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Phytochemical screening and antimicrobial activity of *Momordica charantia* L. and *Morinda lucida* Benth extracts from Benin

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***Momordica charantia* and *Morinda lucida* are Benin's pharmacopeia plants that are used traditionally for the treatment of infectious diseases. This study aims to investigate phytochemical profile and antimicrobial activity of both plants. The dried leaf powder is used for extraction with different solvents by ultrasonication (35 Hz) at room temperature for 2 h. TLC and the method based on coloring and precipitation differential reactions were used for preliminary screening. HPTLC analysis was performed on silica gel 60 F_{254}, 20.0 X 10.0 cm HPTLC plates, with Toluen: Ethyl acetate: Formic Acid: Methanol (3:4:0.8:0.7 v/v) as a mobile phase. The antibacterial and antifungal activities were assessed *in vitro* by the method of macrodillution and solid medium agar diffusion. TLC analysis showed many spots which suggest that both of the plants extracts contain various secondary metabolites. HPTLC revealed the presence of Quercetine, cafeic acid and vanilic acid in the plants' extract. *M. charantia* extracts have shown the largest inhibition diameters (25.00±0.00 mm) and inhibit more strains than *M. lucida* extracts. From all the tested strains, only *P. aeruginosa* was the most sensitive to *M. charantia* extracts with 50% bactericidal effect.**

Key words: Phytochemical screening, antimicrobial activity, *Momordica charantia* and *Morinda lucida*, Benin.

INTRODUCTION

Plants have been for centuries the source of molecules and food for humans and wildlife. One of the surveys conducted by the World Health Organization (WHO) reports that more than 80% of the world's population is still depending on the traditional medicines for various diseases (Atef et al*.,* 2019). In the same line, most of the West African population lives in the rural areas and depends on natural resources for their own subsistence

and for their cash income (Achigan-Dako et al., 2011). Mbuni et al. (2020) also reported that rural dwellers prefer traditional medicines because of their closiness to the traditional healers and the fact that the healers understand their culture and environment as well as their patients. Indeed, these plants are used to treat all kinds of chronic diseases (Petrovska, 2012) among which are infectious diseases.

Today, there are more than 250 types of infections and food poisoning caused by bacteria and fungi (Hernández-Cortez et al., 2017). Of these infections and intoxications, the most frequently isolated pathogenic bacteria are *Staphylococci*, *Pseudomonas*, *Streptococcus* and *Escherichia coli* (Elisha et al., 2017; Hernández-Cortez et al., 2017). Otherwise, Pallavali et al. (2017) and Bassetti et al. (2018) reported that Gram-positive cocci such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus* spp. and Gram-negative bacilli such as *E. coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Proteus* species are the most common pathogenic bacteria isolated from wound infections and are also an important cause of wound infections in diabetic individuals and infected wound following surgeries. These bacteria are also mostly responsible for toxins production. Apart from bacterial toxins, mycotoxins are fungal secondary metabolites that can cause the serious infection (Benedict et al., 2016). Indeed, fungal diseases are severe and have very high morbidity as well as up to 60% mortality for patients diagnosed with invasive fungal infection (Staniszewska, 2020). It is the case of *Candida albicans* with many virulence factors implicated in the invasive diseases, that have become common of human infections worldwide (Nouraei et al., 2020; Köhler et al., 2020).

Indeed, the treatment of infections due to bacteria and phytopathogens requires the use of several methods; the best known are the treatments with synthetic products, which is not without consequences on the environment and human health. Nowadays, there is a phenomenon of resistance of bacteria and fungi to most conventional antibiotics. Antibiotic resistance among bacterial or fungal strains strains is a serious situation. It may be so rapid that the effectiveness of common antibiotics may be lost within a span of 5 years due to genetic changes (Chandra et al., 2017). It therefore seems important to explore other alternative for fighting infectious diseases. An alternative is the use of medicinal plants. Many studies show that medicinal plants contain many biologically active secondary metabolites such as tannins (Chokki et al., 2020), terpenoid (Frezza et al., 2019), alkaloids (Vanderplanck and Glauser, 2018), glycosides (Pertuit et al., 2018), flavonoids, phenols (Frezza et al., 2019; Chokki et al., 2020) and other compounds which display various pharmacological activities: antioxidant, anti-inflammatory, anti-allergic, anti-cancer, analgesic, anti-diabetic, antibacterial, antifungal, antiviral activities (Ksouri et al., 2007; Forni et al., 2019; Senhaji et al., 2020). In the socio-economic and health context of developing countries, including Benin, the study of plants can lead to obtaining adequate and low-cost therapeutic responses, with proven scientific efficacy and optimal cultural acceptability.

Momordica charantia and *Morinda lucida* are frequently used in Benin traditional pharmacopiea against various diseases. *Morinda lucida* Benth., belonging to the family Rubiaceae is a tropical rainforest tree. It is known as xwesué (in Benin) and is one of the most used plants in the preparation of traditional medicines against fever (Lawal et al., 2012). The leaves are used as “oral teas”, which are usually taken orally for the traditional treatment of malaria, and as a general febrifuge, analgesic, laxative and anti-infections (Adeyemi et al., 2014). *M. charantia* (bitter melon) is a tropical and subtropical vine of the family cucurbitaceous widely grown in India, South Asia, China, Africa (Kubola and Siriamornpun, 2008) and particularly in Benin. Leaf aqueous macerate is used without combination with other plants in the treatment of microbial and viral infections (measles virus). Considering the vast potentiality of plants as sources for antimicrobial drugs, the present research aims to carry out preliminary phytochemical screening and evaluates antimicrobial activity of *M. charantia* and *M. lucida* leaf extracts.

MATERIALS AND METHODS

Plant material

M. lucida leaf samples were collected from Agata (06°30'28"N, 002°38'44"E), which is located in the Department of Oueme, Benin, while those of *M. charantia* were collected from Dangbo (06°35'19"N, 002°33'15"E) located in the same department. A voucher specimens No. AAC8100/HNB and No. AAC8101/HNB respectively for *M. lucida* and *M. charantia* were deposited at the Benin national herbarium, University of Abomey-Calavi, Cotonou, Benin. All samples were collected in the morning at 7 am. They were air-dried (23°±2°C) for 15 days before powdered using grinder Retsch type SM 2000/1430/Upm/Smf, Haan Germany.

Preliminary screening

The preliminary phytochemical profiling of leaf powders of *M. charantia* and *M. lucida* to determine the major constituents (nitrogenous, polyphenolic, terpenic compound and glycosides) was done using a qualitative analysis based on coloring reactions and/or precipitation described by Senhaji et al. (2020).

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Preparation of plant extracts

The extracts were prepared with 9 polar solvents [water, water-ethanol 30:70 (v / v) methanol, methanol-HCl 1%, ethanol, acetone, ethyl acetate, dichloromethane] and 2 non-polar solvents (chloroform and petroleum ether). For the polar solvents, 1 g of powder in 100 mL of solvent was subjected to ultrasonication (35 Hz) at room temperature for 2 h. The same operation was carried out with non-polar solvents under reflux system. A total of 24 extracts were thus obtained, 12 per plant. In addition, the residues obtained after the ethyl acetate and petroleum ether extractions were extracted again using methanol and methanol/1% HCl. These extracts are coded Methanol-EA and Methanol/HCl-PE, respectively. Each mixture was filtered through Whatman N° 1 paper (125 mm ø, Cat No. 1001 125) and concentrated under reduced pressure using a rotary evaporator before being oven dried at 40°C. The aqueous extract was lyophilized to dryness. The extraction yields were determined by the ratio between the mass of powder and extract obtained.

Thin layer chromatography (TLC) analysis

TLC of the two plants extracts was carried out using pre-coated silica gel and alumina plate (TLC-grade ; Merck 20 20 cm, 0.2 mm thikness). Each extract was dissolved in the extraction solvent at a concentration of 1 mg/ml and about 2µl of this solution was applied 1 cm from the base of the TLC with cappillar tube. Development of the chromatograms was done in a closed tank in which the atmosphere was saturated with the eluent vapor to separate various constituents of the extract by lining the tank with filter paper wetted with the eluent and dried at the end. Solvent systems used as eluent were (1) toluen-ethyl acetate 9:1, (2) ethyl acetate-formic acid-water 8:1:1, (3) toluen-acetic acid-formic acid 5:4:1, (4) toluen-acetic acid 4:6, (5) toluen-ethyl acetate-formic acid-methanol 3:4:0.8:0.7. TLC spot was visualized under UV light fluorescent at 254 and 366 nm. The best solvent system was used for HPTLC analysis.

High performance thin-layer chromatography (HPTLC)

Chromatography was performed on silica gel 60 F 254, 20.0 X 10.0 cm HPTLC plates manufacturer Merck, with Toluen-Ethyl acetate-Formic Acid-Methanol (3:4:0.8:0.7 v/v) as a mobile phase. The standard (rutin, quercetin, galic acid, tanic acid, cafeic acid, vanilic acid and clorogenic acid) solutions (2.0 µL of 1 mg/mL) were applied to the plates as 7.0 mm bands; samples were applied with CAMAG-Linomat V automated spray on band applicator equipped with a 100 µL syringe and operated with the following settings : band length of 3.0 mm, application rate of 10 s/ µL, migration distance of 80 mm.

Assessment of antimicrobial activity

Organisms and growth conditions

10 reference strains used in this study included $Gram^+$ bacteria (*Staphylococcus aureus* ATCC 29213, *S. epidermidis* T22695, *M. luteus* ATCC 10240, *S. oralis*, *Enterococcus faecalis* ATCC 29212), $Gram^-$ bacteria (*E. coli* ATCC 25922, *Proteus mirabilis* A24974, *Proteus vulgaris* A25015, *Pseudomonas aeruginosa* ATCC 27853) and yeast (*Candida albicans* MHMR). Overnight (18h) cultures were prepared by inoculating 1 mL Muller Hinton broth with 1-2 young colonies of each organism obtained from 24 h-old Muller Hinton Agar cultures. Broths were incubated overnight at 37°C with shaking. Inocula were prepared by diluting overnight cultures in saline to approximately 10^8 cfu mL^{-1} for bacteria and 10^7 cfu mL^{-1} for *C. albicans*. These suspensions were further diluted with sterile saline as required.

Antibiogramme

The disc diffusion method described by Trinh et al. (2020) with slight modifications was used to evaluate the effects of the extracts on the strains. Briefly, two to three sterile paper discs (6 mm in diameter) were lodged, under aseptic conditions, on Mueller Hinton agar Petri dish previously flooded with the appropriate inoculum. The discs were aseptically impregnated with 25 µL of plant extract solution (30 mg/mL) and kept for 15 min at room temperature before incubation at 37°C for 24 h. After the incubation period, the dishes were examined for inhibitory zones. Each sample was performed in triplicate.

Determination of Minimal Inhibitory Concentrations (MIC)

The more effective plant extracts, which exhibited antibacterial activity at 30 $mg.mL^{-1}$, were used to determine their MIC using the macrodilution method described by Dah-Nouvlessounon et al. (2015). Different concentrations of the plant extracts (30, 15, 7.5, 3.75, 1.875, 0.937, 0.468, 0.234, 0.117 and 0.058 $mg.mL^{-1}$) sterilized through Millipore filter were prepared separately in screw tubes. To 1 mL of the above concentrations, 1 mL of the bacterial inoculum (10^6 CFU/mL) was added to obtain 2 mL as a final volume. Culture medium without samples and others without microorganisms were used in the tests as control. Tubes were incubated at 37°C for 18–24 h and growth was evaluated using turbidity measurements. The MIC is the lowest concentration of the compound at which the tested microorganism does not demonstrate visible growth (turbidity).

Dertermination of Minimal Bactericidal/Fungicidal Concentrations (MBC/MFC)

Streaks were taken from the MIC to the highest concentration of plant extracts exhibiting invisible growth, subcultured onto a fresh MH agar medium and incubated at 37°C for 18-24 h. The concentration that yielded no bacterial growth on solid medium was considered the MBC or MFC (Farshori et al., 2013).

Statistical anlysis

Antibiogramme experiment was done in double and data obtained were reported as a mean ± standard deviation (SD). The data were analyzed using Graph Pad Prism 7 software. Differences of p <0.05 were considered significant.

RESULTS

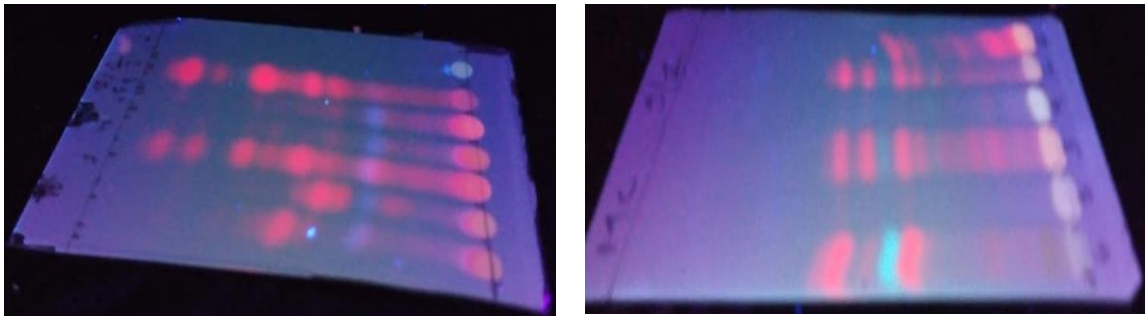
Preliminary phytechemical screening

The preliminary phytochemical analysis performed on the two plants revealed the presence of several secondary metabolites (Table 1). It was noted an uneven distribution of these metabolites from one plant to another. Indeed, 78.57% of the studied secondary metabolites were present in *M. charantia* leaf powder against 71.42% in *M. lucida* leaf powder.

Table 1. Phytochemical constituents of *M. lucida* and *M. charantia* leave powdered samples.

Group of compounds	Class	*M. lucida*	*M. charantia*
Nitrogenous compound	Alkaloids	+	+
	Tanins	+	+
	Flavonoids	+	+
Poly-phenolics compound	Anthocyans	+	-
	Coumarin	+	-
	Quinonics derivate	-	+
	Triterpenoids	+	+
Terpeniques compound	Steroids	+	+
	Cardenolids	+	+
	Saponosids (IM)	+ (152)	+ (213)
	Reducing compounds	+	+
Heterosides	Free anthracénics	-	-
	O-heterosides	-	+
	Mucilags	-	+

+, Presence of secondary metabolite; -, absence of secondary metabolite; IM, Index mouss.



a **b**

Figure 1 . Thin-layer chromatographic separations of *M. lucida* (a) and *M. charantia* (b) extracts.

Thin layer chromatography (TLC) analysis

TLC profiling showed the good separation of the metabolites with system solvent : toluen-ethyl acetate-Formic Acid-Methanol (3:4:0.8:0.7 v/v) used as eluent. As shown in Figure 1, the TLC profiling showed the presence of various components in both plant extracts. After the observations made on the plants powder, the TLC shows the efficiency of the extraction method used to extract the maximum compounds contained in the plants.

HPTLC analysis

Figure 2 shows the HPTLC profil of phenolics compunds standards and tests extracts. The florescence bands of most of the phenolics compunds are not visible at 366 nm wavelength but they are visible at 254 nm. Netherveless, the chromatograms showed that the compound available are satandard; Quercetine Rf = 0.79 was present in all of the test plant extracts. In addition, cafeic acid (Rf = 0.80) and vanilic acid were found in most of the test extracts.

Antimicrobial activity

Sensibility test

The results of the antibiogram are presented in Table 2. The analyses have determined that 50% of *M. charantia* extracts

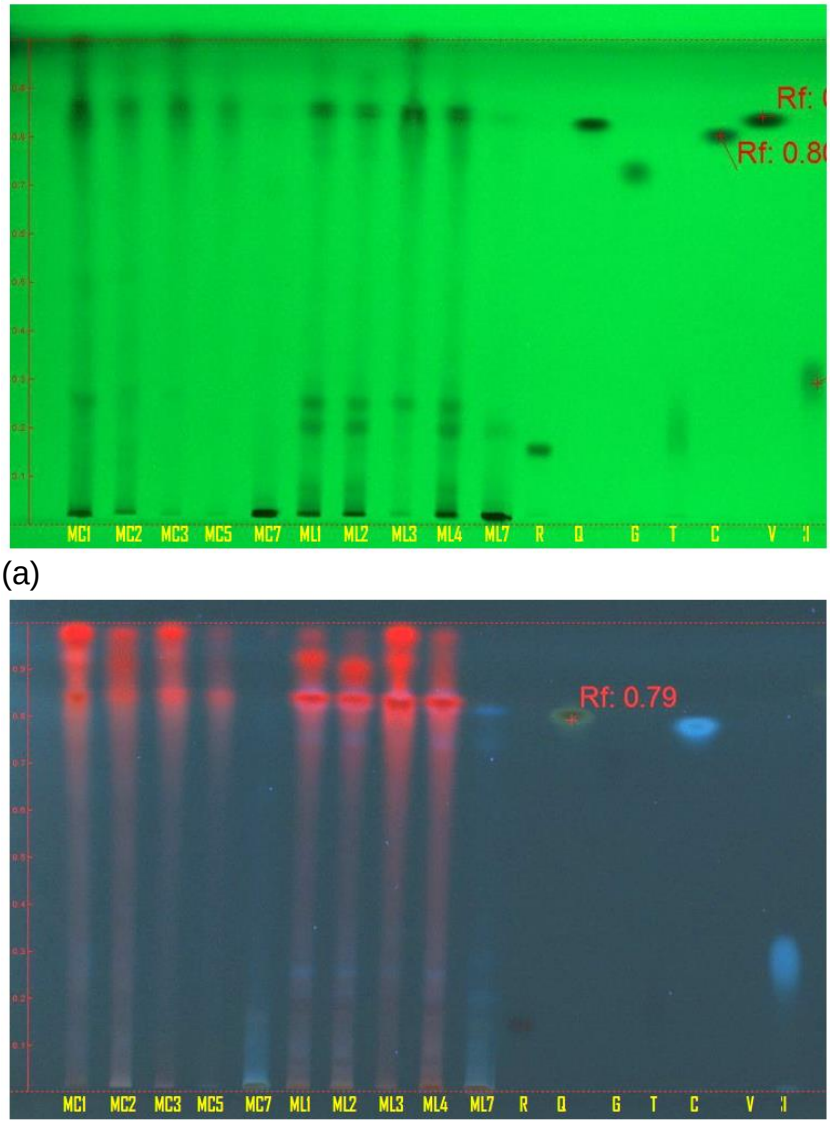


Figure 2. Chromatograms of extracts sample and standard in HPTLC analysis (a) under UV 254 nm, (b) under 366 nm. *M. charantia* (MC1, ethanol; MC2, methanol; MC3, ethyl acetate; MC6, Chloroforme; MC7, water); *M. lucida* (ML1, ethanol; ML2, methanol; ML3, ethyl acetate; ML4, acetone; ML7, water); Standards (R, Rutin; Q, Quercetin; G, acid Galic; T, acid Tanic; C, acid cafeic; V, acid Vanilic; Cl, acid Clorogenic).

inhibit the proliferation of at least one of the tested microorganisms against 66.66% of *M. lucida* extracts. In addition, among the strains sensitive to extracts from both plants, *M. luteus* showed more resistance to all active extracts followed by *P. vulgaris*. Inhibition diameters vary depending on strains and extract types. *M. charantia* extracts have shown the largest inhibition diameters and inhibit more strains than *M. lucida* extracts (Table 2).

For *M. charantia*, the smallest diameter (8.87±0.25 mm) was obtained with chloroform extract on *S. oralis*, while the largest diameter of inhibition (25.00 ± 0.00 mm) was obtained with methanol-EA extract on *E. coli* strain. The analysis of variance ANOVA shows a highly significant variation ($p < 0.0001$) by considering the response of the strains in terms of inhibition diameter and also a significant variation ($p < 0.05$) compared to the power of the extracts in terms of the number of inhibited strains. Regarding *M. lucida*, the smallest inhibition diameter (8.25 ± 0.50 mm) was obtained with the dichloromethane extract on *S. oralis* strain, while the largest diameter (19.28 ± 0.25 mm) was

Table 2. Inhibitory diameter (mm) of the active extracts.

Plant	Extracts	Inhibitory diameter (mm)									
		S. aur	***P. aer***	***P. mir***	***M. lut***	***S. epi***	***P. vul***	***S. ora***	***E. foe***	***E. coli***	***C. alb***
M. charantia	Dichloromethane	16.37±0.48	24.00±0.41	16.00±1.15	ni	15.87±1.18	ni	12.12±0.25	11.25±0.50	14.75±0.96	15±0.00
	Ethyl acetate	ni	17.25±0.47	ni	ni	ni	ni	ni	ni	ni	ni
	Chloroform	23.00±0.00	15.75±0.28	11±0.00	17±0.00	ni	14±0.00	8.87±0.25	10±0.00	ni	11.5±0.00
	Methanol-EA	22.38±0.25	21.25±0.50	12.13±0.25	ni	14.63±0.47	ni	17.75±0.95	24.38±0.47	25.00±0.00	16.50±0.57
	Methanol/HCl	16.88±0.25	20.50±0.57	7.50±0.40	ni	17.00±0.00	ni	21.25±0.57	24.50±0.57	20.00±0.00	16.25±0.28
	Methanol/HCl-PE	16.00±0.00	21.38±0.47	ni	ni	17.63±0.25	ni	20.00±0.00	20.88±0.25	20.50±0.57	15.00±0.00
M. lucida	Methanol/HCl	ni	Ni	ni	ni	10.25±0.50	ni	ni	ni	ni	ni
	Ethyl acetate	ni	13.12±0.25	ni	ni	ni	ni	ni	ni	ni	ni
	Dichloromethane	ni	Ni	9.62±0.25	ni	8.37±0.48	ni	8.25±0.50	14.25±0.50	ni	ni
	Methanol	ni	Ni	13.37±0.25	ni	ni	ni	ni	11±0.00	ni	12.12±0.25
	Acetone	11.00±0.00	Ni	14.13±0.25	ni	15.00±0.00	11.50±0.57	ni	17.13±0.25	ni	11.00±0.00
	Ethanol	ni	Ni	ni	ni	12±0.00	ni	ni	ni	ni	ni
	Methanol/HCl-PE	ni	Ni	ni	ni	ni	ni	ni	ni	19.28±0.25	ni
	Ethanol/water	ni	Ni	ni	ni	08.5±0.57	ni	ni	ni	ni	ni

S. aur, *Staphylococcus aureus*; P. Aer, *Pseudomanas aeruginosa*; P. Mir, *Proteus mirabilis;* M. Lut, *Miccrococcus luteus*; S. Epi, *Staphylococcus epidermidis*; P. Vul, *Proteus vulgaris*; S. Ora, *Streptococcus oralis*; E. foe, *Enterococcus foecalis*; E. Coli, *Escherichia coli*; C. alb, *Candida albicans*; ni, no inhibition.

obtained with the Methanol/HCl-PE extract on *E. coli* strain.

Minimal inhibitory concentration (MIC)

The MIC of the active extracts of the two plants are determined and presented in Table 3. These concentrations vary according to the extract types.

With *M. charantia* extracts, the lowest inhibitory concentration (0.117 $mg.mL^{-1}$) was obtained with the chloroform extract on the *S. aureus* strain while the highest concentration (7.5 mg/mL^{-1}) was obtained with the dichloromethane extract with *P. mirabilis* and *S. oralis*. For each extract type, a significant difference ($p < 0.05$) was observed between the tested strains. For each strain, however, there is a variation in the threshold of significance. Indeed, for the strains *S. epidermidis* and *E. coli*, no difference was observed ($p > 0.05$).

With *M. lucida*, the lowest concentration (0.234 $mg.mL^{-1}$) was obtained with the methanol, acetone and methanol/HCl-PE extracts respectively against *P. mirabilis*, *E. foecalis* and *E. coli* strains. On the other hand, the lowest inhibition (7.5 $mg.mL^{-1}$) was obtained with the dichloromethane and ethanol/water extracts on *S. epidermidis*.

Minimal bactericidal concentration (MBC)

Minimal bactericidal concentrations (MBC) of the active extracts of both plants are presented in Table 4. Like MICs, MBCs vary depending on the types of extract. With *M. charantia*, the lowest concentration (0.234 $mg.mL^{-1}$) was obtained with Methanol-EA extract against *E. coli* while the highest concentration obtained is greater than 30 $mg.mL^{-1}$. This concentration was obtained with the dichloromethane extract on the *P. mirabilis* strain. The lowest inhibitory concentration (0.468 $mg.mL^{-1}$) of *M. lucida* extracts was obtained with the methanol extract. The ratio of the two MIC and MBC parameters showed bactericidal and bacteriostatic effects (Table 4).

Indeed, for *M. charantia*, the dichloromethane extract has a bactericidal effect on 71.42% of the bacterial strains that are sensitive to it, followed

Table 3. Minimal inhibitory concentrations (MIC) of the active extracts of *M. charantia* and *M. lucida*.

Plants	MIC (mg.mL^{-1})									
	Extracts	***S. aur***	***P. aer***	***P.mir***	***M.lut***	***S. epi***	***P. vul***	***S. ora***	***E. foe***	***E. coli***	***C. alb***
M. charantia	Dichloromethane	3.75	1.875	7.5	nd	0.937	nd	7.5	3.75	0.234	0.468
	Ethyl acetate	Nd	0.937	nd	nd	nd	nd	nd	nd	nd	nd
	Chloroform	0.117	0.468	3.75	0.937	nd	0.937	1.875	1.875	nd	1.875
	Methanol-EA	0.234	0.468	3.75	nd	1.875	nd	0.937	0.117	0.234	0.468
	Methanol/HCl	1.875	0.468	3.75	nd	0.937	nd	0.937	0.117	0.468	0.937
	Methanol/HCl-PE	3.75	0.234	nd	nd	0.937	nd	0.468	0.234	0.468	0.937
M. lucida	Methanol/HCl	Nd	nd	nd	nd	1.875	nd	nd	nd	nd	nd
	Ethyl acetate	Nd	1.875	nd	nd	nd	nd	nd	nd	nd	nd
	Dichloromethane	Nd	nd	3.75	nd	7.5	nd	3.75	0.468	nd	nd
	Methanol	Nd	nd	0.234	nd	nd	nd	nd	0.937	nd	0.468
	Acetone	3.75	nd	1.875	nd	0.937	1.875	nd	0.234	nd	3.75
	Ethanol	Nd	nd	nd	nd	1.875	nd	nd	nd	nd	nd
	Methanol/HCl-PE	Nd	nd	nd	nd	nd	nd	nd	nd	0.234	nd
	Ethanol/water	Nd	nd	nd	nd	7.5	nd	nd	nd	nd	nd

S. aur, *Staphylococcus aureus*; P. Aer, *Pseudomanas aeruginosa*; P. Mir, *Proteus mirabilis;* M. Lut, *Miccrococcus luteus*; S. Epi, *Staphylococcus epidermidis*; P. Vul, *Proteus vulgaris*; S. Ora, *Streptococcus oralis*; E. foe, *Enterococcus foecalis*; E. Coli, *Escherichia coli*; C. alb, *Candida albicans*; nd, not determined.

Table 4. Minimal bactericidal concentrations (MBC) of the active extracts of *M. charantia* and *M. lucida.*

Plants	MBC and MFC (mg.mL^{-1})										
	Extracts	***S. aur***	***P. aer***	***P. mir***	***M. lut***	***S. epi***	***P.vul***	***S. ora***	***E. foe***	***E. coli***	***C. alb***
M. charantia	Dichloromethane	7.5*	1.875*	>30	nd	0.937*	nd	7.5*	7.5*	0.937	0.468
	Ethyl acetate	Nd	3.75	nd	nd	nd	nd	nd	nd	nd	nd
	Chloroform	0.937	0.468*	15	1.875*	nd	3.75	15	1.875*	nd	7.5
	Methanol-EA	1.875	1.875	30	nd	15	nd	7.5	0.937	0.234*	3.75
	Methanol/HCl	15	3.75	30	nd	3.75	nd	1.875*	0.468	7.5	15
	Methanol/HCl-PE	15	0.468*	nd	nd	3.75	nd	1.875	1.875	0.937*	7.5
M. lucida	Methanol/HCl	Nd	nd	nd	nd	7.5	nd	nd	nd	nd	nd
	Ethyl acetate	Nd	7.5	nd	nd	nd	nd	nd	nd	nd	nd
	Dichloromethane	Nd	nd	3.75*	nd	30	nd	15	1.875	nd	nd
	Methanol	Nd	nd	0.468*	nd	nd	nd	nd	3.75	nd	3.75
	Acetone	30	nd	15	nd	7.5	7.5	nd	1.875	nd	15
	Ethanol	Nd	nd	nd	nd	3.75*	nd	nd	nd	nd	nd
	Methanol/HCl-PE	Nd	nd	nd	nd	nd	nd	nd	nd	3.75	nd
	Ethanol/water	Nd	nd	nd	nd	>30	nd	nd	nd	nd	nd

With* = bactericidal effects; without* = bacteriostatic effects. S. aur, *Staphylococcus aureus*; P. Aer, *Pseudomanas aeruginosa*; P. Mir, *Proteus mirabilis;* M. Lut, *Miccrococcus luteus*; S. Epi, *Staphylococcus epidermidis*; P. Vul, *Proteus vulgaris*; S. Ora, *Streptococcus oralis*; E. foe, *Enterococcus foecalis*; E. Coli, *Escherichia coli*; C. alb, *Candida albicans*; nd, not determined.

by the chloroform extract which has a bactericidal effect on 42.85% of the sensitive bacteria. In contrast to the ethyl acetate extract which has a bacteriostatic effect, all the other extracts of *M. charantia* showed a bactericidal effect on at least one sensitive bacterial strain. Of all the tested strains, only *P. aeruginosa* was the most sensitive to *M. charantia* extracts with 50% bactericidal effect. On the other hand, *P. mirabilis* strain was the most resistant to all extracts of *M. charantia*.

Contrary to the observations made with *M. charantia*,

P. mirabilis is the strain which presented more sensitivity to *M. lucida* extracts with two bactericidal effects obtained with dichloromethane and methanol extracts. In addition to the ethanolic extract that had a bactericidal effect on the *S. epidermidis* strain, all other *M. lucida* extracts had bacteriostatic effects on all susceptible strains. The tested strains showed more resistance to *M. lucida* extracts than those of *M. charantia*.

DISCUSSION

The extracts used in this study were prepared using ultrasonication. The choice of this method is based on the fact that the mechanical effects of ultrasound induce a disruption of the cell walls. This leads to greater intraparticle penetration of the solvent into the cells, thus facilitating the rapid release of their contents and the acceleration of the kinetics extraction (Landoulsi, 2016). The efficiency of cell disruption and mass transfer are the main factors responsible for the good performance of ultrasound extraction (Romdhane, 1993). Ultrasound has the advantage of considerably reducing the extraction time and increasing the extraction yield (Bourgou et al*.,* 2016). The yields obtained during the extraction varied from one plant to another and according to the solvents. Since for the same solvent yields vary according to the plant, while the same amount of plant powder was extracted with the same amount of solvent under the same conditions, the explanation of the difference would be related to the chemical composition of the plants that would not be the same. Phytochemical screening showed that both plants are source of secondary metabolites. The preliminary screening reveals, in nitrogen compunds group, the presence of alkaloïds. This observation was similar to those made by Adomi and Umukoro (2010) in Nigeria. Tanin and flavonoid found in both plants were reported to have antibacterial, anti fungal, antiviral and antioxydant activity (Leelaprakash et al*.,* 2011; Manandhar et al*.,* 2019). Ndam et al. (2014) in Cameroon and Kazeem et al. (2013) in Nigeria have the same observations. Dandawate et al*.* (2016), De Oliveira et al*.* (2018) and Khatun et al*.* (2020) reported that phytochemical screening of *M. charantia* revealed the presence of secondary metabolite such as alkaloids, flavonoids, , tannins, saponins and terpenoids.

In drug analysis, analytical methods used are diversified and are still being improved to find better solutions in pharmaceutical analysis. In this study, we use also HPTLC technique. HPTLC is an analytical technique based on TLC, but with enhancements intended to increase the resolution of the compounds to be separated and to allow quantitative analysis of the compounds. The usage of HPTLC is well appreciated and accepted all over the world. HPTLC is an ideal screening tool, that not only confirms but also establishes its identity (Wang et al., 2010). A simple and reproducible method using HPTLC was successfully performed for the qualitative and quantitative analysis of medicinal plant (Yadav et al*.,* 2011; Puranik et al*.,* 2010). Therefore, HPTLC analysis showed in our extracts, the presence of some phenolics compunds such as: Quercetine Rf = 0.79 present in all of test plant extracts, cafeic acid (Rf = 0.80) and vanilic acid were found in most of the test extracts. For *M. charantia*, the same observation was made by Shodehinde et al*.* (2016) for quercetin and acid cafeic. In the same way, Thiruvengadam et al*.* (2014) found vanilic acid in *M. charantia* leaf extracts. Phytochemical studies by several authors have shown the presence of several secondary metabolites in the organs of *M. lucida* (Owolabi et al*.,* 2014; Adebayo et al*.,* 2020). The diversity of these secondary metabolites at the level of each plant gives it a wide range of biological activities. Indeed, some authors have shown the traditional use of these plants in the treatment of several diseases such as microbial and viral infections, diabetes, malaria, cancer (Ezuruike and Prieto, 2014; Kumar et al*.,* 2010). Therefore, antimicrobial activity of the extracts of these two plants was evaluated *in vitro* in our study.

The antimicrobial activity has shown that the susceptibility of the microorganisms to tested extracts varies according to the plants and the types of extracts. The variation observed at the plant level is due to the chemical composition of each plant which can be influenced by several factors such as: the soil and pedological conditions that highlight the plant's nutrition (Durand, 2007; Stewart et al*.,* 2001) on which the formation and expression of secondary metabolites depends (Fritz et al*.,* 2006). Moreover, these observations can be explained on the one hand by the conditions and harvesting period of organs. Indeed, some authors (Slimestad and Verheul, 2005; Toor et al*.,* 2006) showed the unequal distribution of secondary metabolites in plant organs between time intervals. These variations are due, among other things, to light and temperature conditions (Riga et al*.,* 2008). Moreover, regarding the extracts, the affinity that the extraction solvents exhibit according to their polarity with the phytomolecule (Bourgou et al*.,* 2016) would be the basis of the intrinsic difference (for the same plant) observed. The extracts inhibited the proliferation of microorganisms tested with inhibition diameters ranging from 7.50±0.40 to 25.00±0.00 mm. The ratio of Minimal Bactericidal Concentrations (MBC) and Inhibition (MIC) according to a previous study (Berche et al*.,* 1991) showed that some extracts have bactericidal effects which shows a good antibacterial activity. The antimicrobial activity observed with these plants is attributed to their chemical composition. In addition, other authors like Naqvi et al*.* (2020) showed promising antibacterial activities, >18.5 ± 0.21 mm zone-of-inhibition against *S. aureus*, and 18.4 ± 0.17 mm zone-of-inhibition against *Escherichia coli* with methanol extract of *M. charantia*. Ale (2020) reported that the aqueous extracts of different parts of *M. lucida*

were found to be effective against *S. aureus*, *B. subtilis*, *E. coli*, and *P. aeruginosa*. In addition, Olawuwo et al. (2020) investigate the *in vitro* antifungal activity of acetone and aqueous extracts of *M. lucida* (Rubiaceae) against ATCC strains of *Aspergillus fumigatus* , *A. flavus, C. albicans* and *Cryptococcus neoformans* as well as clinical isolates of *A. fumigatus* and *C. albicans* and showed that the minimum inhibitory concentration (MIC) of both extracts against tested organisms ranged from 0.11 to 2.50 mg/ml and 0.03 to 2.50 mg/ml after 48 and 72 h respectively. In the present study, MIC obtained with *M. lucida* extracts range from 0.468 to 3.75 mg/ml respectively for methanol and acetone extracts. This similitude between the results shows that *M. lucida* extract has antifungal activity.

Conclusion

From the results obtained, TLC analysis showed many spots that suggest that both plants' extracts contain many secondary metabolites. HPTLC revealed the presence of Quercetine, cafeic acid and vanilic acid in the plant extract. The presence of those compounds confers on these plants the antimicrobial activity. *M. charantia* extracts inhibited the proliferation of the test strains than those of *M. lucida* extracts. The Dichloromethane extract of *M. charantia* displays more bactericidal effect of references strains than those of *M. lucida*. The purified extracts of *M. charantia* and *M. lucida* can be useful both in food conservation and in human medicine.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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