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IN VITRO ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF AQUEOUS EXTRACT FROM *MORINDA MORINDOIDES* (BAKER) MILNE-REDHEAD (RUBIACEAE) LEAVES USED AS ANTIDIARRHOEAL REMEDY IN TRADITIONAL MEDICINE IN DEMOCRATIC REPUBLIC OF CONGO AND ITS ISOLATED CONSTITUENTS

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ABSTRACT
The study reports the antibacterial and antifungal activities in vitro of aqueous extract (decoction) from Morinda morindoides leaves and of its soluble fractions, and isolated flavonoids and iridoids. Results revealed that aqueous extract and its soluble fractions were devoid with antibacterial activity against all selected standard ATCC bacteria strains with minimal inhibitory (MIC) and bactericidal concentrations (MBC) > 500 µg/ml). Against clinical isolates mainly implicated in diarrhea, aqueous extract and its some fractions exhibited good antibacterial and bactericidal activities with MIC and MBC values < 100 µg/ml against a large game of selected bacteria. In some cases, they exhibited moderate or weak activity with MIC and MBC values of 125, 250 or 500 µg/ml according to the case. Among flavonoids, chrysoeriol exhibited antibacterial activity against Bacillus cereus ATCC 4579, Candida albicans ATCC 102301, Escherichia coli ATCC 8739, Mycobacterium fortuitum ATCC 6841, Streptococcus pyogenes ATCC and Staphylococcus aureus ATCC 6538 with MIC and MBC ranging from 62.5 to 500 µg/ml. Quercetin, luteolin showed moderate or weak activity against Bacillus cereus ATCC 4579, Streptococcus pyogenes ATCC 12344, E. colis ATCC 8739 and Pseudomonas aeruginosa ATCC 154452 respectively (MIC and MBC = 250 and 500 µg/ml) while luteolin-7-O-glucoside exhibited moderate activity against Bacillus cereus ATCC4579, C. albicans ATCC 102301, Klebsiella pneumonia ATCC 883 and S. pyogenes ATCC12344 (MIC and MB = 125 or 250 µg/ml), low activity against Proteus vulgaris ATCC1335 and P. aeruginosa ATCC 15445 (MIC and MBC = 500 µg/ml). Iridoids exhibited antibacterial activity with MIC ranging from 7.81 to 250 µg/ml and bactericidal activity with MBC from 15.63 to 500 µg/ml against all selected standard ATCC bacteria. Against clinical isolates, flavonoids chrysoeriol, luteolin, luteolin-7-O-glucoside, kaempferol, kaempferol-3-O-glucoside and quercetin, and iridoids gaertneric acid, acetylgaertneroside, dihydroxygaertneroside, dihydroxymethoxygaertneroside, gartneroside, epoxygaertneroside, epoxymethoxygartneroside, and methoxygaertneroside showed good antibacterial and bactericidal activity (MIC and MBC < 100 µg/ml) or moderate and weak (MIC and MBC = 125, 250 or 500 µg/ml) activity or were inactive (MIC and MBC > 500 µg/ml). All tested samples were devoid with cytotoxic effect against Vero cell lines (CC50 > 100 µg/ml). These results partly indicated that aqueous extract of M morindoides can act as antidiarrhoeal remedy partly by its antibacterial property and this activity is due to the presence of flavonoids and iridoids. Aqueous extract showed good antifungal activity by diffusion method while all isolated constituents were devoid with this biological activity.

KEYWORDS: *Morinda morindoides*, Rubiaceae, leaf, flavonoids, iridoids, antibacterial activity, cytotoxicity.

INTRODUCTION
Morinda morindoides (Baker) Milne-Redh. (Rubiaceae) (Synonym: *Gaertnera morindoides* Bak. or *Morinda confusa* Hutch.) commonly called in vernacular languages as Nkonga bululu in Tshiluba, Nkongo bololo or Nkama meso (literal traduction: plant to or with hundred eyes) in Lingala and Kikongo in Democratic Republic of Congo, is one of the most popular medicinal plants used in villages and towns in this country in traditional medicine to treat various illnesses. An

aqueous decoction of fresh leaves, which is the typical traditional remedy is known by all people and does not require the presence of a tradipractioner for its preparation. It is used for the treatment of diabetes, diarrhea, intestinal worms, rheumatism, amoebiasis, malaria and fever, infectious wounds, cutaneous eruptions, abdominal pains, constipation, hemorrhoids, gastralgia, icteria, rheumatism, blennorragia and dermatological diseases such as mycosis and scabies. It is also employed as oral tonic, stimulant of appetite and against general tiredness in children and adults.[1-4]

Previous investigations on this medicinal plant part have reported some interesting biological activities related to its some traditional uses. These included the *in vitro* anticomplementary,[5-7] the *in vitro* an *vivo* antimalarial,[8-10] antioxidative,[11] cardioinhibitory [12] and immunologic [13] activities.

On the other hand, diarrhea is the passage of liquid or watery stools three or more times per day. It involves an increase in the fluidity, volume and frequency of bowel movements resulting in loss of electrolytes and water. In developing countries, diarrhea is a major problem which reaches morbidity and mortality for malnourished children and is one of the popular diseases causing death of children under five years old. In the world, it is estimated 5-8 million deaths every year [15, 16]. Although it is well known that diarrhea can have an infectious or no infectious origin, in traditional medicine, the cause of the disease is often unknown because of the lack of a specific and precise diagnosis.[17] In addition, some complex factors related to the disease include the living of people in poor sanitation areas and socio-economic status, poor life style environmental conditions and the non-availability of guaranteed conventional medical treatment, largely contribute to the contagion of the disease.[18,19]

Acute diarrhea is mainly caused by some pathogen microorganisms such as *Shigella* sp., *Escherichia coli*, *Staphylococcus aureus*, *Vibrio cholerae*, *Salmonella thyphimurium*, *Bacillus aureus*, *Giardia lambia* and the parasite *Entamoeba histolytica* causing amoebiasis.[15,20,21] These agents cause the influx of water and electrolytes to the lumen and increase the intestinal motility, thereby using water stool and at the end cause diarrhea.[22] It is acquired through the fecal-oral route and by the injection of contaminated water and food with these pathogen microorganisms. In this case, the use of antibiotic therapy alone for the microbial pathogens, usually enterotoxigenic *E. coli*, less often *Shigella*, *Salmonella*, *Giardia* species or *Campylobacter jejeuini* is effective. Nospecific antidiarrheal agents typically do not address the underlying pathophysiology responsible for the diarrhea. Many other antidiarrheal agents act by decreasing intestinal motility and should be avoided for treatment as far as possible in acute diarrheal illness caused by invasive pathogen microorganisms.[23] Diphenoxylate, Atropine and Loperamide are current medicines used to treat diarrhea, but they cause some side effects such as vomit, nausea, intestine obstruction and constipation. In some cases, these agents may mask the clinical picture, delay clearance of microorganisms and increase the risk of systemic invasion by the infectious microorganisms as well as local complications such as toxic megalocon.[23] Although WHO (World Health Organization) recommended formulas for oral rehydratation solutions as ideal, but, there has been great interest in the use of herbal remedies for the treatment of the disease with no or minor side effects.[14]

For this, taking account of the frequent use of some medicinal plant species in traditional medicine for the treatment of diarrhea, scientific investigations in different pharmacological models are nowadays performed to prove their effectiveness to cure diarrhea and some of them were reported to possess interesting antidiarrheal properties *in vitro* and/or *in vivo* tests at different extents.[15,24-29] This activity is also proved by assessment of antibacterial activity of the studied medicinal plant extracts[30-34] tacking account of the infectious origin of the disease.

Thus, the present study was undertaken to evaluate the *in vitro* antibacterial and antifungal activities of aqueous extract from *M. morindoides* leaves and its soluble fractions, and isolated chemical constituents including flavonoids and iridoids, biological activity which can partly explain its claimed antidiarrheal properties by traditional healers. The cytotoxic effect of all samples from *M. morindoides* leaves against Vero cells is also described.

2. MATERIALS AND METHODS

2.1. Plant material

Fresh leaves of *Morinda morindoides* (Baker) Milne-Readh. (Rubiaceae) were collected in Kinshasa, Democratic Republic of Congo (DR-Congo) and the plant was identified in Institut d'Etudes et de Recherches en Agronomie (INERA), Department of Biology, Faculty of Sciences, University of Kinshasa in October 1990. A voucher specimen (MN 04122004MMSL) of the plant had been deposited in the herbarium of this institute. For the present study, a new batch of plant material was collected in the same place in April 2017. Fresh leaves were used in this study since this state of plant material is that used by traditional healers to prepare their remedies according to their daily practices.

Figure 1: *Morinda morindoides* (Baker) Milne-Redh. (Rubiaceae) leaves, fruits and flowers.

2.2. Reagents

Methanol (purity 99.99%), ethylacetate (purity 99.96%) and *n*-butanol (purity 99% extra pure) were purchased from Across Organic (USA) and chloroform (purity 99.99%) was obtained from Fisher Scientific (UK). All solvents were with HPLC grade. Distilled water was used.

2.3. Preparation of extracts, fractions, isolation of flavonoids and iridoids.

20 g of fresh leaves were mixed with 150 ml distilled water and boiled for 30 min at 100°C on a hotplate. The mixture was cooled and filtered. The filtrate was evaporated in *vacuum* to give dried extract denoted as extract AE (12.32g, 61.60%). An amount of extract AE (10 g) was dissolved in 150 ml distilled water, filtered and successively and exhaustively extracted with chloroform, ethylacetate and *n*-butanol. Each fraction was evaporated in *vacuum* yielding corresponding dried residues denoted as extracts AE-1 (1.58, 15.80%), AE-2 (2.16g, 21.60%) and AE-3 (2.46g, 24. 60%) respectively. The residual aqueous phase was also treated as described above yielding a dried residue denoted as extract AE-4 (2.76g, 27.6%). By using 50 g of plant material, this amount was macerated and exhaustively percolated with 80% methanol leading to a dried extract after evaporation in *vacuum* and denoted as extract ME (23.63g). This extract was also fractionated as described for aqueous extract AE. Flavonoids and iridoids were isolated from 80% methanol extract using 1000 mg of plant material by different chromatographic technics and identified by different conventional spectroscopic methods as previously described by.[6,7,40]

2.4. Phytochemical screening

The phytochemical screening of the aqueous extract and its fractions, 80% methanol extract was carried out by TLC on precoated silica gel $60F_{254}$ plates (thickness layer 0.25 mm, Merck, Germany) using different mobile phases and reagents described in the literature for the detecting major phytochemical groups such as alkaloids, phenolic compounds (flavonoids, and anthraquinones), coumarins, steroids and terpenes. The froth test, HCl 0.2N and isoamylic alcohol aftr heating and extraction of the red color with organic solvent, Stiasny's reagent (formol and HCl conc.) were used to identify saponins, anthocyanins, catechic and gallic tannins respectively,[35,36]

2.5. Biological evaluation

2.5.1 Antibacterial testing

The following standard bacteria were used: *Bacillus cereus* ATCC 4579, *Candida albicans* ATCC 102301, *Escherichia coli* ATCC 8739, *Enterobacter cloacae* ATCC 13047, *Klebsiella pneumonia* ATCC 883, *Mycobacterium fortuitum* ATCC 6841, *Streptococccus pyogenes* ATCC 12344, *Proteus vulgaris* ATCC 1335, *Pseudomonas aeruginosa* ATCC 154452, *Staphylococcus aureus* ATCC 6538, fungi *Candida albicans* ATCC 10231, *Aspergillus niger* ATCC 6275 and *A. fumigatus* 1022 from the laboratory of Microbiology of Professor Vanden Berghe, Department of Pharmaceutical Sciences, University of Antwerp, Antwerpen, Belgium. On the other hand, clinical isolates from Cliniques Universitaires du Mont-Amba, Kinshasa, Democratic Republic of Congo, including *Bacillus cereus*, *Escherichia coli*, *Proteus vulgaris Shigella dysenteria*, *Shigella flexneri*, *Salmonella typhimurium*, *Staphylococcus aureus* mainly implicated in diarrhea were also used for the antibacterial testing *in vitro*, and the fungi *C. albicans*, *Aspergillus niger* and *A. fumigatus*.

For the evaluation of antibacterial activity, 2 mg of aqueous extract and its fractions, 80% methanol extract, flavonoids and iridoids were dissolved in 2 ml DMSO to have corresponding stock solutions of 1 mg/ml. These last were diluted in two fold dilutions with TSB (trypcase soy broth) to have a series of test concentrations from 500 to 0.1µg/ml. An inoculum consisting of about each 10^6 microorganism/ml TSB was incubated overnight at 37°C for 24 h. A 1/1000 dilution of this suspension was prepared with the same medium, except the suspension of *M. fortuitum* ATCC 6841 of which the dilution was 1/100. 100 µl of each bacterial dilution was brought into the holes o microtiter plates. Thereafter, 100 µl of each test sample (extracts, fractions and isolated constituents) in decreasing concentrations were added. Each vertical column contain one microorganism dilution. The last column contained 10 µl of TSB and bacteria without test samples as the negative control for normal growth of bacteria. Two other microtiter plates contained Ampicillin and Tetracycline respectively with bacteria were used as positive controls. All microtiter plates were incubated at 37°C in humidified atmosphere for 24 h. The inhibition of bacteria growth was evaluated by comparing with normal bacteria growth in the control holes prepared without test samples. The minimum inhibitory concentration (MIC) was determined as the lowest concentration of the sample that completely inhibited macroscopic growth of bacteria. To determine the minimum bactericidal concentration (MBC), the two

lowest concentrations which inhibited bacteria growth were plated out on a nutrient agar and incubated at 37°C for 24 h. The minimum bactericidal concentration (MBC) is the lowest concentration of an antibacterial agent required to kill a particular bacterium.

The test for fungi was similar to that for bacteria, except for the used medium. A suspension of fungi was prepared from a tube-culture of 3 weeks old in Sabouraud agar and the germs were suspended in 5 ml of sterile distilled water. The suspension was used for inoculation of the holes of microtiter plates (one column, one fungus). The plates were incubated in the dark at room temperature for 7 days, and the fungal inhibition growth was compared to control holes prepared in the same way without test samples. Minimum inhibitory antifungal (MIA) and fungicidal (MFC) concentrations were determined.[37,38]

2.5.2. Cytotoxic evaluation against Vero cells

The procedure used was previously described by.[6] during the evaluation of antiviral activity using different virus growing on Vero cells in microplates with 96 holes.

2.6. Statistical analysis

Results are presented as mean ± standard error of mean (S.E.M). Statistical analysis was carried out using one way analysis variance (ANOVA) followed by Turkey's multiple comparison tests where p value ≤ 0.05 was considered as statistically significant using Graph Pad Parism version 5.03 software.

3. RESULTS AND DISCUSSION

3.1. Qualitative phytochemical screening

Results from photochemical screening of aqueous extract of *M. morindoides* leaves revealed the presence of flavonoids, coumarins, saponins, anthraquinones, terpenes, steroids, proanthocyanins, catechic and gallic tannins. These phytochemical groups were located in fractions according to their solubility. Anthocyanins and cardiotonic glycosides were not detected in our experimental conditions. The presence of alkaloids was doubtful although a positive test with Dragendorff's reagent was obtained and seems to be a false positive test since other compounds such as iridoids isolated from the plant part of this medicinal plant also react with this reagent.[7] In addition, to our knowledge, no alkaloid was never isolated and reported until now from *Morinda* species.

Table 1: Qualitative phytochemical screening.

Chemical groups	Results	Chemical groups	Results
Alkaloids	-	Gallic tannins	++
Anthocyanins	-	Proantocyanidins	++
Anthraquinones	+++	Saponins	++
Coumarins	-	Steroids	+++
Cardiotonic glycosides	-	Sugars	++
Catechic tannins	++	Terpenes	+++
Flavonoids		Aminated compounds	+++

This chemical composition of aqueous extract AE was the same for 80% methanol extract and its fractions. Our results are in good agreement with.[1,3,4]

3.2. Antibacterial and antifungal activities of extracts, fractions, flavonoids and iridoids isolated from *M. morindoides* leaves

Results from the antibacterial and bactericidal activities are presented in Tables 1 and continued. They revealed that aqueous extract of *M. morindoides* leaves and its soluble fractions chloroform, ethylacetate, *n*-butanol and residual aqueous phase were devoid with inhibitory effect on the growth of all selected standard ATCC bacteria strains and the fungi *Candida albicans* ATCC 2301 at the highest tested concentration of 500µg/ml. They were declared inactive against these selected ATCC strains (Table 1).

Table 1: Antibacterial and antifungal activities of aqueous extract, its fractions and isolated flavonoids isolated from *M. morindoides* leaves against standard ATCC bacteria strains and fungi (MIC, MBC and MFC, µg/ml).

Samples	*B. c*		*C.a*		*E.c*		*En.c*		*K.p*	
	MIC	**MBC**	**MIC**	**MFC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
AE and its fractions	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500
Chryoeriol	**250**	**500**	**500**	**500**	**500**	>500	>500	>500	>500	>500
Luteolin	>500	>500	500	>500	**125**	**250**	>500	500	**125**	**250**
L-7-O-Glc	125	250	>500	**250**	**250**	500	>500	>500	**250**	**500**
Quercetin	**500**	**500**	>500	>500	**125**	**250**	>500	>500	>500	>500

B.c: *Bacillus cereus* ATCC 4579, *Candida albicans* ATCC 102301, E.c: *Escherichia coli* ATCC 8739, *Enterobacter cloacae* ATCC 13047, *Klebsiella pneumonia* 13883, *Mycobacterium fortuitum* ATCC 6841, *Streptococccus pyogenes* ATCC 12344, *Proteus vulgaris* ATCC 1335, *Pseudomonas aeruginosa* ATCC

154452, *Staphylococcus aureus* ATCC 6538. AE-aqueous extract, L-7-O-Glc: luteolin-7-O-glucoside.

Table 1: Continued.

Samples	***M.f***		***S.p***		***P.v***		***P.a***		***S.a***	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
AE and its fractions	>500	>500	>500	>500	>500	>500	>500	>500	>500	>500
Chryoeriol	**125**	**250**	**62.5**	**125**	>500	>500	>500	>500	**500**	**500**
Luteolin	>500	>500	>500	>500	>500	>500	**500**	**500**	>500	>500
L-7-O-Glc	**>500**	**>500**	**62.50**	**125**	**500**	**500**	**500**	**500**	**>500**	**>500**
Quercetin	>500	>500	**500**	**500**	>500	>500	**250**	**500**	**250**	**500**
Ampicillin	0.8	1.6	0.4	0.8	5	10	5	10	1.6	3.2
Tetracycline	0.4	0.8	0.8	1.6	10	15	10	15	3.2	6.4

See Table 1

Only methanol 80% extract ME and its ethylacetate soluble fraction rich in flavonoids showed weak antibacterial and bactericidal activities against *B. cereus* ATCC 4579, *M. fortuitum* ATCC 6841 and *S. aureus* 6538 (MIC and MBC = 1 mg/ml), and moderate activity against *S. pyogenes* ATCC 12344 (MIC = 125 and MBC = 250 µg/ml). They were without significant effect against the growth of the remaining selected standard bacteria ATCC (MIC and MBC > 1 mg/ml) and were considered as inactive.[6]

Among flavonoids tested, chrysoeriol showed good antibacterial activity against *Streptococcus pyogenes* ATCC 12344 with MIC value of 62.50 µg/ml and exhibited moderate bactericidal activity against this bacteria with MBC value of 125 µg/ml. In addition, this flavonoid exhibited moderate antibacterial activity against *Bacillus cereus* ATCC 4579, *Escherichia colis* ATCC 8739 and *Mycobacterium fortuitum* ATCC 6841 (MIC = 125 or 250 µg/ml), and exhibited weak bactericidal activity against these ATCC bacteria strains (MBC = 500 µg/ml). Luteolin and its glycoside-7-O-glucoside exhibited moderate or weak antibacterial, and weak bactericidal *activity* against a large game of standard ATCC strains with MIC and MBC values of 125 , 250 or 500 µg/ml according to the case (Table 1 and 2 continued). Our results are in good agreement with.[39]

Quercetin displayed low antibacterial and bactericidal activity against *B. cereus* ATCC 4579 and *S. pyogenes* ATCCC 12344 (MIC and MBC = 500 µ/ml. It also was moderately or weakly active against *E. coli* ATCC 8739, *P. aeruginosa* ATCC 154452 and *S. aureus* with MIC and MBC values of 125 and 250, and 500, µg/ml respectively. Our results are in good agreement with [40-42] for the antibacterial activity of quercetin against some bacteria such as E. *coli*, *P. aeruginosa* and *S. aureus*. These three flavonoids were inactive against the remaining selected standard ATCC bacteria strains.[6]

Other tested flavonoids such as kaempferol and its glycosides kaempferol-3-O-rhamnoside, -3-O-rutinoside and -7-O-neohesperidoside (morindaoside), chrysoeriol-7-O-neohesperidoside, quercetin-3-O-rhanmoside and -3-O-rutinoside were devoid with antibacterial and bactericidal activities against all selected standard ATCC bacteria strains at the highest tested concentration of 500 µg/ml. Particularly, the antibacterial activity of quercitrin is controversial in the literature since some auteurs have reported its antibacterial activity by diffusion method against a large game of bacteria such as *S. aureus*, *E. coli*, *P. aeruginosa*, *P. vulgaris* , *Neissera gonorrhea*, *M. fortuitum* and *K. pneumonia* producing a diameter zone of inhibition greater than 1 cm [43]. But in contrast, recently, this flavonoid glycoside was found to be devoid of antibacterial activity by the same diffusion method against standard ATCC bacteria strains such as *Staphylococcus epidermidis* ATCC 12228, *Staphylococcus haemolyticus* ATCC 2737, *Proteus mirabilis* (MRSA), *Shigella sonnei* ATCC 2531, *Salmonella typhimurium* ATCC 13311 and *Escherichia coli* ATCC 25922 at tested concentrations of 350 and 500 µg/ml.[44] The antibacterial activity of this flavonoid was never reported by dilution method since by this method, it was found to be inactive against a large game of standard bacteria at the highest tested concentration of 500 µg/ml.[6,47] This situation is not particular because it concerns also many other flavonoids. In general, reports of antibacterial activity of flavonoid testings are always confliting, probably owing to inter and intra assay variation in susceptibility testing.[39]

Isolated iridoids from *M. morindoides* leaves exhibited good antibacterial and bactericidal activity.[37,48] against a large game of selected standard ATCC bacteria strains with MIC and MBC < 100 µg/ml. Some of them showed moderate or weak activity against other selected ATCC bacteria (Table 1 and 2 continued). The most active iridoid was epoxymethoxygaertneroside showing good antibacterial and bactericidal activities with MIC and MBC values < 100 µg/ml against all selected standard ATCC microorganisms. It was followed by acetylgaertneroside, dehyromethoxygaertneroside, epoxymethoxygaertneroside and gaertneroside inhibiting 7 selected standard ATCC bacteria growth while expoxygaertneroside, gaertneric acid and methoxygaertneroside exerted their beneficial effects against 6, 5 and 4 standard ATCC bacteria respectively with the same above MIC and MBC values (Table 1 and

2 continued). Moreover, epoxymethoxygaertneroside dehydromethoxygaertneroside, epoxygaertneroside and gaertneroside displayed pronounced antibacterial activity against some standard ATCC bacteria with MIC value of 7.81 µg/ml and pronounced or good bactericidal activity against the same ATCC strains with MBC values of 7.81 or 15.62 µg/ml according to the case (Table 1 and 2 continued). Except the antibacterial and bactericidal activities of epoxymethoxygaertneroside against *S. aureus* ATCC 6538 which were good (MIC and MBC < 100 µg/ml), the antibacterial activity of the remaining other iridoids against *K. pneumonia* ATCC154452, *P. aeruginosa* ATCC154452 and *Staphylococcus aureus* ATCC 6538 was moderate (MIC =125 or 250 µg/ml) while their bactericidal effect was moderate or weak (MIC and MC = 125, 250 and 500 µg/ml respectively) according to the case.

A structure antibacterial-activity relationship for iridoids against selected ATCC bacteria strains revealed that the presence of methoxy group in position C-3' increased the antibacterial and bactericidal activities (epoxymethoxygaertneroside compared to epoxygaertneroside, dehydromethoxygaertneroside compared to dehydrogaertneroside, methoxygaertneroside compared to gaertneroside), an additional epoxy group between C-6 and C-7 positions increased these activities (epoxygaertneroside compared to gaertneroside and acetylgaertneroside), the presence of acetyl-glucosyl or H group in position of C-1 is in favour of the increase of these same activities (acetylglucosylgaertneroside compared to gaertneroside or gaertneric acid compared to gaertneroside). The presence of =O group in C-13 position and methoxy group in C-3' position, methoxy group in C-3' position and epoxy group between C-6 and C-7 positions, methyl group in C-14 and methoxy.

Table 2: Antibacterial activity of iridoids isolated from *M. morindoides* leaves against standard ATCC bacteria (MIC and MBC, µg/ml).

Samples	***B.c***		***C.a***		***E.c***		***En.c***		***K.p***	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Acetylgaertneroside	15.63	31.25	31.25	62.50	31.25	62.50	7.81	15.63	62.50	125
Dehydrogaertneroside	31.25	62.50	62.50	125	62.50	125	15.63	31.25	125	250
Dehydromethoxygaertneroside	15.63	31.25	31.25	62.50	31.25	62.50	7.81	15.63	62.50	125
Epoxygaertneroside	31.25	62.50	62.50	125	31.25	62.50	7.81	15.63	62.50	125
Epoxymethoygaertneroside	7.81	7.81	62.50	125	15.63	31.25	7.81	7.81	62.50	125
Gaertneroside	62.50	62.50	125	250	62.50	125	15.63	31.25	125	250
Gaertneric acid	15.63	31.25	62.50	125	31.25	62.50	7.81	15.63	125	250
Methoxygaertneroside	31.25	62.50	125	250	62.50	125	15.63	31.25	125	250
Samples	**M.f**		**S.p**		**P.v**		**P.a**		**S.a**	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Acethylgaertneroside	7.81	15.63	7.81	15.63	62.50	62.50	250	500	125	250
Dehydrogaertneroside	31.25	62.50	15.63	31.25	3125	62.50	500	>500	250	500
Dehydromethoxygaertneroside	15.63	31.25	7.81	15.63	15.63	31.25	250	500	125	250
Epoxygaertneroside	7.81	15.63	7.81	15.63	31.25	62.50	125	250	125	250
Epoxymethoygaertneroside	7.81	15.63	7.81	7.81	15.63	31.25	125	250	62.50	62.50
Gaertneroside	15.63	31.25	15.63	31.25	62.50	125	250	500	250	500
Gaertneric acid	7.81	15.63	7.81	15.63	62.50	125	125	250	125	250
Methoxygaertneroside	31.25	62.50	15.63	31.25	31.25	62.50	250	500	125	250
Ampicilline	1.5	3.0	3.0	6	0.75	1.5	1.5	3	6	12
Tetracycline	3	6	3	6	1.5	3	1.5	3	6.	12

See Table 1. group in C-3' or again methyl group in C-14 position enhanced the activities i.e dehydromethoxygaertneroside compared to methoxygaertneroside, epoxymethoxygaertneroside compared to gaertneroside, methoxygaertneroside compared to gaertneric acid and gaertneroside compared to gaertneric acid respectively.

When tested against clinical isolates, aqueous extract AE and its fractions AE-1 to AE-1.4 showed good antibacterial and bactericidal activity against some isolated microorganisms with MIC and MBC values < 100 µg/ml.[37,48] This is the case of aqueous extract AE and its fractions AE-2 to AE-1.4. Against other clinical isolates, they displayed moderate or weak activity (MIC and MBC = 125, 250 and 500 µg/ml respectively) according to the case (Table 3). AE-1 soluble fraction only showed good antibacterial activity against *B. cereus* and *S. dysenteria* (IC = 62.50 µg/ml) with moderate bactericidal effect (Table 3) and its antibacterial and bactericidal activities against other clinical isolates were moderate (MIC and MBC = 125 and 250 µg/ml).

Flavonoids chrysoeriol and quercetin exhibited good antibacterial and bactericidal activities against some selected clinical isolates with MIC and MBC values < 100µg/ml and showed moderate (MIC and MBC =125 or 250µg/ml) or weak activity (MIC and MBC = 500

µg/ml) and were inactive (MIC and MBC > 500 µg/ml) against other (Table 3).[37,48] Luteolin, kaempferol and kaempferol-3-O-glucoside showed also good antibacterial and bactericidal activity against a limited range of clinical isolates (MIC and MBC < 100 µg/ml,[37,48] moderate and weak activity (MIC and MBC = 125 and 250 µg/ml) or was inactive against other (Table 3). Our results corroborated well with.[55,56] for the activity of these two flavonoids against some bacteria strains. Luteolin 7-O-glucoside exhibited good antibacterial activity against *B. cereus* and *S. flexneri* with MIC value of 62.50µg/ml and its bactericidal effect against all selected clinical isolates was moderate or weak according to the case (Table 3). It was without effect against the growth of *P. vulg*aris and *S.aureus* (MIC and MBC > 500 µg/ml. Other flavonoid glycosides cited above were inactive against all selected clinical isolates at the highest tested concentration of 500µg/ml. In the present study, 80% methanol extract showed good antibacterial and bactericidal activities against all selected clinical isolates with MIC and MBC values < 100 µg/ml, except its bactericidal activity against *E. coli* which was moderate (MBC= 125 µg/ml) (Table 3). Its activities against some standard ATCC strains and all clinical isolates were higher compared to aqueous extract AE (Table 3).

Based the antifungal activity, by dilution methods, AE and its isolated constituents were devoid with antifungal activity against standard ATCC fungi *C. albicans* ATCC 10231, *Aspergillus niger* ATCC 6275 and *A. fumigatus* ATCC 1022 at the highest tested concentration of 500 µg/ml. All flavonoid aglycones and glycosides and iridoids were devoid with antifungal activity against these standard ATCC fungi.[6] But by dilution method, against the same clinical isolated fungi, aqueous extract AE produced a diameter zone of inhibition of 25 to 35 mm depending to tested concentration.[6] Only the 80% methanol extract and its chloroform soluble fraction showed good antifungal activity with MIC value of 62.5 µg/ml and moderate fungicidal activity with MFC of 125 µg/ml against *M. canis* ATCC 9563. They were however, devoid with effect against the growth of the remaining fungi (MIC and MFC > 500µg/ml). However, they showed good antifungal activity against clinical isolated fungi *C. albicans*, *Aspergilus niger* and *A. fumigatus* with MIA and MFC values < 100µg/ml.[6] Other iridoids isolated from various medicinal plants were also previously reported to exhibit antifungal activity by diffusion method against *Saccharomyces cervisiae*.[49]

In other previous studies, aqueous, acetatic and hexanic extracts from *M. morindoides* collected in Daola (Ivory Coast) were reported to be active against clinical isolates *Staphylococcus aureus* and *Pseudomonas aeruginosa* with a dose-dependent effect since their activity was found when tested at concentrations from 1.95 to 32.25 mg/ml.[51,52] had reported that acetatic extract form *M. morindoides* leaves was active against eight standard *Escherichia coli* strains among which *E.coli* ATCC 25922, ATCC 8739 and 2361, and against tree known serotypes *E. coli* O26 H6, O142 K86 and O126 B16 isolated in water and hospital with CMI values recorded from 3.75 and 15 mg/ml and CMB values from 7.5 to 30 mg/ml.

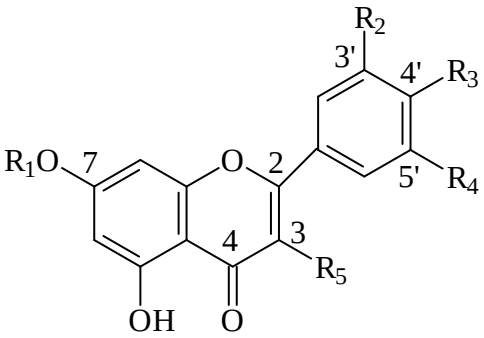
On the other hand, aqueous extract and its soluble fractions from *M. morindoides* leaves collected in Kinshasa (1987) in Zaire, actually Democratic Republic of Congo (DR-Congo), were also tested for their potential antifungal activity by diffusion method. Results indicated that aqueous and its fractions exhibited interesting antifungal activity against clinical isolates *C. albicans Aspergillus fumigatus* and *niger* by producing diameter zones of inhibition from 15 to 35 cm according to the tested concentration [53]. But, when tested against standard ATCC fungi, results revealed that aqueous extract and its soluble fractions were inactive against ATCC fungi *Candida albicans* ATCC 3269, *Aspergillus fumigatus* ATCC 41256, *Microscoporum canis* ATC 12479, *Epidermophyton flocosum* ATCC 23156 and *Trichohyton rubrum* ATCC 35674 by dilution method at the high tested concentration of 500µg/ml.

In other previous studies**,** extracts and fractions from *M. morindoides* collected from Guinea was reported to exhibit antifungal activity on clinical isolate *Crytococcus neoformans* with CMI of 0.78 µg/ml and IC_{50} = 0.08 ±0.02µg/ml.[53,54] Total aqueous extract, ethanol 70% and residual aqueous phase of this extract, from *M. morindoides* leaves collected in Ivory Coast were reported to be active against *Vibrio cholera* with MIC values of 1.92, 2.70 and 6.25 mg/ml and MBC of 15, 5 and 25 mg/ml respectively.[55] Extracts and fractions of *M. morindoides* collected in the same country were found to be active against clinical isolates fungi *Aspergillus fumigatus* and *Candida albicans* with IC_{50} respective values of 0.04 and 0.151 mg/ml.[56,57] The ethylacetate, ethanol, aqueous and ethanol-water extracts from *M. morindoides* leaves collected also in Ivory Coast had reported to have positive effects on the growth of clinical isolate fungus *Aspergillus fumigatus* which was inhibited with respective IC_{50} values in order of Inter 1.25, < 6.1, < 12.47 and < 300 µg/ml.[63,64]

Table 3: *In vitro* antibacterial activity of *M. morindoides* extracts, fractions and flavonoids against clinical isolates (MIC and MBC in µg/ml).

Samples	*B. cereus*	*E. coli*	*P. vulgaris*	*S. aureus*	*S. dysenteria*	*S. flexneri*	*S. sonnei*
	MIC MBC	MIC MBC	MIC MBC	MIC MBC	MIC MBC	MIC MBC	MIC MBC
AE	62.5 125	31.25 62.50	125 250	250 500	31.25 62.50	15.75 31.25	31.25 62.50
ME	32.25 62.50	62.50 125	15.75 31.25	125 250	31.25 62.50	15.75 31.25	15.75 31.25
AE-1	62.5 125	125 250	>500 >500	125 250	62.5 125	250 250	125 250
AE-2	15.75 31.25	31.25 62.50	125 250	125 250	62.5 125	31.25 62.50	15.75 31.25
AE-3	31.25 62.5	62.50 125	>500 >500	125 250	31.25 125	125 250	62.5 250
AE-4	31.25 62.50	>500 >500	>500 >500	>500 >500	31.25 62.50	31.25 62.50	>500 >500
Chrysoeriol	31.25 62.50	62.50 125	>500 > 500	>500 >500	31.25 62.50	15.62 31.25	31.25 62.50
Kaempferol	31.25 62.50	62.50 125	>500 >500	>500 >500	31.25 62.50	31.25 62.50	62.50 125
K-3-O-Glc	62.50 125	62.50 125	>500 >500	>500 >500	125 250	62.50 125	125 250
Luteolin	62.25 125	125 250	>500 >500	>500 >500	62.25 125	31.25 62.50	62.50 125
L-7-O-Glc	62.25 125	125 250	>500 >500	>500 >500	125 250	62.50 125	31.25 62.50
Quercetin	31.25 62.50	62.50 125	>500 >500	62.50 125	62.50 125	31.25 62.50	31.25 62.50
Tetracycline HCl	6.25 12.5	12.5 12.5	12.5 25	0.78 1.56	0.78 1.56	0.78 1.56	3.12 6.25

See Table 1.AE; aqueous extract, ME: 80% methanol extract, AE-1, AE-2, AE-3 and AE-4: chloroform, ethylacetate, n-butanol and residual aqueous phase from the partition of aqueous AE extract, B. cereus: Bacillus cereus, E. coli: Eschericha coli, S. dysenteria : Shigella dysenteria, S. flexneri: Shigella flexneri, S. sonnei: Shigella sonnei, S. thiphy: Salmonella thiphymurium Interestingly,[58,59] had maked a series of soaps based hexanic extract from M. morindoides leaves collected in Daloa (central west region of Ivory Coast) which had shown antifungal effect against clinical isolated fungi A. fumigatus. C. albicans. Trychophyton mentagrophytes and T. rubrum. These soaps showed dose-dependent decrease in the number of fungi colonies with increase in the tested extract concentrations of the soap. T. mentagrophytes was the most sensitive strain while C. albicans was the most resistant, irrespective of the soap type. T. rubrum and A. fumigatus presented intermediate sensitivity in relation to tested strains. The IC50 values of basic soap were ranged from 2.78±0.01 to 10.41±0.01 mg/ml while those of the plant extract varied between 2.44 to 6.50 mg/ml. Basic soap and plant extract had the same minimal inhibitory concentration (MIC) of 31.25 µg/ml, but IC50 data indicated that the one containing plant extract was more effective.[59] The same soaps had also reported to inhibit the growth of resistant bacteria such as Staphylococcus aureus and Pseudomonas aeruginosa, responsible of cutaneous infections with IC50 values of 3.05±0.68 and 3.15±1.25 mg/ml and a common MIC value of 62.50 mg/ml. For soaps, the best counting of fungi colonies was observed with 31.25 mg/ml. Flavonoids isolated by.[6,7] were considered by,[59] to be likely to have played a major role in the antifungal activity of the extract since they are lipophilic due to the presence to a phenyl chain. These antifungal studies had demonstrated that M. morinodoides leaves possess antifungal activity which can explain its current use for the treatment of dermatologic infections such mycosis and scabies in traditional medicine. In addition to other evaluated biological activities of M. morindoides leaves extracts mentioned above,[60] had reported the cytotoxic effect induced by M. morindoides leaf fractions including toluene, methyl tert-butyl ether, ethylacetate, n-butanol and water against human and murine leukemia cells mediated trough the induction of apoptosis.

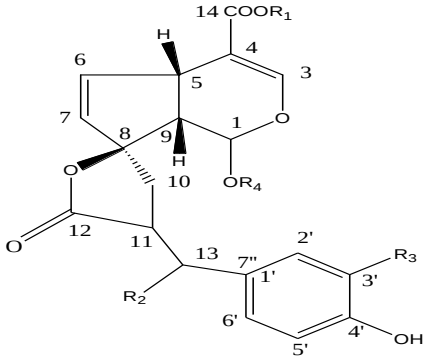


Flavonoid names	R_1	R_2	R_3	R_4	R_5
1. Apigenin	H	H	OH	H	H
2. Apigenin-7-O-Glc	Glc	H	OH	H	H
3.Chrysoeriol	H	H	OH	OCH_3	H
4.Chrysoeriol-7-O-neohesp	Rha-(1→2)-Glc	H	OH	OCH_3	H
5. Luteolin-7-O-Glc	Glc	H	OH	OH	H
6. Kaempferol	H	H	OH	H	OH
7.Kaempferol-3-O-Rha	H	H	OH	H	Rha
8.Kaempferol-3-O-Rut	H	H	OH	H	Rha-(1→6)-Glc
9. Kaempferol-7-O-neohesper	Rha-(1→2)-Glc	H	OH	H	OH

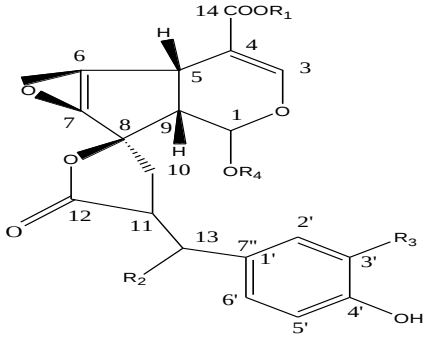
10. Quercetin	H	OH	OH	H	OH
11. Quercetin-3-O-Rha	H	OH	OH	OH	O-Rha
12. Quercetin-3-O-Rut	H	H	OH	OH	Rha-(1→6)-Glc
13.Quercetin-7,4' dimethyl-ether	OCH_3	H	H	OCH_3	OH

Glc; glucoside, Rha; rhamnoside, Rut: rutinoside, neohesp: neohesperidoside

Fig. 2: Structures of antibacterial flavonoids isolated from *M. morindoides* leaves



	R_1	R_2	R_3	R_4	
1	CH_3	OH	H	Glc	: Gaertneroside
2	CH_3	OH	H	6-acetyl-Glc	: Acetylgaertneroside
3	CH_3	=O	H	Glc	: Dehydrogaertneroside
4	CH_3	=O	OCH_3	Glc	: Dehydromethoxygaertneroisde
5	H	OH	H	Glc	: Gaertneric acid
6	CH_3	OH	OCH_3	Glc	: Methoxygertneroside



	R_1	R_2	R_3	R_4	
7	CH_3	OH	H	Glc	: Epoxygaertneroside
8	CH_3	OH	OCH_3	Glc	: Epoxymethoxygaertneroside

Glc: glucoside

Figure 3: Structures of antibacterial iridoids isolated from *M. morindoides* leaves

Based on the effect of antibacterial activity of these tested extracts and isolated compounds, according to Marmonier cited by.[61] who proposed that, an extract is bacteriostatic when the rapport MBC/MIC $\geq$ 4 and bactericidal if MBC/MIC $\leq$ 4, the present reported data can be advanced that extracts and isolated compounds exerted significant bactericidal effects since this rapport is between 0.5 and 2 $\leq$ 4. Although the obtained data from this study demonstrated antibacterial properties of these selected samples from *M. morindoides* leaves, their effects were still lower compared to Ampillicine and Tetracycline used as antibiotic reference products.

In general, flavonoids and iridoids with different chemical structures from various medicinal plants, are some natural products among other, reported in many investigations to exhibit antibacterial activity.[40,42,43,62-64] evaluated by different methods. This effect largely contributes to the support and justification of the use of some medicinal plants in traditional medicine for the treatment of various infections.

4. CONCLUSION

The results from this study clearly demonstrate that aqueous extract and its different soluble fractions were

devoid with antibacterial and antifungal activity against selected standard ATCC strains. But, they showed good, moderate or weak antibacterial and bactericidal activities against clinical isolates according to the case. Some flavonoids isolated from *M. morindoides* leaves were also found to exhibit moderate antibacterial and bactericidal activities against standard ATCC bacteria and showed good or moderate activity against clinical isolates according to the case. Interesting, good antibacterial and bactericidal activities were shown by iridoids against all selected standard bacteria ATCC strains. Extracts also showed good antifungal activity against some fungi. Thus, the use of this medicinal plant part in traditional medicine for the treatment of diarrhea can be explained partly by its antibacterial activity demonstrated *in vitro* test in the present study. This activity is related to the presence of flavonoids and iridoids considered as active principles.

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