that were used by traditional healers to treat diarrhea. However, further investigations should be envisaged in order to guaranty their quality and their efficacy.

5. Authors' Contribution
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.

6. Conflict of Interest Disclosure
None

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Table 1: Ethnobotanical information of selected medicinal plant species for phytochemical analysis in Niger, West Africa

Family	Scientific name	Local Name (Hausa)	PPU
Anacardiaceae	*Lannea acida* A. Rich.	Faru	Bk
Mimosaceae	*Acacia nilotica* Linn.	Bagaruwa	Po
Burseraceae	*Boswellia dalzielli* Hutch.	Hano	Bk
Combretaceae	*Combretum micranthum* G. Don.	Geza	Lf
Anacardiaceae	*Sclerocarya birrea* (A. Rich.) Hochst	Dania	Bk
Olacaceae	*Ximenia americana* Linn.	Tsada	Bk
Mimosaceae	*Prosopis Africana* (R. Br.) Guill & Perr.	Kiriya	Bk
Combretaceae	*Combretum nigricans* var. elliotii (Engl. & Diels) Aubrév.	Tsiriri	Ap

Abbreviation: PPU, Plant Part Utilized; Bk, Bark; Po, Pods; Lf, Leaf; AP, Aerial part

Table 2: Results of phytochemical screening of 8 medicinal plants

	Plant part	Solvent	Al		Fl	Sa	Ta	Te	St	Ph
			Mayer	Dragendorff						
Lannea acida A. Rich.	Bk	Me	+	+	+	-	+	+	+	+
		Et	+	+	-	+	+	-	-	+
		Aq	-	-	-	-	+	-	-	+
Acacia nilotica Linn.	Po	Me	+	+	+	+	+	+	+	+
		Et	-	+	+	+	+	+	+	+
		Aq	-	-	-	+	+	-	+	+
Boswellia dalzielli Hutch.	Bk	Me	+	+	+	+	+	+	+	+
		Et	+	+	+	+	+	-	+	+
		Aq	+	+	+	-	+	-	-	+
Combretum micranthum G. Don.	Le	Me	+	+	+	+	+	+	+	+
		Et	+	+	+	+	+	+	+	+
		Aq	+	+	+	+	+	-	-	+
Sclerocarya birrea (A. Rich.) Hochst	Bk	Me	-	-	+	+	+	+	+	+
		Et	-	-	+	+	+	+	+	+
		Aq	-	-	+	-	+	+	-	+
Ximenia americana Linn.	Bk	Me	+	+	+	+	+	+	Traces	+
		Et	+	+	+	+	+	+	Traces	-
		Aq	-	-	+	+	+	+	-	+
Prosopis Africana (R. Br.) Guill & Perr.	Bk	Me	+	+	+	+	+	+	+	+
		Et	+	+	+	+	+	+/-	+	+
		Aq	+	+	+	+	+	+	+	+
Combretum nigricans var. elliotii (Engl. & Diels) Aubrév.	Ap	Me	+	-	+	+	+	+	+	-
		Eth	+	+	+/-	+	+	+	+	+
		Aq	+	+	-	-	+	+	-	-

Abbreviation: Bk : Bark ; Po : Pods ; Lf : Leaf; Ap : Aerial part ; Me: methanol; Et: ethanol; Aq: aqueous; Al: alkaloid; Fl: flavonoids; Sa: saponins; Ta : tannins ; St : steroids ; Te : terpenoids ; Ph : phenols

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