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Anti-diarrheal activity of *Mangifera indica* L. (Anacardiaceae) leaf extracts, an antimicrobial plant of the Beninese flora

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Abstract : The present study aims to evaluate the anti-diarrhoeal activity of extracts of *Mangifera indica* leaves, a plant used in traditional medicine for its antimicrobial properties. Thus, the extracts were tested on 10 reference strains and 9 clinical strains isolated from diarrhoeal stools by agar diffusion method and determination of the antimicrobial capacity. Phytochemical screening was used to determine the major chemical groups present in the extracts by staining and precipitation reactions. Apart from the two reference strains (*P. mirabilis* and *S. oralis*) and the four clinical strains (*K. pneumoniae, E. coli, P. aeruginosa* and *K. oxytoca*) which developed resistance to the extracts, the extracts generally showed antibacterial activity on both reference and clinical strains and the ethanolic extract seemed to be more effective. The presence of major chemical groups like tannins, flavonoids, anthocyanins, reducing compounds, quinone derivatives found in the extracts of the leaves of the plant could confer the known activity of this plant.
Keywords : *Mangifera indica*, reference strains, clinical strains, phytochemical screening.

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Introduction

At the present time when humanity is facing different kinds of diseases (microbial, parasitic, viral, cardiovascular etc.) and when the management of health issues is proving to be a real societal problem, especially in developing countries, a recourse to available local resources would be a real solution to deal with these scourges (Mangambu *et al*., 2014 ; Toklo, 2021). Thus, several plants are used not only for health care in traditional medicine but also for research of new active molecules following one of the greatest global public health challenges which is only the resistance of pathogens to conventional antimicrobials (Mezouar et al., 2014). While diarrheal diseases cause several million deaths each year worldwide (Field, 2003; Ahomadegbé et al., 2018) and are the leading cause of death in children under 5 years of age in developing countries (Parashar et al., 2003), the present study was undertaken on an antidiarrheal medicinal plant (Ahomadegbe 2021) for its valorization. From the plant kingdom and family Anacardiaceae, *M. indica* is a large tree that can grow to 10-25 meters in height, with a crown diameter of 20 meters. Native to eastern India, it grows in all tropical regions (Akoègninou *et al.*, 2006). Different parts of the plant are used in traditional medicine to treat various diseases such as diarrhea, dysentery, anemia, asthma, bronchitis, cough, hypertension, insomnia, rheumatism, toothache, leucorrhea, hemorrhage, hemorrhoids (Shah *et al.*, 2010; Adjanohoun *et al.*, 1991; Adjanohoun *et al.*, 1989).

The present study aims specifically at evaluating the infectious anti-diarrheal power of two extracts of the plant on reference strains and clinical strains isolated from infectious diarrheal stools.

Materials and Methods

Plant material

The plant material consists of *M. indica* leaves collected in Abomey-Calavi in the Atlantic department in the south of the country from a mango tree located in the yard of a concession behind the UAC. A sample was identified from the specimens at the National Herbarium of the University of Abomey-Calavi. Once harvested, the leaves were dried at room temperature for two weeks from the sun at the laboratory. They were then ground into powder using a grinding machine.

Microbial strains

The extracts were tested on ten (10) reference strains are:

- Gram negative (-) bacteria: *Escherichia coli* ATCC 25922, *Escherichia coli* O157, *Proteus mirabilus* A24974, *Pseudomonas aeruginosa* ATCC 27853;
- Gram positive (+) bacteria: *Enterococcus faecalis* ATCC 29212, *Micrococcus luteus* ATCC 10240, *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* T22695, *Streptococcus oralis* ;
- Yeast: *Candida albicans* MHMR.

They were also tested on nine (9) clinical strains (all gram negative bacteria) isolated from diarrheal stools and stored at the Bacteriology section of the National Laboratory of the Ministry of Public Health (LNMSP). The clinical strains consisted of *Citrobacter freundii, Escherichia coli, Klebsiella oxytoca, Klebsiella pneumoniae, Klebsiella rhinoscleromatis, Pseudomonas aeruginosa, Pseudomonas ogzinabitans, Salmonella choleraesius and Shigella flexneri*.

Preparation of extracts

Aqueous extracts: The aqueous extract was obtained by decoction. Thus, 100 g of the leaf powder was boiled in 1000 mL of distilled water for 30 minutes. The mixture was filtered through Whatman paper and the filtrate was then evaporated under vacuum at 45 °C to obtain the extract with a yield of 8.33%.

Ethanol extracts: 100 g of leaf plant powder was extracted with 1000 mL of ethanol for 72 h. The obtained mixture was filtered through Wathman paper and then evaporated under vacuum at 45 °C to obtain the dry extract with a yield of 9.41%. The extraction was performed three times in succession.

Determination of phytochemical constituents

The method used in this work is that of Houghton *et al*. (1998) used by Toklo *et al.* (2019). It is a qualitative analysis based on staining and/or precipitation reactions performed on the extracts of the leaves of the plant.

Evaluation of antimicrobial activity

Sensitivity test

The *in vitro* antimicrobial activity of the different extracts obtained was demonstrated by the solid-state diffusion method (Anani *et al.*, 2000). The sterile discs of 5mm diameter were placed with the help of a pair of tweezers previously flamed under aseptic conditions, on plates already flooded with bacterial cultures. The discs were aseptically impregnated with 30 µl of the supernatant of the crude plant extract stock solution, which was prepared 24 h prior to the manipulation from 50 mg of crude extract in 1 mL of sterile distilled water. The dishes were then left for 15-30 min at room temperature for pre-diffusion of the substances before being incubated at 37°C in the oven for 24 h. The diameters of any inhibition zones were measured using a graduated ruler (Doughari *et al.*, 2007) after 24 of incubation.

Determination of the Minimum Inhibitory Concentration MIC

It was determined by the liquid macro dilution method described by Delarras (1998) and used by Dah-Nouvlessounon *et al*. (2015) with visual appreciation of the growth of microorganisms. One milliliter of sterile distilled water was introduced into a series of 11 test tubes numbered T1 to T11. One milliliter of stock solution of 50 mg/mL plant extract was added to tube T1 from which successive dilutions (half) were made up to tube T10 to have concentrations of 25 mg/mL, 12.5 mg/mL, 6.25 mg/mL, 3.125 mg/mL, 1.562 mg/mL, 0.781 mg/mL, 0.391 mg/mL, 0.195 mg/mL, and 0.098 mg/mL. All tubes (T1 to T11) inoculated with 1mL of inoculum (10^6 CFU/mL) were incubated at 37°C for 24 h and examined for bacterial growth as indicated by turbidity. Tube T11 is considered as a control in case of a very high activity of the extract. The MIC of an extract against a given strain is the lowest concentration showing no visible growth of the germ to the naked eye.

Determination of the Minimum Bactericidal Concentration MBC

It was determined by plating all tubes starting from the MIC against the highest concentrations on MH agar medium and incubated at 37°C for 24 h (Sina *et al.*, 2021). Upon observation, the lowest concentration of the extract that does not allow the bacteria to survive (no growth) corresponds to the Minimum Bactericidal Concentration (MBC) (Doughari *et al.*, 2007)

Determination of Antimicrobial Potency (AP)

It was determined by the ratio between the Minimum Bactericidal Concentration and the Minimum Inhibitory Concentration (MBC/MIC). Its value highlights the bactericidal effect of the extract if it is less than or equal to 4 ($PA \leq 4$) and then the bacteriostatic effect if it is greater than 4 ($PA > 4$) (Okou *et al.*, 2018).

Statistical analysis

All experiments were performed in triplicate and the resulting data were reported as mean ± Standard Error with Excel spreadsheet. These data were analyzed using GraphPad Prism.5 software. Differences were considered significant with $P < 0.05$.

Results

Phytochemical screening

The phytochemical screening allowed the identification of large chemical groups in the plant extracts and the results obtained are recorded in table 1 below.

Table 1: Results of phytochemical screening of aqueous and ethanolic extracts of *M. indica* leaves

Metabolites	*M. Indica*	
	Aqueous extract	Ethanolic extract
Alkaloids	+	+
Tannins	+	+
Flavonoids	+	+
Anthocyanins	+	+
Leucoanthocyanins	-	+
Quinone derivatives	+	+
Saponosides	+	-
Triterpenoids	-	-
Steroids	-	-
Cardenosides	-	-
Cyanogenic derivatives	-	-
Mucilage	+	+
Coumarins	+	-
Reducing compounds	+	+
Free Anthracene	-	-
O-heterosides with reduced genuins	+	-
C-Heterosities	+	-

Legends: - = absent; + = présent

Evaluation of antimicrobial activities of extracts inhibition diameters of extract-sensitive strains

In 24 hours of incubation, the diameters of the inhibition zones of the strains vary according to the bacteria and the type of extract. Figures 1 and 2 show that the sensitivity of the reference strains varies according to the type of extract. The ethanolic extract seems to be more effective than the aqueous extract. We note at the level of the reference strains, more sensitive bacteria and resistant bacteria. *S. epidermidis* is sensitive to both extracts with an inhibition diameter varying between 19 and 20 mm respectively for the aqueous and ethanolic extracts. While *P. mirabilis* and *S. oralis* seem to be the most resistant strains to our extracts. For the strains isolated from diarrheal stools (Figure 2), the ethanolic extract was able to inhibit the growth of 5 different strains out of the 9 while the decocted extract was able to inhibit the growth of two strains. The highest inhibition diameter (23.67 ± 0.66 mm) was obtained with the ethanolic extract on *P. ogzynabitans* and the lowest inhibition diameter (11.00 ± 0.57 mm) was obtained with the aqueous extract on *K. rhinoscleromatis.*

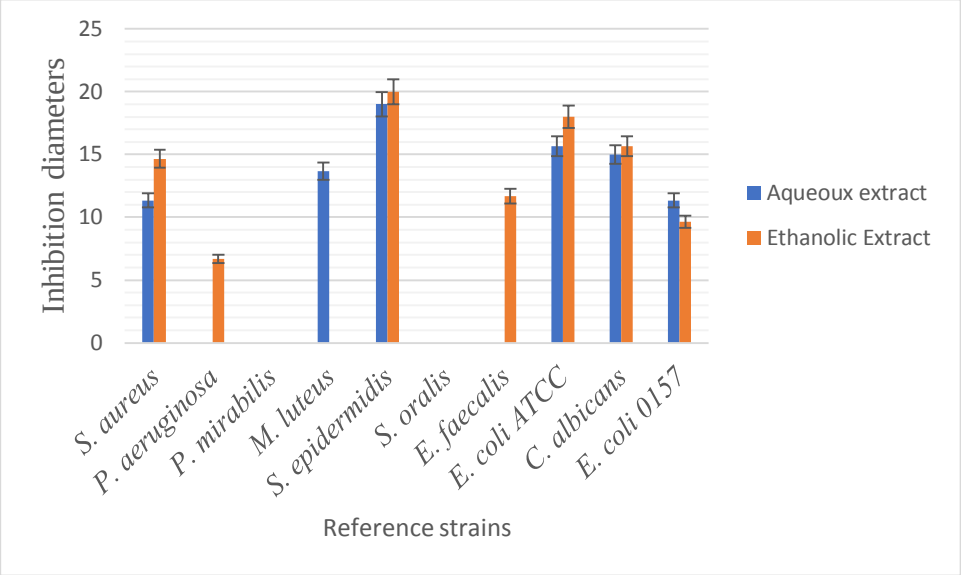

Figure 1: Inhibition diameter of extracts of *M. indica* on reference strains

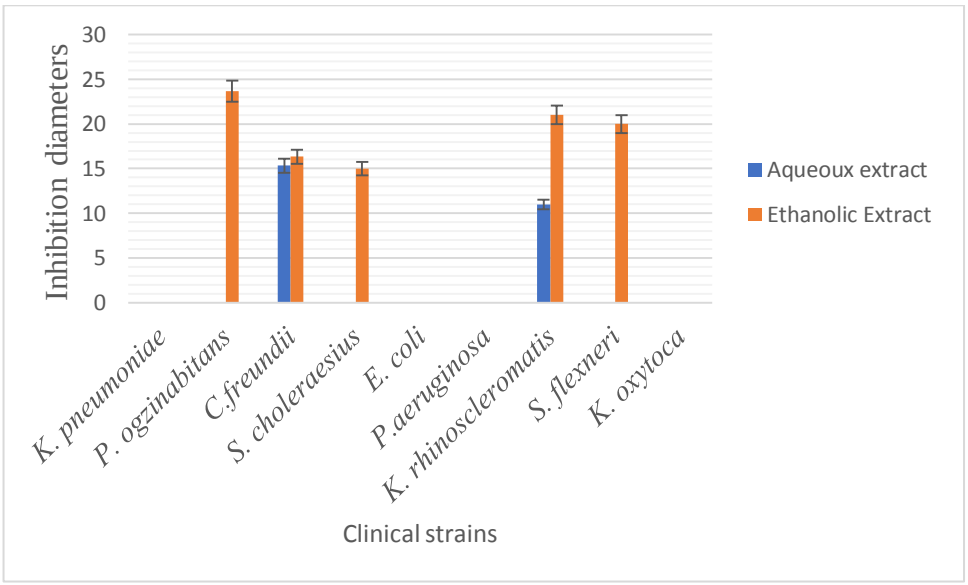

Figure 2: Inhibition diameter of extracts of *M. indica* on clinical strains

Minimum Inhibitory Concentrations (MIC) and Maximum Bactericidal Concentrations (MBC)

The MICs and BMCs of aqueous and ethanolic extracts of *M. indica* leaves, on reference strains and strains isolated from diarrhoeal stools are shown in Table 2 below.

The minimum inhibitory concentration of the aqueous extract varies between 1.563 and 3.125 mg/mL for reference strains and between 0.391 and 6.25 mg/mL for clinical strains. With the ethanolic extract, we have a MIC varying between 0.391 and 3.125 mg/mL for both reference and clinical strains.

Bactericidal and bacteriostatic effect of extracts on susceptible strains

Table 2 shows the different effects of *M. indica* leaf extracts on reference and clinical strains. It appears that with the reference strains, the aqueous extract of *M. indica* showed a bactericidal effect on *S. aureus, M. luteus, S. epidermidis* while the ethanolic extract of *M. indica* exerted a bactericidal effect on *S. epidermidis* and *E. coli ATCC 25922* and a bacteriostatic effect on *S. aureus, E. faecalis*, and *C. albicans*. Similarly, with strains

isolated from diarrheal stools, the ethanolic extract showed a bactericidal effect on *P. ogzinabitans* and a bacteriostatic effect on *S. choleraesius* and *S. flexneri*.

Table 2: MIC in mg/mL of aqueous and ethanolic extracts of *M. indica*, acclimatized in Benin on reference strains and strains isolated from diarrheic stools

	Souches	*MIC*		*MBC*		*MBC/MIC*	
		aqueous	**ethanolic**	**aqueous**	**ethanolic**	**aqueous**	**ethanolic**
Reference strains	*S. aureus* ATCC 29213	3.125	0.391	3.125	3.125	1	8
	P. aeruginosa ATCC 27853	-	1.563	-	> 50	ND	ND
	P. mirabilis A24974	-	-	-	-	ND	ND
	M. luteus ATCC 10240	1.563	-	3.125	-	2	ND
	S. epidermidis T22695	1.563	3.125	3.125	6.25	2	2
	S. oralis	-	-	-	-	ND	ND
	E. faecalis ATCC 29212	-	3.125	-	25	ND	8
	E. Coli ATCC 25922	1.563	3.125	> 50	12.5	ND	4
	C. albicans MHMR	3.125	3.125	> 50	25	ND	8
	E. Coli 0157 : H7ATCC	1.563	0.781	> 50	> 50	ND	ND
Clinical strains	*Klebsiella pneumoniae*	-	-	-	-	ND	ND
	Pseudomonas ogzinabitans	-	1.563	-	6.25	ND	4
	Citrobacter freundii	0.391	0.781	> 50	> 50	ND	ND
	Salmonella choleraesius	-	0.391	-	50	ND	128
	Escherichia coli	-	-	-	-	ND	ND
	Pseudomonas aeruginosa	-	-	-	-	ND	ND
	Klebsiella rhinoscleromatis	6.25	3.125	> 50	> 50	ND	ND
	Shigella flexneri	-	1.563	-	12.5	ND	8
	Klebsiella oxytoca	-	-	-	-	ND	ND

Legend: - = inactive; ND = Not Determine.

Discussion

The present study was undertaken to evaluate the anti-diarrheal activity of extracts of leaves of *M. indica*, a medicinal plant of the Benin flora. Thus, the chemical composition and the evaluation on different reference and clinical strains were evaluated with the decocted and ethanolic extracts. It appears that the ethanolic extract seems to present a better yield than the aqueous extract which can be partly explained by the difference in polarity of the solvents used. Qualitative phytochemical screening of both extracts revealed the presence of different classes of compounds such as alkaloids, tannins, flavonoids, anthocyanins, quinone derivatives, mucilages, reducing compounds (Table 1) with a difference in a few chemical families present in either extract. These results corroborate those obtained by Divyalashmi *et al*. (2017); Sneha *et al*. (2016) and then Kaur *et al*. (2015) who assessed the phytochemical screening of different organs and extracts of *M. indica* differently. On the other hand in the leaf extracts collected from Nigeria, the absence of tannins was noticed by Yakubu *et al*. (2015). This difference in chemical composition could be explained by the phenomenon of chemotype (different geographical origins of the plants studied: Benin and Nigeria) or by the extraction method used. Antibacterial activity was carried out with the two extracts of *M. indica* leaves on ten (10) reference strains and nine (9) clinical strains isolated from diarrheal stools. The results indicate that with the reference strains, the extracts not only inhibit the growth of gram positive strains (*S. aureus* ATCC 29213, *S. epidermidis* T22695, *M. luteus* ATCC 10240, *E. faecalis* ATCC 29212) but also gram-negative strains (*E. Coli* ATCC 25922, *E. Coli* 0157 : H7ATCC, *P. aeruginosa* ATCC 27853) plus a yeast (*C. albicans* MHMR). The best activities were obtained on *S. epidermidis* (20 mm), *E. coli* ATCC (18 mm) and *C. albicans* (15.66 mm) with the ethanolic

extract which seems to have a better activity than the decocted extract. Furthermore, the reference strains developed more resistance to the aqueous extracts than to the ethanolic extracts. This can be partly explained, subject to isolation and structural identification in the extracts, by the fact that the content of the active principle would be higher in the ethanolic extract.

Similarly, antibacterial activity was carried out on nine (9) clinical strains isolated from diarrheal stools. Only the growth of two strains including *C. freundii* (15.33 mm) and *K. rhinoscleromatis* (11 mm) could be inhibited with the aqueous extract of *M. indica*. While the ethanol extract of the plant showed the best inhibitions on five clinical strains only of gram negative. These were *P. ogzinabitans* (23.66 mm), *C. freundii* (16.33 mm), *S. choleraesius* (15 mm), *K. rhinoscleromatis* (21 mm) and *S. flexneri* (20 mm). The ethanol extract showed bactericidal effect on *P. ogzinabitans* and bacteriostatic effect on *S. choleraesius* and *S. flexneri*. These results are superior to those Mustapha *et al.* (2014) and Sneha *et al*. (2016) who worked in Nigeria and India respectively and showed that the extracts showed no inhibitory effect on *S. aureus* and other strains tested. This could be explained by the fact that they tested the extracts at a lower concentration (30 mg/mL) than ours (50 mg/mL). Similarly, Doughari *et al*. (2008) showed an absence of activity in aqueous extracts at concentrations ranging from 50 to 250 mg/mL. This result obtained by these authors could be explained by the absence of tannins and flavonoids, two different secondary metabolites suspected to be responsible for anti-diarrheal properties. Our results also corroborate those obtained by Omotayo *et al.* (2022), who showed that methanolic extracts of *M. indica* leaves and bark inhibited the growth of bacterial strains (*Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae, Salmonella spp, Escherichia coli, Proteus mirabilis*) isolated from wastewater samples. On the other hand our results are inferior to those obtained by Alo *et al*. (2012) who obtained with the ethanolic extract of *M. indica* at a low concentration of 0.8 mg/mL a good inhibition on clinical isolates of *Salmonella typhi*.

The results of the present study on different reference and clinical strains as well as those obtained in the literature can partially confirm the antimicrobial effect of this plant and the interest given to its use in traditional medicine.

Conclusion

The present study has demonstrated the anti-diarrheal activity of *M. indica* leaf extracts. The ethanolic extract seems to show a more interesting activity than the decocted extract and the secondary metabolites present in these extracts could justify their efficacy on reference and clinical microbial strains. However, further studies are necessary for the identification and isolation of the active principle in the process of valorization of this plant.

Conflict of Interest

Authors declare no conflict of interest.

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