Journal of Biosciences and Medicines, 2024, 12, 101-113
https://www.scirp.org/journal/jbm
ISSN Online: 2327-509X
ISSN Print: 2327-5081

Phytochemical Characterization and Evaluation of the Antibacterial Activity of Leaf Extracts of *Mitragyna inermis* (Willd.) O. Ktze on the *in Vitro* Growth of Clinical Strains *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* Involved in Gastro Enteritis

Monon Kone[1*], Youssouf Zanga Traore[1], Tidiane Kamagate[1], Ahmont Landry Claude Kablan[2], Abdoulaye Toure[1], Karamoko Ouattara[3,4], Adama Coulibaly[3]

[1]Department of Biochemistry-Genetics, Training and Research Unit Biological Sciences, Peleforo Gon Coulibaly University, Korhogo, Côte d'Ivoire
[2]Department of Mathematics-Physics-Chemistry, Training and Research Unit Biological Sciences, Peleforo Gon Coulibaly University, Korhogo, Côte d'Ivoire
[3]Department of Biology and Health, Training and Research Unit of Bioscience, University of Félix Houphouët-Boigny, Abidjan, Ivory Coast
[4]Department of Microbiology and Molecular Biology, Training and Research Unit of Agriculture, Fisheries Resources and Agro-Industry, University of San Pedro, San Pedro, Côte d'Ivoire
Email: *konemonon2017@gmail.com

How to cite this paper: Kone, M., Traore, Y.Z., Kamagate, T., Kablan, A.L.C., Toure, A., Ouattara, K. and Coulibaly, A. (2024) Phytochemical Characterization and Evaluation of the Antibacterial Activity of Leaf Extracts of *Mitragyna inermis* (Willd.) O. Ktze on the *in Vitro* Growth of Clinical Strains *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* Involved in Gastro Enteritis. *Journal of Biosciences and Medicines*, **12**, 101-113.
https://doi.org/10.4236/jbm.2024.126011

Received: March 30, 2024
Accepted: June 11, 2024
Published: June 14, 2024

Abstract

Gastroenteritis constitutes a group of diarrheal diseases of infectious origin, responsible for absenteeism from work, morbidity and mortality, especially among aged people. This study aimed to evaluate the antibacterial activity of *Mitragyna inermis* extracts on the in vitro growth of *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* involved in gastroenteritis. Phytochemical screening was carried out using two distinct methods. The detection of phytochemical compounds by tube coloring and chromatography on a thin layer of silica gel. The sensitivity of organisms was evaluated by the agar well method. The dilution method in liquid medium coupled with spreading on Mueller Hinton agar helped determine the CMB/MIC activity ratios. The investigations show that the extract has the best extraction yield (75.86% ± 0.20%) compared to the aqueous macerated (61.8% ± 0.08%) and decocted (66.6% ± 0.12%). These extracts contain several phytochemical compounds such as flavonoids, polyphenols, tannins, alkaloids, saponosides,

coumarins and sterols and terpenes. These substances are endowed with biological activities and could be at the origin of antibacterial activity observed with *M. inermis* extracts. The analysis of antibacterial activity showed that the germs are sensitive to the extracts with inhibition diameters ranging from 8.30 ± 0.53 to 17.87 ± 0.58 mm. The ethanolic extract was the most active with diameters varying from 15.07 ± 0.62 to 17.87 ± 0.58 mm on all the germs tested. *E. coli* and *S. aureus* were the most sensitive germs to the extracts. *P. aeruginosa* was the least sensitive germ. Activity reports indicate that the extracts exert bactericidal activity on *E. coli* and *S. aureus* but bacteriostatic activity on *P. aeruginosa*. These results justify the use of *M. inermis* leaves in a traditional environment to treat gastroenteritis.

Keywords

Phytochemical Characterization, Antibacterial Activity, *Mitragyna inermis*, Gastro Enteritis

1. Introduction

Traditional medicine has experienced renewed interest in recent years and occupied an important place in the health policy of several developing countries. According to the World Health Organization (WHO), nearly 80% of African populations use traditional medicine for primary health care [1]. These populations, mainly rural, use traditional medicine as an alternative to problems of accessibility to care, medications and the high costs of health services. The reasons linked to the phenomena of multi-resistance of pathogens to active substances [2] [3] [4] and the manifestation of severe side effects, even toxic in certain cases, encourage the use of traditional medicine [5] [6]. Certain molecules such as metronidazole, widely prescribed in the case of intestinal disorders, cause headaches and nausea. It is believed to be carcinogenic and teratogenic [7]. Non-steroidal anti-inflammatory drugs manifest side effects such as lesions, gastrointestinal irritation and renal toxicity while the use of opiates is reserved for severe pain because of their adverse side effects (respiratory depression, dependence, sweating) [8]. The consequences of the development of pharmaceutical industry have led researchers to undertake work relating to the pharmacological actions of medicinal plants [9]. It is in this context that we got interested in *Mitragyna inermis* used in traditional medicine to treat several pathologies. Indeed, *M. inermis* is a species widely used by healers, but differently depending on geocultural access [9]. The barks are often prescribed for gravida-puerperal conditions, stomach aches, dysentery, bilharzia, diabetes and urinary pain [10]. It is an antimalarial, an antispasmodic, a cardiotonic and anticholesteremic. The most used parts are the leaf and the bark of the trunk, removed by scraping the epidermis and subterm, which would be, after drying and reduction into powder, a healing agent for large wounds [10].

2. Material and Methods

2.1. Material

2.1.1. Plant Material

The plant material consists of powdered leaves of *Mitragyna inermis* (Willd.) O. Ktze. Harvested in Boyo commune of Kouto, Bagoué region (northern Ivory Coast) and identified at the National Floristic Center of the Felix Houphouët Boigny University of Abidjan.

2.1.2. Reagents and Extraction Solvents

The developers and reagents used for phytochemical screening in tubes and by Thin Layer Chromatography (TLC) are: Sulfuric vanillin, Dragendorff (KOH) at 5% ($AlCl_3$) at 1%, ($FeCl_3$) at 2%. Distilled water and 96% ethanol were used as extraction solvents.

2.2. Methods

2.2.1. Harvest of the Plant

The leaves of *Mitragyna inermis* were harvested, then washed and then dried in ambient air at laboratory temperature. The dry leaves were ground using an electric grinder until a fine powder was obtained.

2.2.2. Preparation of Total Extracts

The extractions by maceration (aqueous and ethanolic) were carried out according to the method described by [11] and the decoction was made according to [12].

2.2.3. Preparation of Selective Extracts

A volume of 20 mL of each total extract was exhausted by successive fractionations with (3 × 10 mL) hexane (C_6H_{14}), dichloromethane (CH_2Cl_2) and ethyl acetate (AcOEt). The different selective organic fractions were concentrated in an oven then stored in the refrigerator at 4°C. These fractions were used for phytochemical screening [13].

2.2.4. Calculation of Extraction Yield

The total extracts (aqueous, decocted and ethanolic) were weighed in order to calculate the yield (Yield) of each extraction according to the formula below and then reduced into percentage.

2.2.5. Phytochemical Characterization of Extracts

The screening of secondary metabolites of the extracts was carried out on the one hand by means of coloring tests in test tubes according to the analytical techniques described by [14], [15] [16] and on the other hand on TLC plate following the methods described by [17].

2.2.6. Evaluation of Antibacterial Activity

1) Preparation of culture medium

The preparation of TBX, EPT Baird Parker, Cetrimide agar, Nutritive Agar

and Müller-Hinton culture media was carried out following the manufacturer's instructions.

2) Verification of the purity of bacterial species

Under sterile conditions, a characteristic colony isolated on the specific culture medium is taken using a platinum loop then spread in streaks on a dish containing the nutrient agar. The box is then incubated at 37˚C for 24 hours.

3) Preparation of the inoculum

Using a Pasteur pipette, a colony aged 18 to 24 hours is taken and placed in a test tube containing 10 mL of sterile physiological water. The mixture is compared to the Mac Farland 0.5 control. This solution constitutes the 100% stock solution.

4) Inoculum counting

The enumeration of the inoculum is carried out by a 10th dilution from stock solution 10^0. The different decimal dilutions obtained as well as the stock solution 10^0 are inoculated using a calibrated loop of 2 µL by radial streaks on an agar Mueller Hinton antimicrobial free. This box constitutes box A. It is incubated at 37˚C for 24 hours.

5) Preparation of the concentration range of extracts

The concentration range was prepared according to [18].

The concentration range of the plant extract from 64 mg/mL to 4 mg/mL was prepared in screw test tubes using the double dilution method. Thus, five (5) test tubes marked T_1 to T_5 were used. The first tube (T_1) contains 10 mL of distilled water to which 0.6 g of plant extract has been added. The second tube (T_2) contains 5 mL of distilled water as do all the other tubes (T_3 to T_5). The contents of the tube marked T_1 are shaken vigorously mechanically. When the mixture becomes homogeneous, half of the contents of this tube (5 mL) is transferred to tube T_2. The latter is also stirred in the same way as before and half of the content is transferred to tube T_3 and so on just as at T_5. Half of the content of the last tube is discarded into a jar provided for this purpose. The range thus prepared was sterilized in an autoclave at 121°C for 15 min.

6) Seeding the concentration range of extracts

Seeding the concentration range is carried out by adding 1 mL of the content of each tube of the concentration range to 1 mL of the inoculum (100% stock solution), around the Bunsen burner flame.

The Growth Control (TC) tube will contain 2 mL of the inoculum.

The Sterility Control (TS) tube will contain 2 mL of sterile culture medium.

7) Sensitivity test

The sensitivity of the strains to plant extracts was carried out by the diffusion technique in agar medium. Mueller Hinton medium was inoculated by swabbing. Using a sterile cookie cutter, wells of approximately 6 mm in diameter are made in the 4 mm thick agar. Each well received 80 µL of the substance to be tested at concentrations 16 and 8 mg/mL. After 15 min of diffusion at laboratory temperature, the Petri dishes are incubated at 37˚C for 18 to 24 h. The presence or absence of an inhibition zone was observed. The results were interpreted ac-

cording to [19].

8) Determination of the antibacterial activity ratio (MIC and CMB)

Antibacterial parameters were carried out according to [18].

Minimum Inhibitory Concentration (MIC)

The MIC is determined from the spiked concentration range by observing the test tubes with the naked eye. Cloudy media indicates the growth of the culture and clear media is evidence that growth has been inhibited. The MIC corresponds to the smallest concentration inoculated without growth visible to the naked eye.

Minimum Bactericidal Concentration (MBC)

The MBC is determined by subculturing all experimental tubes in the seeded concentration range without growth visible to the naked eye. This subculturing constitutes box B. The CMB is defined by comparison of box A and box B. It corresponds to the smallest concentration of box B whose number of colonies is less than or equal to the number of colonies of the dilution 10^{-4} from box A.

2.2.7. Statistical Analysis of the Results

The results were analyzed using Statistica software version 7.1 and Microsoft Excel 2010. All data were expressed as mean ± standard deviation. The significance of the differences between the treated groups was evaluated by the Fischer test carried out at the 5% threshold. Differences were considered significant for $p < 0.05$.

3. Results and Discussion

3.1. Extraction Yield

The extraction yield, mass and color of the different extracts of *M. inermis* are presented in Table 1. The ethanolic extract obtained the highest extraction yield with 75.86% while the aqueous extract recorded the lowest extraction yield (61.8%). Ethanol would be the solvent that extracts the most compounds from *M. inermis* leaves compared to water.

3.2. Selective Extracts

Fractionation of the total extracts made it possible to obtain three selective extracts from each total extract. Leaching the extracts with hexane gave MA_{Hex} (hexane fraction of the total aqueous extract of *M. inermis*), MD_{Hex} (hexane fraction of the decocted total extract of *M. inermis*) and ME_{Hex} (hexane fraction of the total ethanolic extract of *M. inermis*). These extracts were used for the detection of sterols and terpenes. As for dichloromethane and ethyl acetate, their leaching made it possible to successfully obtain MA_{DCM} MD_{DCM} and ME_{DCM} and $MA_{Acetatic}$ $MD_{Acetatic}$ and $ME_{Acetatic}$. These different extracts were used for the detection of secondary metabolites of interest other than sterols and terpenes.

3.3. Phytochemical Screening by TLC

3.3.1. Screening after Staining in Test Tubes

Table 2 presents the results of the phytochemical screening of the total extracts of

M. inermis leaves. These results indicate the presence of polyphenolic compounds such as total polyphenols, flavonoids, tannins, saponins, sterols and terpenes, quinones and alkaloids. These compounds are more concentrated in the decocted extract compared to the aqueous and ethanolic extract. The ethanolic extract contains fewer polyphenolic compounds than the aqueous and decocted extract. It does not contain saponins and quinones. The results of this tube screening were reinforced by the thin layer chromatography method. The complexity of tube observation linked to the coloring of plant extracts sometimes reveals false positive or negative results. Detection by thin layer chromatography makes it possible to elucidate the results in test tubes. Thus, Table 3 indicates the results of the detection of tannins and phenolic acids. The gray color indicates the presence of tannins while the green color indicates the presence of phenolic acid. In view of these results, the $MA_{Acetatic}$ extract contains a single spot of tannins at Rf (0.95) and four spots of phenolic acids. As for the $ME_{Acetatic}$ extract, it contains two molecules of tannins with Rf (0.72 and 0.97) and three molecules of phenolic acid with Rf (00; 0.06 and 0.27). The $MD_{Acetatic}$ extract contains as many tannins as phenolic acid. $MD_{Acetatic}$ extract contains more tannins compared to other extracts. *Mitragyna inermis* leaf extracts contain more phenolic acids than tannins. The detection of alkaloids was also carried out in the visible. All the extracts showed spots of orange color on a yellow background testifying to the presence of alkaloids (Table 4). The detection of flavonoids was carried out in the visible. All extracts showed yellow-colored spots except the ME_{DCM} extract. This extract recorded spots of green colors testifying to the presence of flavonoid at RF = 0.81 and 0.95 (Table 5). Regarding the detection of coumarins, Table 6 presents the results obtained. All the plates were found to be yellow in the visible, thus indicating the presence of coumarin. The observation of this same plate under UV λ = 366 nm, presented a blue coloring characteristic of coumarins with the MA_{DCM} extract at RF = 0.95 and with the MD_{DCM} extract at RF = 0.25; 0.76 and 0.86.

Table 1. Extraction yield of the different extracts of *Mitragyna inermis*.

Types of Extraction	Solvents	Yield (%)	Color Extracts
Maceration	Aqueous	61.8 ± 0.08	Yellow
Maceration	Ethanolic	75.86 ± 0.20	Black
Decoction	Decocted	66.6 ± 0.12	Brown

Table 2. Phytochemical characterization of *Mitragyna inermis* leaves.

Extracts	Sterols and Polyterpenes	Polyphenols	Flavonoids	Tannins	Quinones	Alkaloids	Coumarin	Saponosides
Aqueous	+	+	+	+	+	+	+	+
Decocted	+	+	+	+	+	+	+	+
Ethanolic	+	+	+	+	−	+	+	−

Table 3. Detection of tannins and phenolic acids, in the H_2Cl_2/AcOEt/ CH_3COOH (1:3.5:1) (V/V/V) developer from acetate-ethyl extracts (AcOEt).

Extracts	R_f (in the visible color, at UV color): possible compound
MA (AcOEt)	0.95 (gray, –): tannins; 0.45 (green, –): Phenolic acid; 0.27 (green, –): Phenolic acid; 0.06 (green, –): Phenolic acid; 00 (green, –): Phenolic acid
ME (AcOEt)	0.97 (gray, –): tannins; 0.72 (gray, –): tannins; 0.27 (green, –): Phenolic acid; 0.06 (green, –): Phenolic acid; 00 (green, –): Phenolic acid
MD (AcOEt)	0.95 (gray, –): tannins; 0.76 (green, –): Phenolic acid; 0.65 (gray, –): tannins; 0.52 (green, –): Phenolic acid; 0.4 (gray, –): tannins; 0.3 (green, –): Phenolic acid; 0.18 (gray, –): tannins; 0.13 (green, –): Phenolic acid

Table 4. Detection of alkaloids in the CH_2Cl_2/AcOEt/C_6H_{14} (2:2:1) (V: V: V) developer from dichloromethane extracts (CH_2Cl_2).

Extracts	R_f (in the visible color, at UV color): possible compound
MA (CH_2Cl_2)	0.97 (orange, –): alkaloids; 0.88 (orange, –): alkaloids
ME (CH_2Cl_2)	0.97 (orange, –): alkaloids
MD (CH_2Cl_2)	0.97 (orange, –): alkaloids; 0.88 (orange, –): alkaloids; 0.81 (orange, –): alkaloids

Table 5. Detection of flavonoids in the developing CH_2Cl_2/AcOEt/C_6H_{14} (2:2:1) (V/V/V) from dichloromethane extracts (CH_2Cl_2).

Extracts	R_f (in the visible color, at UV color): possible compound
MA (CH_2Cl_2)	0.38 (yellow, –): flavonoids; 0.3 (yellow, –): flavonoids; 0.2 (yellow, –): flavonoids; 0.1 (yellow, –): flavonoids;
ME (CH_2Cl_2)	0.95 (green, –): flavonoids; 0.81 (green, –): flavonoids; 0.71 (yellow, –): flavonoids; 0.3 (yellow, –): flavonoids; 0.2 (yellow, –): flavonoids; 0.12 (yellow, –): flavonoids;
MD (CH_2Cl_2)	0.93 (yellow, –): flavonoids ; 0.82 (yellow, –): flavonoids ; 0.7 (yellow, –): flavonoids

Table 6. Detection of coumarins in the CH_2Cl_2/AcOEt/C_6H_{14} (2:2:1) (V/V/V) developer from dichloromethane extracts (CH_2Cl_2).

Extracts	R_f (in the visible color, at UV color): possible compound
MA (CH_2Cl_2)	0.97 (yellow, –): coumarins; 0.56 (yellow, –): coumarins; 0.46 (yellow, –): coumarins; 0.35 (yellow, –): coumarins; 0.95 (–, bleu): coumarins; 0.76 (–, yellow): coumarins; 0.65 (–, yellow): coumarins;
ME (CH_2Cl_2)	0.97 (yellow, –): coumarins; 0.92 (yellow, –): coumarins; 0.85 (yellow, –): coumarins; 0.76 (yellow, –): coumarins; 0.73 (yellow, –): coumarins; 0.6 (yellow, –): coumarins; 0.85 (–, yellow): coumarins; 0.73 (–, yellow): coumarins, 0.6 (–, yellow): coumarins; 0.5 (–, yellow): coumarins
MD (CH_2Cl_2)	0.95 (yellow, –): coumarins; 0.85 (yellow, –): coumarins; 0.72 (yellow, –): coumarins; 0.86 (–, blue): coumarins; 0.76 (–, blue): coumarins; 0.72 (–, yellow): coumarins; 0.25 (–, blue): coumarins.

3.3.2. Sensitivity of Germs with Regard to the Extracts

The results of the bacterial sensitivity test to the extracts are grouped in **Table 7**. The largest inhibition diameters were obtained at the concentration of 16

mg/mL. The germs of *E. coli* and *S. aureus* were sensitive to all extracts of *Mitragyna inermis*, however, *P. aeruginosa* was sensitive to the ethanolic extract but resistant to aqueous and decocted extracts since the inhibition diameters were less than 8 mm. At the highest concentration (16 mg/ml) and by comparison of the inhibition diameters between the germs at the level of each extract, there is no significant difference between the inhibition diameters of the aqueous extract at the level of *E. coli* and *S. aureus*. A similar observation was made between the inhibition diameters of the ethanolic extract at the level of *P. aeruginosa* and *S. aureus*. However, a comparison of the inhibition diameters of the extracts at the level of each germ gave more or less different results. There is a significant difference between the inhibition diameters of the aqueous, decocted and ethanolic extracts at the level of the germs of *S. aureus* and *P. aeruginosa*. A significant difference was also noted in the inhibition diameters of the ethanolic extract and the two other extracts at the level of *E. coli*. The most sensitive germ to *Mitragyna inermis* extracts was *E. coli* and *P. aeruginosa* was the least sensitive. All germs (*E. coli*, *S. aureus* and *P. aeruginosa*) were sensitive to the ethanolic extract with the largest inhibition diameters (17.87 ± 0.58 mm, 15.77 ± 1.44 mm and 15.07 ± 0.62 mm) respectively at the concentration of 16 mg/mL. These results were confirmed by the determination of the antibacterial parameters (Table 8, Table 9, Table 10). The ethanol extract showed a bactericidal effect on *S. aureus* and bacteriostatic effect on *E. coli*. As for the other extracts, they had a bactericidal effect on *E. coli* only. Their effects on other germs have not been determined based on the concentration range of this study. The ethanolic extract was therefore the most active extract.

Table 7. Inhibition diameters of the leaves extracts of *Mitragyna inermis*.

Extracts	Extracts Aqueous		Decocted		Extracts Ethanolic	
Concentrations (mg/mL)	16	8	16	8	16	8
E. coli	13.30 ± 0.60[a]	12.07 ± 1.29	12.43 ± 0.38[a]	11.10 ± 0.80	17.87 ± 0.58[f]	15.87 ± 1.42
S. aureus	12.33 ± 0.44[a]	9.87 ± 0.18	10.33 ± 0.44[e]	8.30 ± 0.53	15.77 ± 1.44[b]	14.13 ± 0.98
P. aeruginosa	7.60 ± 0.47[d]	6.67 ± 0.56	0.00 ± 0[c]	0.00 ± 0	15.07 ± 0.62[b]	12.57 ± 0.38

Mean ± standard deviation of 3 trials.

Table 8. Antibacterial parameters of the decocted extract.

	Antibacterial parameters of the extract			
Samples	CMI (mg/mL)	CMB (mg/mL)	CMB/CMI	Interpretation
E. coli	8	32	4	Bactericidal
S. aureus	16	Nd	Nd	-
P. aeruginosa	16	Nd	Nd	-

Table 9. Antibacterial parameters of the ethanolic macerated extract.

	Antibacterial parameters of the extract			
Samples	CMI (mg/mL)	CMB (mg/mL)	CMB/CMI	Interpretation
E. coli	2	16	8	Bactericidal
S. aureus	8	32	4	Bactericidal
P. aeruginosa	8	Nd	Nd	-

Table 10. Antibacterial parameters of the aqueous macerated extract.

	Antibacterial parameters of the extract			
Samples	CMI (mg/mL)	CMB (mg/mL)	CMB/CMI	Interpretation
E. coli	8	32	4	Bactericidal
S. aureus	16	Nd	Nd	-
P. aeruginosa	16	Nd	Nd	-

4. Discussion

The use of traditional medicine has known renewed interest in recent years and occupied a prominent place in the health policy of several developing countries. The present study showed that ethanol is the solvent that best extracts the active ingredients from the plant studied with an extraction yield of 75.86 ± 0.20%. These results are consistent with those of [20]. However, different results were obtained with water using leafy branches of *Mitragyna inermis* [21]. This difference may be due to the extraction method, the extraction conditions, and the chemical composition of the part of the plant studied. Phytochemical tube and thin layer chromatography screening revealed that the three extracts of *Mitragyna inermis* are rich in tannins, flavonoids, alkaloids, coumarin and phenolic acid. Similar results were obtained by [9]. These authors showed that polyphenols, flavonoids and catechic tannins are present in the methanolic, infused and decocted extracts but absent in the chloroform extract of *Mitragyna inermis* leaves. Saponosides are present only in the decoction while sterols, triterpenes and alkaloids were present in all extracts [9].

Thin layer chromatography revealed the presence of several types of molecules from the family of tannins, flavonoids, coumarins, alkaloids, phenolic acid by the presence of several spots at various Rfs. These results help to justify that the TLC method highlights a large number of bioactive compounds present in plant extracts [13] [22]. This phytochemical profile is also comparable to that reported for *Acacia nilotica* extracts [23]. Tannins, flavonoids, sterols and triterpenes which are most involved in antibacterial activities [23] were highlighted in the extracts of the present study. The antibacterial analysis of *Mitragyna inermis* extracts showed that all the germs studied were sensitive to the ethanolic extract of *Mitragyna inermis* with inhibition diameters ranging from 15.07 ± 0.62 to 17.87

± 0.58 to the concentration of 16 mg/mL. The aqueous macerated and the decoction obtained similar inhibition diameters but lower than those of the ethanolic extract. This difference may be linked to the extraction method and/or the extraction solvent used [24] [25]. The results obtained with the aqueous macerated and the decoction are substantially identical both in terms of the sensitivity of the germs and the antibacterial parameters. This indicates that the extraction temperature did not degrade the phytochemical composition of this plant. Similar results were recorded with total and freeze-dried extracts of the same plant [26]. These authors showed that *Staphylococcus aureus* MRSA germs were sensitive to total and hyophilized extracts of *Mitragyna inermis*. The difference in sensitivity observed could be due to the difference in structure of the germs. The CMB/MIC activity report showed that all *Mitragyna inermis* extracts exhibit bactericidal or bacteriostatic activity on the growth of *E. coli* and *S. aureus*. The antibacterial activity of the extracts may be due to the presence of flavonoids, tannins, polyphenols and coumarins. These phytochemicals act by either modifying the bacterial cell membrane, acting as an antimetabolite, or inhibiting nucleic acid, protein, and/or cell wall synthesis [27].

5. Conclusion

This study established a scientific basis for the traditional use of *Mitragyna inermis*, a plant widely used in traditional medicine in the north of the Ivory Coast, in the treatment of gastroenteritis and gastric ulcers. It has allowed the characterisation of the main phytochemical groups of the extracts using two different methods to evaluate the antibacterial effects of the aqueous decocted, aqueous macerated and ethanolic macerated leaves. The findings revealed that ethanol extracts the active ingredients from *M. inermis* leaves better than water. Phytochemical characterization showed that *M. inermis* extracts contain several phytochemical compounds such as flavonoids, tannins, polyphenols, alkaloids, coumarins and sterols and terpenes. These compounds could be the basis of the antibacterial activity observed. These results could justify the use of *M. inermis* leaves by local populations as an alternative to primary health care, especially in cases of gastroenteritis.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Koné, D. (2009) Enquête ethnobotanique de six plantes médicinales maliennes-Extraction, Identification d'alcaloïdes-Caractérisation, Quantification de polyphenols: Etude de leur activité Antioxydante. Thèse de Doctorat, Université Paul Verlaine de Metz-UPV-M (France)/Université de Bamako Faculté des Sciences et Techniques de Bamako, Bamako.

[2] Akoua, K., Guessennd, N., Gbonon, V., Faye-Ketté, H. and Dosso, M. (2004) Me-

thicillin Resistance of Staphylococcus in Abidjan 1998-2001: A New Problem. *Médecine et maladies Infectieuses*, **34**, 132-136. https://doi.org/10.1016/j.medmal.2003.12.001

[3] Guessennd, N., Bremont, S., Gbonon, V., Kacou-N'Douba, A., Ekaza, E., Lambert, T., Dosso, M. and Courvalin, P. (2008) Résistance aux quinolones de type qnr chez lez entérobactéries productrices de bêta-lactamases à spectre élargi à Abidjan en Côte d'Ivoire. *Pathologie Biologie*, **56**, 439-446. https://doi.org/10.1016/j.patbio.2008.07.025

[4] Chaudhary, A.S. (2016) A Review of Global Initiatives to Fight Antibiotic Resistance and Recent Antibiotics Discovery. *Acta Pharmaceutica Sinica B*, **6**, 552-556. https://doi.org/10.1016/j.apsb.2016.06.004

[5] Talbaoui, A., Jamaly, N. and Aneb, M. (2012) Chemical Composition and Antibacterial Activity of Essential Oils from Six Moroccan Plants. *Journal of Medicinal Plants Research*, **6**, 4593-4600. https://doi.org/10.5897/JMPR10.078

[6] Bouyahya, A., Bakri, Y., Et-Touys, A., Talbaoui, A., Khouchlaa, A., Charfi, S., Abrini, J. and Dakka, N. (2017) Résistance aux antibiotiques et mécanismes d'action des huiles essentielles contre les bactéries. *Phytothérapie*. https://doi.org/10.1007/s10298-017-1118-z

[7] Calzada, F., Yépez-Mulia, L. and Aguilar, A. (2006) *In Vitro* Susceptibility of *Entamoeba histolytica* and *Giardia lamblia* to Plants Used in Mexican Traditional Medicine for the Treatment of Gastrointestinal Disorders. *Journal of Ethnopharmacology*, **108**, 367-370. https://doi.org/10.1016/j.jep.2006.05.025

[8] Schenone, S., Brullo, C., Bruno, O., Bondavalli, F., Ranise, A., Filippelli, W., Rinaldi, B., Capuano, A. and Falcone, G. (2006) New 1,3,4-Thiadiazole Derivatives Endowed with Analgesic and Anti-Inflammatory Activities. *Bioorganic and Medicinal Chemistry*, **14**, 1698-1705. https://doi.org/10.1016/j.bmc.2005.10.064

[9] Konkon, N.G., Simaga, D., Adjoungova, A.L., Nguessan, K.E., Zirihi, G.N. and Kone, B.D. (2006) Étude phytochimique de *Mitragyna inermis* (willd.) o. Ktze (Rubiaceae), plante à feuille antidiabétique. *Journal of Medicinal Plants Research*, **2**, 279-284.

[10] Kerharo, J. and Adams, J.G. (1974) La pharmacopée sénégalaise traditionnelle plantes médicinales et toxiques. Edition Vigot et frères, Paris.

[11] Kone, M., Ouattara, K., Gnahoue, G., Ouattara, A. and Coulibaly, A. (2013) Study Ethnopharmacological and Phytochemical Screening of Some Plants Involved in the Treatment of Abdominal Infections in the Department of Kouto (Côte d'Ivoire). *Scholars Journal of Applied Medical Sciences*, **1**, 56-61.

[12] Korpe, D.A., Isère, D., Sahin, F.I., Cabi, E. and Habiral, M. (2012) High-Antibacterial Activity of Urtica spp. Seed Extracts on Food and Plant Pathogenic Bacteria. *International Journal of Food Sciences and Nutrition*, **64**, 355-362.

[13] Ouattara, L.H., Kabran, M.G.R., Kadja, A.B., Tano, B.M., Mamyrbekova-Bekro, J.A. and Békro, Y.A. (2016) Etude phytochmique et activite anti-oxydante d'extraits de plantes de Côte d'Ivoire utilisées dans le traitement traditionnel des hemorroïdes. *Internationnal Journal of Innovation and Applied Studies*, **15**, 881-893.

[14] Ladyguina, E.Y., Safronich, L.N., Otriacheva, V.E., Balandina, I.A., Grinkevich, N.I., Sorokia, A.A., Glizin, V.I., Molodojnikova, L.M., Mitin, Y.S., Samilina, I.A. and Ermakova, V.A. (1983) Khimicheskii analiz lekarstvenii rastenii, Moskva vischaya chkola.

[15] Dohou, N., Yamni, K., Tahrouch, S., Idrissi, H.L.M., Badoc, A. and Gmira, N. (2003) Screening Phytochimique d'une endémique ibéro-marocaine *Thymelaea*

lithroïdes. *Bulletin de la Societé de Pharmacie de Bordeaux*, **142**, 61-78.

[16] Békro, Y.A., Janat, A.M.B., Boua, B.B., Fézan, H., Tra, B. and Ehouan, E.E. (2007) Etude ethnoboatnique et screening phytochimique de *Caesalpinia benthamiana* (Baill) Herend et Zarucchi (Caesalpiniacaeae). *Science et Nature*, **4**, 217-225. https://doi.org/10.4314/scinat.v4i2.42146

[17] Kabran, G.R.M., Ambeu, N.C., Mamyrbékova, J.A. and Békro, Y.A. (2011) CCM d'extraits sélectifs de 10 plantes utilisées dans le traitement traditionnel du cancer du sein en Côte d'Ivoire. *European Journal of Scientific Research*, **63**, 592-603.

[18] Kone, M., Toure, A., Bolou, G.E.-K., Ouattara, A., Ouattara, K. and Coulibaly, A. (2019) Antibacterial Activity of 04 Medicinal Plant on the *in Vitro* Growth of Multi-Resistant Strains Involved in Diarrhea in the Department of Kouto (Ivory Coast). *European Scientific Journal*, **15**, 41-49. https://doi.org/10.19044/esj.2019.v15n30p41

[19] Ponce, A.G., Fritz, R., Del, V. and Rouras, I. (2003) Antimicrobial Activity of Essential Oils on the Native Microflora of Organic Swiss Chard. *LWT—Food Science and Technology*, **36**, 679-684. https://doi.org/10.1016/S0023-6438(03)00088-4

[20] Mogue, I., Bayaga, H., Tembe, E.F., Njinkio, N.B., Tamanji, V., Tabi, Y.O., Ngono, M.R., Gonsu, K.H., Mbacham, F.W. and Fokunang, N.C. (2021) Étude Phytochimique et Évaluation de l'Activité Anti Salmonella Typhi des Extraits de Combretum Micranthum (Combrétaceae). *The Journal of Medicine and Biomedical Sciences*, **22**, 1-6.

[21] Adama, D., Koffi, K., Haidara, M., Togola, A., Sanogo, R., Essien, K., Aklikokou, K., A., Diallo, D. and Gbeassor, M. (2016) Activité analgésique de quatre plantes utilisées dans la prise en charge traditionnelle du paludisme au Mali et au Togo. *International Journal of Biological and Chemical Sciences*, **10**, 1342-1349. https://doi.org/10.4314/ijbcs.v10i3.34

[22] Adiza, A. (2007) Etude d'une recette traditionnelle, des écorces de tronc de *Sclerocarya birrea* Hosch et d'Uapaca togoensis Pax utilisées dans le traitement du diabète. Thèse de doctorat en Pharmacie, Université des sciences techniques et des technologies de bamako, Mali.

[23] Coulidiati, K.V.N., Ouedraogo, L., Belemnaba, M., Nitiema, M., Gambo, A.G.G., Ouedraogo, K.T., Traore, W.L.M., Kabré, R., Sonde/Boly, S., Ilboudo, M., Lompo, S., Ouedraogo and Guissou, I.P. (2019) Effet gastroprotecteur et potentiel mécanisme d'action antiulcéreuse d'extraits de fruits de *Acacia nilotica var adansonii* (Guill. et Perr.) O. Ktze (Mimosaceae). *Sciences et Technique*, **42**, 121-135.

[24] Bagré, I., Bahi, C., Ouattara, K., Zirihi, G.N., Djaman, A.J., Coulibaly, A. and N'guessan, J.D. (2011) Étude botanique et exploration de l'activité antifongique de *Morinda morindoides* (Baker) Milne-Redh. sur la croissance *in Vitro* de *Cryptococcus neoformans*. *Phytothérapie*, **9**, 136-141. https://doi.org/10.1007/s10298-011-0612-y

[25] Moroh, J.L.A., Bahi, C., Djè, K., Loukou, Y.G. and Guédé-Guina, F. (2008) Étude de l'activité antibactérienne de l'extrait acétatique (EAC) de *Morinda morindoides* (Baker) milne-redheat (Rubiaceae) sur la croissance *In-Vitro* des souches d'*Escherichia coli*. *Bulletin de la Société Royale des Sciences de Liège*, **77**, 44-61.

[26] Traore, L., Yves-Alain, B., Jean-Luc, P., Mamyrbeva-Bekro, J.A. (2015) Study of Crude Extracts from *Cassia sieberiana* Root Bark and *Khaya grandifoliola* Trunk Bark. Phytochemical Screening Quantitative Analysis and Radical Scavenging Activity. *International Journal of Current Pharmaceutical Research*, **7**, 22-26.

[27] Halilu, A.M., Obtober, M.E., Balogun, M. and Namrita, L. (2013) Studies of *in Vitro* Antioxidant and Cytotoxic Activities of Extracts and Isolated Compounds from *Parinari curatellifolia* (Chrysobalanaceae). *Journal National Sciences Resource*, **3**, 149-154.