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Plants used against diarrhea in traditional medicine of Man, western Côte d'Ivoire: Inventory and phytochemical screening

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Abstract

Diarrhea is a public health problem in Côte d'Ivoire. To contribute to the fight against the disease, this study was undertaken in the city of Man from January 2 to May 31, 2024 with traditional practitioners in the city. It allowed us to interview 88 people practicing in this field of traditional medicine. The study inventoried 30 plants belonging to 17 botanical families with a dominance of Fabaceae and Euphorbiaceae. The leaves, leafy stems, roots and whole plants are the parts of the plants used in anti-diarrheal treatments. These plant organs are prepared by decoction, maceration, kneading and trituration. The medicinal recipes are administered as decoction orally and as purge anally. The plant species cited during this study are: *Amphicarpaea bracteata* (FCe = 0.58) and *Ocimum gratissimum* (FCe = 0.49). A phytochemical screening was carried out on the aqueous extracts of these two plants to search the scientific basis of their anti-diarrheal properties. These tri-phytochemical tests indicated the presence of sterols, polyterpenes, polyphenols, flavonoids, alkaloids and saponins. These chemical groups could justify the use of these plants in traditional medicine. These two plant species should therefore be the subject of more in-depth pharmacological tests for the implementation of new molecules against diarrhea.

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Introduction

Current knowledge places the beginning of humanity at 7 million years ago (Guy, 2009). Since then, man has used plants (Ta *et al*., 2023). This observation is linked to the use of plants in various areas of life, such as housing, food and health (N'guessan, 2008). The relationships between man and plants have led to the emergence of several sciences: ethnobotany, ethnomedicine, ethnopharmacology. Ethnopharmacology is the interdisciplinary scientific study of all materials of plant, animal or mineral origin and the knowledge or practices related to them, which vernacular cultures implement to modify the states of living organisms for therapeutic, curative, preventive or diagnostic purposes (Fleurentin, 2012). It is also called traditional medicine which is based on empirical knowledges and pratices. Today, the use of plants for health is widespread not only in developing countries but also in developed societies (Doh *et al*., 2023). Like other countries, the traditional medicine of Côte d'Ivoire, offers remedies for all human pathologies even if they are curable for modern medicine. The conditions treated are diverse: asthma, diabetes, high blood pressure and especially diarrhea, the subject of this study. Diarrhea is a transit disorder characterized by soft or liquid stools, in abnormally high quantities or with an increased frequency of occurrence (Randremanana, 2012). It can be caused by bacterial, viral or parasitic infections. Diarrhea is the third leading cause of death at any age (Assogba, 2012) and the 5th leading cause of premature death in the world (WHO, 2014). In Côte d'Ivoire, diarrhea is a public health problem. However, the management of diarrheal diseases is limited by the inaccessibility of certain populations to hospitals and the high cost of pharmaceutical drugs (Ambé *et al*., 2015). In Man, the site of this study, diarrheal diseases are the 2nd cause of consultation after malaria. Faced to this worrying health situation, the use of plants becomes an appropriate solution. This study is part of this perspective. It is therefore a contribution to the search for new plants of anti-diarrheal properties. It lists the plants proposed by traditional practitioners of Man against diarrhea and seeks the scientific basis for their use through a phytochemical screening.

Materials and methods

Study environment

Man is located in the west of Côte d'Ivoire, exactly 600 km from Abidjan (economic capital). It is one of the largest cities in the West of Côte d'Ivoire, located at 7°24'0"N latitude and 7°33'0"W longitude (Fig. 1).

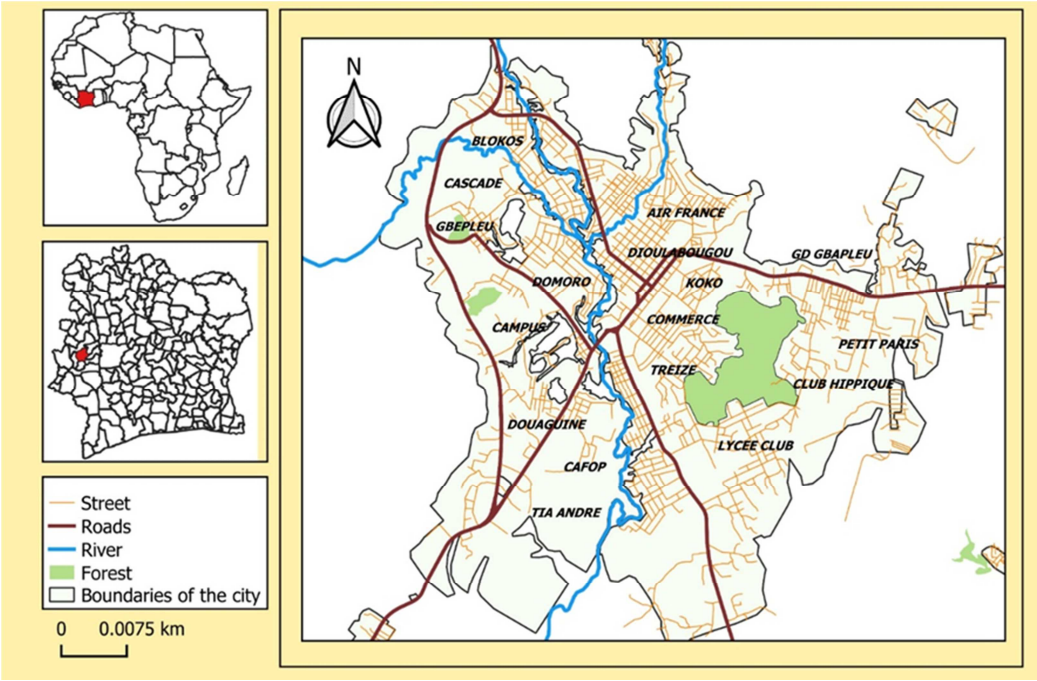

Fig. 1. Geographical location of the city of man in Côte d'Ivoire and Africa

Material of ethnopharmacological survey

The biological material includes all the plants and plant organs encountered among traditional practitioners.

The technical material consists of a survey sheet containing the questionnaires, a pruning shears for collecting plant samples, plastic bags and old newspapers for storing samples. A digital camera and laptop for images, data entry and analysis.

Equipment for phytochemical screening

In this section, the aqueous plant extracts constituted the plant material.

For the technical equipment, we used an oven at 40°C, to obtain dry extracts from the decoction. An electric balance was used to weigh. We had a water bath at 37°C and a water heater. We also used spatulas, cotton wool as a filter, a trituration rod and tongs. Kitchen equipment was also necessary to obtain the decoctions.

Phytochemical screening required the use of several reagents corresponding to the different chemical groups: Stiasny's reagent, Bornstraëgen's reagent, Burchard and Dragendorff's reagents, sodium acetate, sodium acetate, ferric chloride and various other chemicals.

Method of *ethnopharmacological survey*

The investigations took place in the city of Man from January 2 to May 31, 2024 with traditional practitioners in the city. We used the directory of the National Traditional Medicine Program of Côte d'Ivoire (PNMT-CI) to contact traditional practitioners. Out of a total of 97 people recognized by the PNMT-CI in the city, 88 agreed to reveal their ethnomedicinal knowledge. All respondents were interviewed on the basis of a questionnaire relating to plants involved in the treatment of diarrheal diseases. The survey questionnaire relates to plants and plant organs used in the treatment of diarrhea, preparation techniques and the administration route of medicinal recipes.

Collection and identification of plant material

Several field trips were carried out to find and collect samples of plants indicated by traditional practitioners. All the samples collected were identified by the botanical team of Man University in Côte d'Ivoire.

Frequency of species citation (FCs)

The species citation frequency (FCs) refers to the ratio between the number of respondents who mentioned the species and the total number of people interviewed during the survey (Sidio and N'Guessan, 2019). The mathematical formula for the species citation frequency is established as follows according to the same authors:

$$FCs = \frac{\text{Number of people who cited the item(n)}}{\text{Number of people surveyed (N)}}$$

Phytochemical screening method

The most cited plants were subjected to tri-phytochemical tests. They are: *Amphicarpaea bracteata* and *Ocimum gratissimum* (Fig. 1&2).

Fig. 2. *Amphicarpaea bracteata* (Fabacea)

Fig. 3. *Ocimum gratissimum* (Lamiaceae)

Preparation of aqueous extracts

Aqueous extracts were obtained from the decoction of two plants. We made the decoction of the leafy stem of *Amphicarpaea bracteata* and the leaves of *Ocimum gratissimum*. To obtain decoctions, we followed a classic method (Aké-Assi *et al*., 2015). For each plant, we boiled 800g of fresh material in 2 liters of water for 45 min in a kitchen pot with a capacity of 4 liters. The decoction obtained (1 liter) was filtered to obtain 600 ml. This volume of filtrate was concentrated to 25 ml on a sand bath to obtain the dry extract of each plant.

Phytochemical screening

The tri-phytochemical tests were carried out according to an already established protocol (Ta *et al*., 2021).

Sterols and polyterpenes detection

The characterization of these chemical groups was done by the Liebermann reaction. We evaporated to dryness, without carbonizing the residue, in a capsule on the sand bath, 5 ml of the solution. The residue was then dissolved in 1 ml of acetic anhydride and the solution obtained was poured into a test tube. Finally, we poured 0.5 ml of sulfuric acid along the test tube and observed the solution. The appearance at the interphase of a purple or violet ring, turning blue then green, indicated a positive reaction.

Polyphenols detection

The ferric chloride reaction ($FeCl_3$) was used to highlight polyphenols. To 2 ml of each solution, we added a drop of 2% ferric chloride alcoholic solution. Ferric chloride causes, in the presence of polyphenolic derivatives, the appearance of a more or less dark blue-black or green coloration, attesting to the presence of polyphenols.

Flavonoids detection

The highlighting of flavonoids was carried out from the Cyanidin reaction. It consisted of evaporating to dryness in a capsule, 2 ml of each solution and letting it cool. The residue is taken up in 5 ml of half hydrochloric alcohol. The solution is poured into a test tube in which we added 2 to 3 magnesium shavings and observed a release of heat. A pink-orange or sometimes purplish coloration was obtained. We finally added 3 drops of isoamyl alcohol which intensifies the coloration in the presence of flavonoids.

Tannins detection

The search for catechic tannins was carried out using Stiasny's reagent. Five (5) ml of each extract were evaporated to dryness. After adding 15 ml of Stiasny's reagent to the residue, the mixture was kept in a water bath at 80°C for 30 min. The observation of a precipitate in large flakes characterized the catechic tannins. For gallic tannins, we filtered the previous solution. The filtrate is collected and saturated with sodium acetate. The addition of 3 drops of FeCl3 caused the appearance of an intense blue-black color which indicates the presence of gallic tannins.

Quinone substances detection

For combined quinonic substances, we carried out a preliminary hydrolysis. The experiment consisted of hydrolyzing the solutions to characterize all the quinone substances and their derivatives. For this, we evaporated to dryness 2 ml of each solution in a capsule, triturated the residue in 5 ml of hydrochloric acid at 1/5, kept the solution obtained in the boiling water bath for 30 min. We then, after cooling, extracted the hydrolysate with 20 ml of chloroform in a test tube, collected the chloroform phase in another tube and added 0.5 ml of ammonia. The appearance of a color ranging from red to purple attests to the presence of quinones.

Alkaloids detection

The search for alkaloids was done using Dragendorff's reagent and Burchard's reagent. For this, we evaporated to dryness in a capsule, 6 ml of each solution, took up the residue in 6 ml of 60° alcohol and divided the alcoholic solution into 2 test tubes. After this step, we added 2 drops of Dragendorff reagent to the first tube and 2 drops of Burchard reagent to the second tube. In the first

tube, the appearance of a orange precipitate indicated the presence of alkaloids and in the second tube, the observation of a precipitate or a creamy-white reported a positive reaction.

Saponosides detection

Saponosides were detected by assessing the abundance of mosses after shaking. We poured 15 ml of each extract into a test tube, shaken vigorously for 10 seconds and left to stand for 10 min. The persistence of the foam at a height of 2 to 3 cm was proof of the presence of saponosides.

Statistical analysis of data

The statistical analysis concerned the FCs values. It made it possible to make a hierarchical classification dendrogram using SPSS 20 software. To construct the dendrogram, the plants were coded. This code takes into account only the first three letters of the genus and the first two letters of the species to designate a plant (Aké *et al.*, 2019). A plant named *Alchornea cordifolia* is coded Alcco.

Results

Inventoried plants and therapeutic uses

Ethnopharmacological investigations relating to anti-diarrheal plants have made it possible to inventory 30 plant species belonging to 17 families with a predominance of Fabaceae (16.66%) and Euphorbiaceae (10%). The parts of the plants used in treatments are: leaves (63.33%), whole plants (16.66%), leafy stems (10%) and roots (10%). These plant organs are prepared by decoction (72.97%), kneading (13.51%), trituration (8.10%) and maceration (3.33%). The medicinal recipes are administered as a drink and as an anal purge. All these informations are recorded in Table 1.

Table 1. Inventoried plants and medicinal characteristics

Plant species	Botanical families	Organs used	Preparation techniques	Administration methods
Alchornea cordifolia	Euphorbiaceae	Root	Trituration	Drink
		Leaf	Decoction	Purge
Allamanda cathartica	Apocynaceae	Leavy stem	Decoction	Drink
Amphicarpaea bracteata	Fabaceae	Leavy stem	Decoction	Drink
Centrosema virginianum	Fabaceae	Whole plant	Decoction	Drink
Clausena lansium	Rutaceae	Leaf	Maceration	Drink
Cnestis ferruginea	Connaraceae	Leaf	Decoction	Purge
			Trituration	
Combretum indicum	Combretaceae	Leaf	Decoction	Drink
Combretum micranthum	Combretaceae	Leaf	Decoction	Drink
Cyperus eragrostis	Cyperacea	Whole plant	Decoction	Drink
Cyperus erectus	Cyperaceae	Whole plant	Decoction	Drink
			Kneading	Purge
Erigeron sumatrensis	Asteraceae	Leaf	Decoction	Purge
Euphorbia hirta	Euphorbiaceae	Leaf	Decoction	Drink
			Kneading	Purge
Griffonia simplicifolia	Fabaceae	Leaf	Decoction	Drink
Heliotropium indicum	Boraginaceae	Leaf	Decoction	Drink
			Kneading	Purge
Mangifera indica	Anarcadiaceae	Root	Decoction	Drink
Markhamia lutea	Bognoniaceae	Leaf	Decoction	Drink
Megathyrsus maximus	Poaceae	Whole plant	Decoction	Drink
Mezoneuron benthamianum	Fabaceae	Root	Decoction	Drink
Mimosa pudica	Fabaceae	Whole plant	Decoction	Drink
			Kneading	
Mitracarpus hirtus	Rubiaceae	Leavy stem	Decoction	Drink
Morinda citrifolia	Rubiaceae	Leaf	Decoction	Drink
Newbouldia laevis	Bognoniaceae	Root	Decoction	Drink
Ocimum gratissimum	Lamiaceae	Leaf	Decoction	Drink
Parquetina nigrescens	Apocynaceae	Leaf	Trituration	Purge
Passiflora foetida	Passifloraceae	Whole plant	Decoction	Drink
Phyllantus acidus	Euphorbiaceae	Leaf	Decoction	Drink

Pneumatopteris pennigera	Thelypteridaceae	Leaf	Decoction	Drink
Solanum mauritianum	Solanaceae	Leaf	Decoction	Drink
Solenostemon monostachyus	Lamiaceae	Leaf	Decoction	Drink
Synedrella nodiflora	Asteraceae	Leaf	Decoction	Drink

Frequency of citation for plant species (FCs)

The calculated FCs constituted two groups of plants represented by a dendrogram (Fig. 4). Group 1 is composed of *Amphicarpaea bracteata* (FCs = 0.58) and *Ocimum gratissimum* (FCs = 0.49).

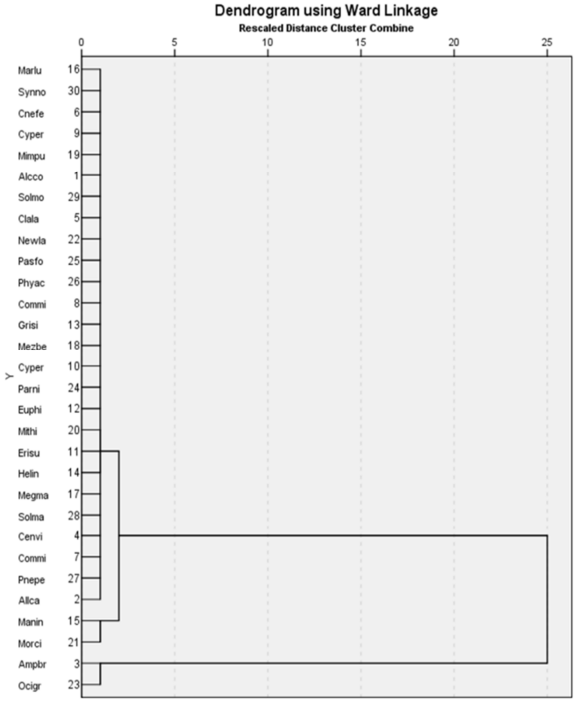

Fig. 4. Dendrogram of hierarchical classification for inventoried plants according to FC

These two plants are the most used against diarrheal diseases in the traditional pharmacopoeia of Man. The 28 other plants forming group 2 were rarely cited by the traditional practitioners interviewed.

Phytochemical screening

The results of the phytochemical screening are recorded in Table 2. Sterols and polyterpenes are present in the extracts of the 2 plants in average proportions while flavonoids and polyphenols are abundant. Concerning tannins, they are found only in the extracts of *Amphicarpaea bracteata*. Quinone substances are absent in the plant extracts. The two plant extracts contain alkaloids D while alkaloids B are present only in *Ocimum gratissimum*. In connection with saponins, they are abundant in *Amphicarpaea bracteata* and moderately present in *Ocimum gratissimum*.

Table 2. Phytochemical screening results

Chimical groups		Extract of *Amphicarpaea bracteata*	Extract of *Ocimum gratissimum*
Sterols et polyterpenes		+	+
Polyphenols		++	+
Flavonoids		++	++
Tannins	Gallic	–	–
	Catechic	–	+
Quinone substances		–	+
Alcaloids	Dragendorf (D)	+	+
	Burchard (B)	–	++
Saponins		++	+

Discussion

The study identified 28 plant species used against diarrhea in traditional medicine of Man. These plants belong to 17 families with a high representation of Fabaceae and Euphorbiaceae. These two plant families were listed by Ambé *et al.* (2015) in a similar study conducted in Abidjan (southern of Côte d'Ivoire). The most used plant organs in the preparation of medicinal recipes are leaves (63.33%). This result corroborates that of Lawaly *et al.* (2015) who indicated the dominance of these organs in anti-diarrheal recipes in Niger. Furthermore, the high use of leaves in medicinal recipes could be explained by the easy accessibility of these organs (N'Guessan *et al.*, 2009). Decoction is the most recommended preparation technique. This is a result consistent with the study of Béné *et al.* (2016), who showed that decoction is the most requested method of preparation in traditional medicinal recipes in Côte d'Ivoire. In addition, decoction collects the most active ingredients and reduces the toxicity of plants (Soro *et al.*, 2021).

In relation to FCs, the interviewees of this study regularly cited two plants. They are: *Amphicarpaea bracteata* and *Ocimum gratissimum*. In the Ivorian literature *O. gratissimum* has already been revealed as an anti-diarrheal plant (Aké-Assi, 2011). However, the use of *A. bracteata* in the treatment of diarrheal diseases is a revelation of this study.

The phytochemical tests indicated that the two plants analyzed are rich in sterols, polyterpenes, flavonoids, alkaloids and saponosides. Polyphenols and flavonoids are antibiotics (Kouadio *et al.*, 2021). These chemical groups could effectively fight against diarrheal diseases because bacteria sometimes cause diarrhea.

Conclusion

This study is a contribution to the search for solutions against diarrheal diseases. It was carried out in Man (a city in the west of Côte d'Ivoire) with the participation of traditional healers. It allowed to question 88 people and to identify 30 plants divided into 17 botanical families with a dominance of Fabaceae and Euphorbiaceae. The leaves, leafy stems, roots and whole plants are the parts of the plants used in anti-diarrheal treatment. These plant organs are prepared by decoction, maceration, kneading and trituration. The medicinal recipes are administered in decoction orally and in purge by anus. The plant species regularly evoked are: *Amphicarpaea bracteata* (FCs = 0.58) and *Ocimum gratissimum* (FCs = 0.49). A phytochemical screening was carried out on the aqueous extracts of these two plants to expose the scientific basis of their use against diarrhea. These phytochemical tests indicated the presence of sterols, polyterpenes, polyphenols, flavonoids, alkaloids and saponins. These chemical groups could be responsible of the anti-diarrheal properties of these plants. These two plant species (*Amphicarpaea bracteata* and *Ocimum gratissimum*) should therefore be the subject of more in-depth pharmacological tests for the implementation of new molecules against diarrhea.

Acknowledgments

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