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)

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Abstract

This work aims at evaluating *in vitro* the antibacterial effects of aqueous and hydroethanolic extracts of leaf macerates, *Manilkara multinervis*, *Waltheria indica* root bark, *Securrinega virosa*, and *Anogeissus leiocarpa* stem bark. These four medicinal plants are traditionally used to treat diarrhea in the canton of North-Niéné (Department of Kouto, Côte d'Ivoire). The antibacterial activities of the various extracts from these plants were carried out on multi-resistant strains (*Escherichia coli* BLSE, *Shigella flexneri* BLSE, *Staphylococcus aureus* meti-R). The methodology consisted of extracting the drugs with a 70% hydroalcoholic solvent and distilled water. Agar diffusion and dilution methods were used for susceptibility testing and determination of CMI and CMB parameters. Agar diffusion and dilution methods were used. By the diffusion method, all four plants were found to be active on at least one of the bacteria tested. The ethanolic extract of *M. Multinervis* was the most active by inducing a diameter of 15 mm on the growth of *S. aureus* meti-R. As for the dilution method, the ethanolic extracts of *W. indica* and *M. Multinervis* showed bactericidal effects on both *S. aureus* at 6.25 mg/mL and 3.125 mg/mL

respectively as well as on all other 100 mg/mL germs. Only *M. Multinervis* recorded the highest activity. This important activity was demonstrated on *S. aureus* meti-R with a minimum bactericidal concentration of 3.125 mg/mL. These results confirm the traditional use of these plants in the management of diarrheal diseases in the Department of Kouto.

Keywords: Medicinal plants, Diarrheal diseases, Antibacterial activity, Department of Kouto

1-Introduction

Diarrheal diseases are nowadays an important cause of morbidity and mortality in the human species. They are much more prevalent in developing countries, Africa and especially south of the Sahara (Adjuik et al. 2006). In Côte d'Ivoire, the prevalence is 19% in rural areas compared to 15% in urban areas (Brama et al. 2014). Diarrheal diseases are reported to be the second leading cause of morbidity after malaria in children under five in Côte d'Ivoire (Adjuik et al. 2006). This high rate in Africa and particularly in Côte d'Ivoire is certainly linked to several factors, including non-compliance with good hygiene practices, poor food security conditions and inaccessibility to drinking water (Attia et al. 2013). Several infectious agents are responsible for diarrhea. Among these agents, the emergence of conventional antibiotic-resistant agents is reported to be the basis for several treatment failures (Nascimento et al. 2000). In order to eradicate this scourge, many researchers have shown the importance of bioactive compounds isolated from plants and their antimicrobial activities without major side effects in humans (Rocio et al. 2007). The results of this research therefore suggest the introduction of herbal medicines. With this in mind, the study focused on *Manilkara multinervis*, *Waltheria indica*, *Securrinega virosa* and *Anogeissus leiocarpa*. Indeed, these four medicinal plants are commonly used by the populations of the canton of North-Niéné (Department of Kouto, Côte d'Ivoire) as supplements to pharmaceutical drugs or sometimes as the only alternative to relieve diarrhea. These plant species of such great importance for the health of populations deserve to be scientifically studied in order to verify their antidiarrheal properties and thus justify their traditional use.

2-Material and Methods

2.1-Biological material

The plant material consisted of leaves (*Manilkara multinervis*), root bark (*Waltheria indica*, *Securrinega virosa*) and trunk bark (*Anogeissus leiocarpa*).

As for the bacterial material, it consisted of 03 multi-resistant bacterial strains, including 02 producers of broad spectrum β-lactamase (*Escherichia*

coli BLSE, *Shigella flexneri* BLSE,) and 01 methicillin-resistant (*Staphylococcus aureus* Meti-R). They were provided by the Laboratory of Bacteriology and Virology of the Pasteur Institute of Côte d'Ivoire.

2.2-Preparation of plant extracts

The well-dried plant samples are reduced to powder. Then, 200 g of fine powder is carefully homogenized in 1L of distilled water or in 70% ethanol using a rotary shaker for 24 hours. The homogenate obtained was first wrung out in a square of cloth, then filtered successively twice on hydrophilic cotton and once on Whatmann n°1 filter paper. The filtrate obtained was then evaporated in the VENTICEL oven at 50°C (Zirihi et al. 2003). Several powders of different colours were obtained.

2.3-Determination of the antibacterial activity of plant extracts

2.3.1-Preparation of bacterial inoculum

The bacterial inoculum was prepared from an isolated 18-hour colony in 10 mL Mueller Hinton broth (MHB) and incubated for 3 to 5 hours at 37°C to obtain a pre-culture. A volume of 0.1 mL was collected and added to 10 mL of MHB twice concentrated. This bacterial suspension is evaluated at about 10^6 cells/mL and constitutes the 10^0 dilution or pure inoculum.

2.3.2-Preparation of the concentration range of plant extracts

The concentration range of the plant extract was prepared in seven test tubes numbered from 1 to 7 by the double dilution method according to a geometric progression of 1/2 reason. In a series of eight hemolysis tubes numbered C_1 to C_8, 1mL of pure inoculum was introduced. Then, 1mL of plant extract was added to the tubes according to the prepared concentration range. This distribution of plant extract was made so that 1mL of 200 mg/mL plant extract was transferred into the C1 tube. Tube C_2 received 1 mL of 100 mg/mL and so on until tube C_7 received 1mL of the 3.125 mg/mL solution. The C_8 tube received instead of the plant extract, 1 ml of sterile MHB which was used as a growth control. As a result of the volume/volume dilution achieved, the concentration in the tubes was reduced by half. These tubes were incubated at 37°C for 24 hours.

2.3.3-Sensitivity test (well method)

The agar diffusion technique was used to study the sensitivity tests. Mueller Hinton medium, poured and dried in a petri dish, was flooded with 3 mL of inoculum. Then, using a sterile die, wells about 6 mm in diameter were drilled into the agar. Each well received 80 µL of the test substance at a concentration of 100 mg/mL. The Petri dishes were incubated at 37°C for 24 hours, after 30 minutes of diffusion at laboratory temperature. The presence

or absence of an inhibition zone was observed and the inhibition diameter was measured. Ciprofloxacin (5 µg) was used as a control.

2.3.4-Antibacterial parameters MIC and MBC

The Minimum Inhibitory Concentration (MIC) was the lowest concentration of the plant extract for which there is no growth visible to the naked eye after 24 hours of incubation. Its determination was made by observing the disorder induced by the growth of germs present in each tube. From the MIC, the lowest concentration that allows only 0.01% of bacteria in suspension at the start in 24 hours to survive corresponds to the MBC. It is determined by spreading on a solid medium of 2 µL of the content of each tube with a concentration greater than or equal to the MIC.

2.3.5-Statistical analyses:

The data were processed using Graph Pad Prism 5.0 software (Microsoft, United States). The statistical analysis of the results was performed using variance analysis (Anova One-Way) followed by the Tukey test for the comparison between the activity of the aqueous and ethanolic extract at 100 mg/mL and that of the antibiotic (5 µg/mL). The value of the averages is accompanied by the standard error on the mean (mean ± SEM). The probability values $P < 0.05$ were considered significant (***).

3-Results and Discussion

3.1-Extraction yields of plant extracts

The extraction yields of the various extracts varied from 3.25 to 19.71 (%). These values are recorded in Table 1. In view of these results, *A. leiocarpa* and *M. multinervis* obtained the highest extraction yields regardless of the solvent used. A comparison of solvent yields indicates that, 70% ethanol produced the highest extraction yield with an average of 9.39% compared to 8.70% observed with distilled water. This indicates that phytoconstituents of the plants studied in this study are more soluble in hydroalcoholic solutions than pure water. Similar results were obtained by Bagré et al. (2007) and Touré et al. (2011) during the extraction of *Morinda morindoides* Baker (Rubiaceae). These results suggest that 70% ethanol is a good solvent for extracting the active ingredients from medicinal plants.

Table 1: Extraction yields of extracts from selected plants.

Plants	**Aqueous extracts**		**Hydroethanolic extracts 70%**	
	Yields (%)	Appearance/Colour	Yields (%)	Appearance/Colour
A. leiocarpa	19.71±0.62	Crystals / Black	11.56±0.20	Powder/Black
M. multinervis	7.03±0.82	Powder/Black	10.32±0.05	Powder/Black
S. virosa	3.25±0.91	Powder/Black	5.91±0.21	Crystals/Black
W. indica	4.82±0.06	Crystals / Black	9.78±0.41	Pellet/Brown

3.2-Determination of antibacterial parameters

The results of the inhibition diameters after the sensitivity tests are presented in Table 2. It appears that each of the four plants has an activity on the growth of at least one of the germs tested. The inhibition diameters varied from 7 to 15 mm. The smallest diameters (7; 8 and 9 mm) were obtained with aqueous extracts of *S. virosa*, *A. leiocarpa* and *W. indica* on *E. coli* BLSE and *S. flexneri* BLSE. Compared to the aqueous extract, ethanolic extracts had diameters greater than 10 mm. However, an extract is considered active when it induces an inhibition zone greater than or equal to 10 mm (Ponce *et al*. 2003). Thus, according to the results obtained and Ponce *et al*. (2003), all ethanol extracts from the plants studied were active on the growth of all bacterial germs tested. This reflects in this study that ethanolic extracts were more active than aqueous extracts. As a result, ethanol 70% would concentrate the active ingredients of plants better than water. Similar inhibition diameters were obtained by Yala *et al*. (2016), in the study of *Eryngium foetidium*. On the other hand, contrary results have been observed by Monogalo *et al*. (2016). Indeed, these authors obtained during the *W. indica* study that the aqueous extract induced a larger inhibition diameter (13 mm) than the ethanolic extract (11mm) on the growth of *E. coli*. The discrepancy between the results of the two studies can be explained by several factors, including the climatic conditions in the study areas (high temperature, high sun exposure, drought and salinity). These factors are thought to stimulate the biosynthesis of secondary metabolites (Falleh *et al*., 2008). In addition to these factors, the nature of the bacterial strain studied could also be added. Overall, the largest diameters (13 and 15 mm) were induced by the aqueous and ethanolic extracts of *M. multinervis* on the growth of *S. aureus* Meti-R. This bacterial germ was the most sensitive to the plant extracts studied. However, the inhibition diameters induced by all the plant extracts tested remain smaller than those of ciprofloxacin, whose inhibition zones are very significantly larger than those of the plant extracts studied.

Table 2: Inhibition diameters of the different studied plant extracts (mm)

Plants	Extracts	*E. coli* BLSE	*S.aureus* Meti-R	*S. flexneri* BLSE
Anogeisus leiocarpa	Aqueous	9±0.14	11±0.10	9±0.10
	Ethanolic	10±0.10	12±0.14	10±0.41
Securrinega virosa	Aqueous	7±0.16	10±0.17	8±0.20
	Ethanolic	10±0.12	11±0.10	10±0.12
Waltheria indica	Aqueous	9±0.51	12±0.17	10±0.14
	Ethanolic	12±0.71	12±0.17	10±0.14

Manilkara multinervis	Aqueous	10±0.11	13±0.71	10±0.17
	Ethanolic	12±0.80	15±0.04	12±0.21
	Cipro(5 µg/mL)	20±0.06***	25±0.16***	19±0.15***

Cipro: Ciprofloxacin (5µg). BLSE: Beta-Lactamase with Extended Spectrum.

The analysis of the results mentioned in Table 3 shows that aqueous extracts induce a low antibacterial power on the in vitro growth of the strains studied compared to 70% ethanol extracts. However, antibacterial parameters (MIC and MBC) vary from one extract to another and from one strain to another. The lowest MIC values (3.125 and 6.25 mg/mL) were obtained with aqueous and ethanolic extracts of *W. indica* and *M. multinervis* on the growth of *S. aureus* Meti-R. These MIC values are generally consistent with those of the inhibition diameters. Extracts that have induced a large inhibition zone have the smallest MIC on the corresponding strains. In contrast, in the case of the aqueous extracts of *S. virosa*, *A. leiocarpa* and *W. indica*, their activities on *E. coli* BLSE and *S. flexneri* BLSE gave the smallest diameters (7; 8 and 9 mm) while their MIC was low (25, 25 and 12.5 mg/mL). This suggests that the aqueous extracts of *S. virosa*, *A. leiocarpa* and *W. indica* do not diffuse well in the agar. It is therefore necessary to couple solid and liquid tests. the results of this study are similar to those obtained by Biyiti *et al*. (2004). These authors, during the research of the antibacterial activity of four Cameroonian medicinal plants, reached the same conclusions as those of this study. In addition, the MBC/MIC activity reports mentioned in Table 3 allow for a better characterization of the effect of each extract on the microorganisms tested according to the method described by Konan *et al*. (2014). For these authors, a substance is said to be bactericidal when the MBC/MIC ratio is ≤ 2, and bacteriostatic when this ratio is > 2, which shows that: the aqueous and ethanolic extracts of *M. multinervis* have a bactericidal effect on all the germs tested. As for the aqueous and ethanolic extracts of *S. virosa* and *A. leiocarpa*, they have bacteriostatic activity on the growth of these same strains. Unlike the extracts of the other 3 plants, the strong activities of *M. multinervis* extracts could be explained by the difference in concentration of the different chemical groups present in these extracts but also by their nature. Indeed, through a triphytochemical study, Koné *et al*. (2013) proved that these plants are composed of flavonoids, tannins, sterols and saponins whose microbicidal properties have already been found by several authors. The difference in the biomolecular composition of the extracts could be the basis for the difference in biological activities observed between the plants studied. In general, the Gram (+) strain recorded a MBC/MIC activity report ≤ 2 while the Gram(-) strains obtained one or more MBC/MIC activity reports ≥ 2. These results

indicate that Gram (+) bacteria are more sensitive to plant extracts than Gram (-) bacteria. The results of the work of this study are in agreement with those of Soundararajan *et al*. (2012). These authors showed that extracts of *Elaeis guineensis* showed better activity on Gram (+) bacteria than on Gram (-) bacteria. Several studies have shown that the difference in activity between bacterial strains could be explained by the difference in the parietal composition of the two types of bacteria. Gram (+) bacteria consisting exclusively of a thick wall of peptidoglycan, seem to be more favourable to the penetration of phytomolecules that would easily reach their intracellular targets. On the other hand, Gram (-) bacteria with their outer membrane composed of phospholipids that would interfere with molecules, especially hydrophobic compounds, make it difficult for them to pass through the wall (Tian *et al*. 2009).

Table 3: Antibacterial parameters of the different studied plant extracts

Plants	Parameters	*E. coli* BLSE		*S. aureus* Meti-R		*S. flexneri* BLSE	
		Aq.E	Eth.E	Aq.E	Eth.E	Aq.E	Eth.E
Anogeisus leiocarpa	MIC	25	12.5	25	12.5	12.5	12.5
	MBC	100	100	100	50	100	50
	MBC/MIC	4	8	4	4	8	4
Securrinega virosa	MIC	25	25	25	25	25	25
	MBC	100	100	100	100	100	100
	MBC/MIC	4	4	4	4	4	4
Waltheria indica	MIC	25	25	6.25	3.125	25	12.5
	MBC	100	100	12.5	6.25	100	50
	MBC/MIC	4	4	2	2	4	4
Manilkara multinervis	MIC	50	25	6.25	3.125	50	25
	MBC	100	50	12.5	3.125	100	50
	MBC/MIC	2	2	2	1	2	2

MIC: Minimum Inhibitory Concentration, **MBC:** Minimum Bactericidal Concentration, **Aq.E:** Aqueous Extract, **Eth.E:** Ethanolic Extract

Conclusion

This work has highlighted the antibacterial properties of various extracts of *Manilkara multinervis*, *Waltheria indica*, *Securrinega virosa* and *Anogeissus leiocarpa* on germs potentially involved in diarrhea in humans. The results obtained would justify the traditional use of the plant in the control of a large number of diarrheal diseases. However, if all the plant extracts in this study showed either a bactericidal or bacteriostatic effect, the best antibacterial activity is the ethanolic extract of *M. multinervis*. Indeed, the extracts of *M. multinervis*, presented bactericidal effects on the growth of all the germs tested. It would therefore be interesting to undertake in-depth

studies on the ethanolic extract of *M. multinervis* in order to isolate the different molecules with antibacterial activity contained in this extract, to study the toxicity and then to elaborate an improved traditional medicine.

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