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"In vitro" antimicrobial activity of extracts from the leafy stems of *Momordica charantia Linné* (Cucurbitaceae) on some multi-resistant microbial strains

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Abstract

Objective. This study aimed to explore the "*In Vitro*" antimicrobial activity of extracts of leafy stems of *M. charantia* on a few multi-resistant germs.

Methods: Phytochemical screening of *M. charantia* leafy stem powder was carried out by the methods of colorimetry and thin layer chromatography followed by the search for larval cytotoxicity. The sensitivity test by the solid medium diffusion method and the search for resistance genes were carried out on *E. coli* ATCC25922 then on *K. pneumoniae*, *K. oxytoca*, and *E. coli* isolated from hospital samples. Flavonoids, alkaloids, stetol-terpenes and saponosides were identified in the powder of the leafy stems of *M. charantia*.

Results: No cytotoxic effects were observed in *Artemia salina* at the LC 50 of 6.25 mg/ml. With the exception of Ciprofloxacin, Ertapenem and Ceftriaxone which showed respective resistance rates of 60%, 90% and 90%, absolute resistance, i.e. 100%, was observed against Ampicillin, Aztreonam, and Augmentin. The resistance genes present in the bacterial strains studied were SHV, TEM, CTX-M1 and CTX-M15. The sensitivity tests carried out indicate that the aqueous and ethanolic extracts were active on the strains tested with respectively average inhibition diameters of between 9 ±1 and 14 ±1 mM then between 9 ±1 and 12 ±1 mM.

Conclusion: This study revealed antimicrobial activity of each of the aqueous and ethanolic extracts of the leafy stems of *M. charantia* of the multidrug-resistant bacterial strains studied.

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Keywords: *M. charantia;* Resistance genes; Sensitivity test; Antimicrobial activity; Bacterial multi-resistance

1. Introduction

Infectious diseases caused by germs pathogens threaten human health worldwide. During the 20th century, antibiotics contributed to the considerable reduction in mortality linked to infectious diseases which were the leading cause of death in the 1940s [1]. However, the massive use of antibiotics, particularly in human and animal health, has over the years caused selection pressure on bacterial populations [2]. This situation causes the development and diffusion of a diversity of resistant strains, often responsible for repeated therapeutic failures, and today known as one of the threats with complications for global health. In addition to prolonged hospitalization times and exorbitant health expenses, antibiotic resistance significantly increases population mortality [3]. O'Neill Jim estimated that by 2050, more than 10 million people per year would have died from infections linked to multidrug-resistant bacteria and this would once again become the leading cause of death worldwide [4]. This worrying situation makes antibiotic resistance a major public health issue. With a view to the research and development of new, more effective molecules, phytotherapy offers very interesting answers to this problem given that many plant extracts have shown inhibitory activity on several bacterial strains presenting resistance to antibiotics [2; 5]. *Momordica charantia* (*M. charantia*) is a plant with multiple medicinal properties used in the preparation of remedies in many countries. This plant has been reported to possess innumerable biological activities including anthelmintic, antibacterial, antidiabetic, anti-inflammatory, and antioxidant [6]. *M. charantia* is therefore known to contain antibacterial compounds, but scientific evidence of their potential to combat multi-antibiotic-resistant germs remains poorly documented. This study focused on the *In Vitro* antimicrobial activity of extracts from the leafy stems of *M. charantia* L. on some multi-resistant germs.

2. Material and method

2.1. Plant material

The plant material consisted of the leafy stems of *M. charantia*. The fresh leafy stems of *M. charantia* were harvested during the month of December 2020 in Porto-Novo, a town located approximately 32 kilometers from Cotonou.

2.2. Bacterial material

The bacterial material used consisted of a reference strain of *E. coli* ATCC25922 and 24 bacterial strains from fecal sludge from septic tanks of the Ouémé-Plateau Departmental University Hospital. These strains were stored at -36 °C in the Applied Microbiology and Pharmacology of Natural Substances Research Unit. *Artemia salina* shrimp eggs were used to carry out the larval cytotoxicity test. The molecular genetic markers used are recorded in the table below

Table 1 Molecular genetic markers sought

Genes	Primers	Size 5'-3'	References
TEM	TEM F	ATGAGTATTCAACATTTCCGC	[7]
	TEM R	CAATGCTTAATCAGTGAGG	[7]
SHV	SHV F	AAGATCCACTATCGCCAGCAG	[7]
	SHV R	ATTCAGTTCCGTTTCCCAGCGG	
CTX-M1	CTX-M1 F	GGTTAAAAAATCACTGCGTC	[7]
	CTX-M1R	TTGGTGACGATTTTAGCCGC	
CTX-M15	CTX-M15 F	CACACGTGGAATTTAGGGACT	
	CTX-M15R	GCCGTCTAAGGCGATAAACA	
IMP	IMP F	GGAATAGAGTGGCTTAATTC	[8]
	IMP R	GGTTTAACAAAACAACCACC	
VIM	VIM F	GCACTTCTCGCGGAGATTG	[9]
	VIM R	CGACGGTGATGCGTACGTT	

GES	GES F	GCAATGTGCTCAACGTTCAAG	[10]
	GES R	GTGCCTGAGTCAATTCTTTCAAAG	
NDM	NDM F	GGCCACACCAGTGACAATATCA	[11]
	NDM R	CAGGCAGCCACCAAAAGC	
KPC	KPC F	GCCGCCAATTTGTTGCTGAA	[12]
	KPC R	GCCGGTCGTGTTTCCCTTT	
OXA 48	OXA 48 F	TGTTTTTGGTGGCATCGAT	[13]
	OXA 48 R	GTAAMRATGCTTGGTTCGG	
QNR A	QNR AF	AGGATTCTCACGCCAGGATT	[14]
	QNR AR	CCGCTTTCAATGAAACTGCAA	

TEM=Temoniera; SHV=Sulfhydryl Variable; CTX-M1=Cefotaximase Munich 1; CTX-M15=Cefotaximase Munich 15; IMP=Imipenemase; VIM=Verona Integron-encoded Metallo-β-lactamases; GES=Guyana Extended Spectrum; NDM=New Delhi Metallo-β-lactamases; KPC = *Klebsiella pneumoniae* carbapenemase; OXA 48= Oxacillinase-48; QNR A=Quinolone Resistance A

2.3. Methods

2.3.1. Collection of material

After harvest, the leafy stems were rinsed with distilled water and then dried in the laboratory at an average temperature of 25 °C. Once dried, the leafy stems of *M. charantia* were ground and powdered. Then, the powder obtained was subjected to aqueous and ethanolic extraction. The bacterial material was composed of stool samples and waste water taken from the pits and cesspools of the Ouémé-Plateau Departmental University Hospital Center in Porto-Novo. Shrimp larvae eggs of *Artemia salina* and the molecular genetic markers were purchased commercially. Once the shrimp eggs were purchased, these eggs were dissolved in salted water then left to stir for 24 hours to allow the young larvae to hatch.

2.3.2. Phytochemical screening

The search for the major chemical groups contained in the leaf extracts of *M. charantia* was carried out by a summary qualitative phytochemical analysis based on coloring tests developed by EL-Haoud and colleagues [15].

2.3.3. Thin-layer chromatography

Thin layer chromatography made it possible to demonstrate the existence of chemical groups contained in the powder of the leafy stems of *M. charantia*. In addition to the dry powder, some chemical compounds previously demonstrated in the powder extract of leafy stems of *M. charantia* using the coloring tests were used as reference substances when carrying out the TLC (Ref. Alkaloids, Ref. Flavonoids, Ref. Tannins and Ref. Anthocyanins) conform to the method described by Adounkpè and collaborators [16].

2.4. Evaluation of larval cytotoxicity

As part of our work, the protocol used by Houmènou and colleagues was used [17]. The dose-response data were logarithmically transformed and the Lethal Concentration 50 (LC50) was determined by a polynomial regression study. To interpret these results, correlation grids associating the degree of toxicity CL50 proposed by Mousseux and used by Dehou and colleagues were taken as reference [18].

2.5. Realization of the antibacterial activity of *M. charantia* extracts

The selected bacterial strains were re-isolated on Mueller Hinton and confirmed by standard bacteriology methods [19]. The antibiogram was carried out following the method described by the antibiogram committee of the French Society of Microbiology (2020). Seeding using the Kirby and Bauer method and placing antibiotic disks. A swab soaked in the inoculum was inoculated onto the Mueller Hinton agar plate by passing the swab two or three times over the entire surface of the medium, rotating each time over the entire surface of the medium, each time rotating the box of 60 °C, to ensure uniform seeding. The plates were dried for 15 minutes at 37 °C [20]. The antibiotic disks were placed under aseptic conditions and left for 30 min at room temperature and then incubated at 37 °C for 24 h. After 24 hours of

incubation, the diameters of the inhibition zones were observed and measured using a flat millimeter ruler on the back of the Petri dishes.

2.6. Search for molecular genetic markers

2.6.1. DNA extraction and repair of the Master Mix

A few young pure 24-hour colonies from each strain were added to 200 µL of distilled water. The mixture was mixed by vortex and then centrifuged at 3000 g for 15 min. The supernatant was collected and the pellet was eliminated [21]. The preparation of the reaction medium was carried out following the manufacturer's instructions (Taq DNA Polymerase kit with Standard Taq Buffer from Biolabs). Genes involved in the production of β lactamase such as: TEM, SHV, CTX-M1, CTX-M15, IMP, VIM, GES, NDM, KPC, OXA and QNR A were searched. The reaction medium is summarized in Table II. The quantity of water was adjusted so that the total reaction medium was 25 µL.

Table 2. Composition of the Master Mix

Composition	2.5 µL RXN	Final concentration
10X standard Taq Reaction Buffer	2.5 µL	1X
10 mM dNTPs	0.5 µL	200 µM
10 µM Foward Primer	0.5 µL	0.2 µM (0.05-1µM)
10 µM Reverse Primer	0.5 µL	0.2µM (0.05-1µM)
Taq DNA polymerase	0.125 µL	1,25 units / 50 µL PCR
DNA	3.0 µL	<1,000 ng
Nuclease free Water	25.0 µL	-

Taq DNA polymerase =Taq DNA Polymerase kit with Standard Taq Buffer from Biolabs; RXN = abbreviation to generally refer to a mixture of the reactants necessary for a chemical reaction to take place.

2.6.2. Amplification and migration

The amplification conditions are recorded in Table IV. After amplification, the products resulting from the PCR were migrated on 1.5% agarose gel. The amplified DNA mixture and blue juice were deposited in the wells previously dug in the agarose gel against a size marker and negative and positive controls. The migration was launched for 30 min at 110V. The sizes of the different genes were read under trans-illuminator.

Table 3 PCR parameter

Amplification steps		Amplification conditions		
		Bla TEM, SHV, CTXM-1, CTXM-15	Bla IMP, VIM, KPC, GES, OXA 48, NDM	QNR A
Initial denaturation		94 °C/4min	95 °C/5min	95 °C/10min
Cycle	Denaturation	93 °C/30s	95 °C/20s	95 °C/30s
	Hybridization	55 °C/30s	49 °C/45s	51 °C/30s
	Elongation	72 °C/40s	72 °C/30s	72 °C/15s
Final elongation		72C/4min	72 °C/5min	72 °C/5min
Number of cycles		30	35	40

2.6.3. Evaluation of the antibacterial activity of M. charantia extracts

Preparation of aqueous and ethanolic extracts

The aqueous and ethanolic extracts were made using the protocol written by Agbanpkè and colleagues (2016). Thus, 50g of *M. charantia* powder were dissolved in 500mL of distilled water for the aqueous extract and in 500mL of Ethanol 96° for the ethanolic extract. Each preparation was stirred continuously for 72 hours before being filtered with

Whatman 1 paper. The extracts thus produced were dried and the paste obtained was stored in vials at 4 °C for further work [19]. The total aqueous and ethanolic extracts previously obtained were used to prepare solutions with a concentration equal to 100 mg/ml, which were sterilized by filtration on 0.22 µm millipore membranes.

Determination of the sensitivity of bacterial strains to *M. charantia* extracts

Taking into account our inclusion criteria, 11 strains were selected for carrying out antimicrobial activity. These are 5 strains of *K. oxytoca*, 4 strains of *K. pneumoniae*, a strain of multi-resistant *E. coli* and a strain of *E. coli* ATCC25922.

- Inoculum preparation and sensitivity testing by well diffusion

A portion of each 24-hour pure colony from the identified Mueller Hinton medium was emulsified in 5 ml of physiological water to obtain a turbidity of 0.5 on the McFarland scale. Each inoculum was inoculated by swabbing onto Petri dishes containing Mueller Hinton agar. Using the tip of the sterile Pasteur pipette, wells of 6 mm in diameter were dug. Then using a cone and a micropipette, a volume of 50 µL of each *M. charantia* extract was deposited in the previously dug wells. A well containing sterile distilled water will serve as a negative control. The Petri dishes were left for 1 hour at room temperature for pre-diffusion of the substances, before being incubated at 37°C in the oven for 18 hours [19]. The test was repeated three times. After the incubation period, the plates were examined by measuring the diameters of the inhibition zones. The antibacterial activity of the extracts was determined from these diameters of inhibition zones around the wells.

Determination of antimicrobial activity in liquid and solid media

The determination of the minimum inhibitory concentration was carried out by the 96-well microplate method. The Minimum Bactericidal Concentration was determined by seeding the contents of the first well to the MIC well on Mueller Hinton agar medium and incubated at 37 °C for 18 to 24 hours. On observation, the lowest concentration of the extract which does not allow any bacteria to survive corresponds to the minimum bactericidal concentration. The Antibiotic Power is the ratio R = CMB/MIC. It is bactericidal when $R \leq 2$ and bacteriostatic when $4 \leq R \geq 8$ [19].

2.6.4. Data processing and analysis

The results of the sensitivity tests of the bacterial strains compared to the extracts of *M. charantia* were collected and recorded in the Excel 365 spreadsheet then the means of the standard deviations were calculated.

3. Results

3.1. Phytochemical screening

Phytochemical screening made it possible to determine the large families of chemical compounds contained in the powder of leafy stems of *M. charantia*. The results are recorded in Table 4. Phytochemical analysis revealed the presence of compounds such as flavonoids, alkaloids, stetol-terpenes and saponosides.

Table 4 Chemical groups present in the powder of the leafy stems of *M. charantia*

Metabolites/samples	***M. charantia* powder**
Tannins	Absence
Flavonoids	Presence
Anthocyanins	Absence
Leuco-anthocyanins	Absence
Alkaloids	Presence
Mucilages	Absence
Reducing compounds	Absence
Stetol-terpenes	Presence
Saponosides	Presence

3.2. Thin layer chromatography results

Figure 1a Photos of the chromatograph seen under UV light at 256 nm

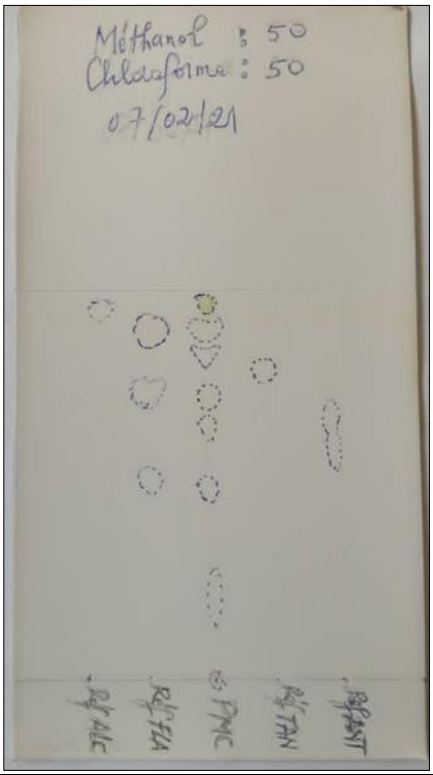

Figure 1b Photos of the chromatograph seen after marking the spots observed with UV

Legend: PMC: *M. charantia* powder, Ref. ALC: Ref. Alkaloids, Ref. FLA: Ref. Flavonoids, Ref. TAN: Ref. Tannins and Ref. ANT: Ref. Anthocyanins.

Reading the chromatograph using 256 nanometer UV light showed that all the substances placed on the thin layer chromatography plate had migrated (fig.1a). The leafy stem powder of *M. charantia* showed at least 8 spots or spots clearly visible under 256 nm UV light and numbered from Spot_1 to Spot_8 from the top to the bottom of the TLC plate. The Alkaloids reference presented three spots in the solvent system Methanol – Chloroform (50:50)

Table 5 Front ratios of the spots appearing after migration in the Methanol – Chloroform solvent system (50:50)

Powder/ Reference substances	Spot	Migration Front Report (Rf)
M. charantia powder	Spot_1	0.91
	Spot_2	0.96
	Spot_3	0.87
	Spot_4	0.72
	Spot_5	0.67
	Spot_6	0.52
	Spot_7	0.36
	Spot_8	0.00
Reference substances	Ref. ALC	0.91 / 0.72 / 0.53*
	Ref. FLA	0.96
	Ref. TAN	0.85
	Ref. ANT	0.65

Ref. ALC: Ref. Alkaloids, Ref. FLA: Ref. Flavonoids, Ref. TAN: Ref. Tannins and Ref. ANT: Ref. Anthocyanins.

Based on the principle that two spots having the same frontal relationship in the same solvent system have a high probability of being identical, thin layer chromatography confirms that the powder of leafy stems of *M. charantia* does indeed contain flavonoids and alkaloids and that tannins and anthocyanins are not represented. These data are consistent with the coloring and identification of chemical groups tests carried out previously. Furthermore, spot 8 having appeared at the point of deposition of the *M. charantia* powder, it would certainly correspond to another (or even several other) chemical groups which could not be displaced in the Methanol – Chloroform solvent system. (50:50) used.

Table 6. Comparison of the frontal ratios of the spots appearing in the *M. charantia* powder to those of each of the reference substances used

Spot	Rf	Interpretations
Spot 1	0.91	Spot 1 is a group of Alkaloids
Spot 2	0.96	Spot 2 is a group of Flavonoids
Spot 3	0.87	Spot 3 does not correspond to any reference substance used
Spot 4	0.72	Spot 4 is a group of Flavonoids
Spot 5	0.67	Spot 5 does not correspond to any reference substance used
Spot 6	0.52	Spot 6 does not correspond to any reference substance used
Spot 7	0.36	Spot 7 does not correspond to any reference substance used
Spot 8	0.00	Spot 8 does not correspond to any reference substance used
Ref FLA	0.91 / 0.72 / 0.53*	Reference Flavonoids
Ref ALC	0.96	Reference Alkaloids
Ref TAN	0.85	Reference Tannins
Ref ANT	0.65	Reference Anthocyanins

Legend: Rf = Frontal report, Ref. ALC=Ref. Alkaloids, Ref. FLA=Ref. Flavonoids, Ref. TAN=Ref. Tannins and Ref. ANT=Ref. Anthocyanins

3.3. Cytotoxicity results of *M. charantia* extracts on *Artemia saliva* larvae

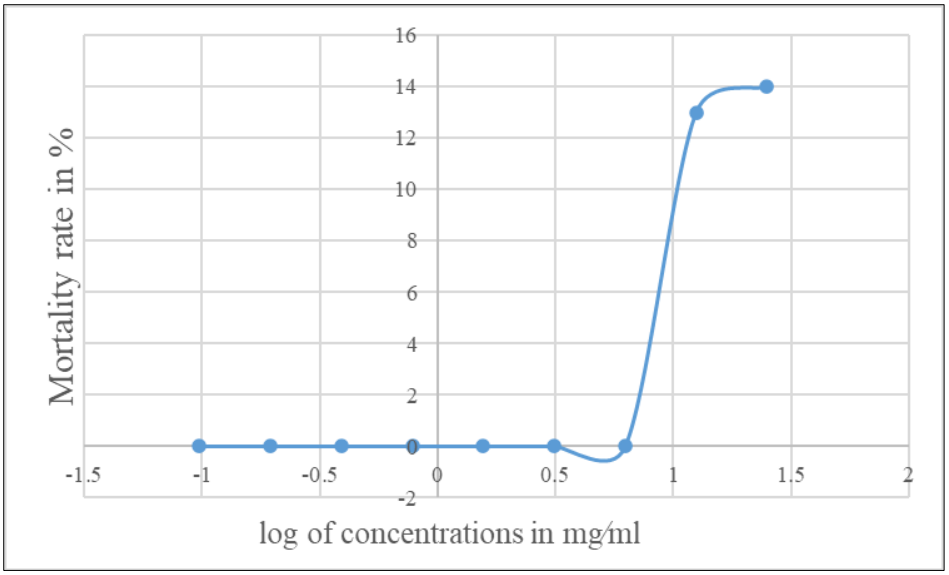

Figure 2. Dose response curve of larval cytotoxicity of extracts from the leafy stems of *M. charantia*

Cytotoxicity of extracts from the leafy stems of *M. charantia* towards Artemia larvae salina has been evaluated. An evolution of the CL50 was observed from the logarithmic curve obtained by the mortality rate as a function of the log concentrations (mg/ml). Figure 2 shows the logarithmic regression curve which represents the relationship between

larval mortality and aqueous extract concentration. Through this figure, as the concentration of the extract increased, the number of surviving larvae decreased. The semi-lethal concentration LC50 is 6.25 mg/ml, which is higher than the upper limit of toxicity of 0.1 mg/ml according to Mousseux (1995). This sensitivity follows a dose-response relationship (Figure 2).

3.4. Resistance profile of selected bacterial strains

Of the 24 bacterial strains selected, 10 bacterial strains meet our inclusion criteria. All of these 10 multi-resistant bacterial strains showed strong resistance to all the classic antibiotics tested and had at least one resistance gene.

Table 7. Resistance profile of selected bacterial strains

Bacterial strains	Origin	Identifiers	Antibiotic resistance profile	Resistance genes found
K. oxytoca	Sediment	KB_1	AMP ^{R} CIP ^{R} ATM ^{R} ETP ^{R} CRO ^{R} AMC ^{R}	OXA-48, TEM
	Sediment	Ko_2	AMP ^{R} CIP ^{R} ATM ^{R} ETP ^{S} CRO ^{R} AMC ^{R}	SHV, TEM
	Eau use	Ko_3	AMP ^{R} CIP ^{R} ATM ^{R} ETP ^{R} CRO ^{R} AMC ^{R}	CTX-M1, CTX-M15, SHV
	Water	Ko_4	AMP ^{R} CIP ^{S} ATM ^{R} ETP ^{R} CRO ^{R} AMC ^{R}	SHV, TEM
	Sells	Ko_5	AMP ^{R} CIP ^{R} ATM ^{R} ETP ^{R} CRO ^{R} AMC ^{R}	SHV
K. pneumoniae	Sediment	Kp_1	AMP ^{R} CIP ^{S} ATM ^{R} ETP ^{R} CRO ^{R} AMC ^{R}	CTXM-15, SHV, TEM
	Selles	Kp_2	AMP ^{R} CIP ^{S} ATM ^{R} ETP ^{R} CRO ^{S} AMC ^{R}	SHV, TEM
	Eau use	Kp_3	AMP ^{R} CIP ^{R} ATM ^{R} ETP ^{R} CRO ^{R} AMC ^{R}	CTX-M1, CTX-M15, SHV, TEM
	Selles	Kp_4	AMP ^{R} CIP ^{I} ATM ^{R} ETP ^{R} CRO ^{R} AMC ^{R}	TEM
E. coli	Used water	Ec_M	AMP ^{R} CIP ^{R} ATM ^{R} ETP ^{R} CRO ^{R} AMC ^{R}	CTX-M1, CTX-M15, SHV
E. coli ATCC25922	Reference strain	Ec_R	None	None

I: Intermediate; S: Sensitive; A: Resistant; AMP: Ampicillin; AMC: Amoxicillin + Clavulanic acid; CRO: Ceftriaxone; ETP: Ertapenem; ATM: Aztreonam; CIP: Ciprofloxacin; Kp: *Klebsiella pneumoniae*; Ko: *Klebsiella oxytoca*; Ec: *Escherichia coli*

3.5. Antimicrobial activity

A total of 11 strains were used, namely 4 strains of *K. pneumoniae*, 5 strains of *K. oxytoca* and 2 strains of *E. coli*. One strain was multi-resistant and the second reference strain was used to evaluate antibacterial activity. The aqueous and ethanolic extracts exhibit an inhibitory effect on the multi-resistant strains tested with an inhibition diameter of between 9.0 **±**1.0 and 14.0 **±**1.6 mm for the aqueous extract and 9.0 **±**1.0 to 12 **±**1 .1 mm for the ethanolic extract

Table 8 Mean ± Standard deviation of inhibition zone diameters of M extracts

Bacterial strains	Identifiers	Average of zone diameters inhibition in mm	
		M. charantia	
		E_H $_2$ O	**E_Et**
K. pneumoniae	Kp_1	0.0 ±0.0	10.0 ±1.0
	Kp_2	10.0 ±1.0	12.0 ±1.1
	Kp_3	10.0 ±1.0	0.0 ±0.0
	Kp_4	14.0 ±1.6	0.0 ±0.0
K. oxytoca	Ko_1	0.0 ±0.0	9.0 ±1.0
	Ko_2	10.0 ±1.0	0.0 ±0.0
	Ko_3	9.0 ±1.0	0.0 ±0.0

	Ko_4	0.0 ±0.0	0.0 ±0.0
	KB_5	9.0 ±1.0	9.0 ±1.0
E. coli	Ec_M	0.0 ±0.0	9.0 ±1.0
E. coli ATCC25922	Ec_R	9.0 ±1.0	10.0 ±1.0

E_H2O: Aqueous extract; E_Et: Ethanolic extract; Kp: *Klebsiella pneumoniae*; Ko: *Klebsiella oxytoca*; Ec_M: Multi-resistant *Escherichia coli*; Ec_R: Reference *Escherichia coli*

Table 9 MIC, CMB and Pa of the different extracts on multi-resistant bacterial strains

Bacterial strains	**Identifiers**	**Parameters of the antibacterial activities of the different extracts: MIC, MBC (mg/ml) and Pa**					
		Aqueous extracts			**Ethanol extracts**		
		MIC	**MBC**	**Pa**	**MIC**	**MBC**	**Pa**
K. pneumoniae	Kp_1	-	-	-	25	100	4
	Kp_2	50	100	2*	50	100	2*
	Kp_3	100	-	-	-	-	-
	Kp_4	50	100	2*	-	-	-
K. oxytoca	Ko_1	-	-	-	50	50	1*
	Is_2	50	100	2*	-	-	-
	Ko_3	100	-	-	-	-	-
	Is_4	-	-	-	-	-	-
	Ko_5	50	100	2*	50	100	2*
E. coli	Ec_M	-	-	-	100	-	-
E. coli ATCC25922	Ec_R	50	100	2*	25	50	2*

E_H 2 O: Aqueous extract; E_Et: Ethanolic extract; Pa: Antibiotic potency (MBC/MIC); MIC: Minimum inhibitory concentration; MBC: Minimum Bactericidal Concentration; Kp: *Klebsiella pneumoniae*; Ko: *Klebsiella oxytoca*; Pa with*: Bactericidal power; Pa without*: Bacteriostatic power

4. Discussion

The present work aimed to explore the *In Vitro* antimicrobial activity of extracts from the leafy stems of *M. charantia* L. on some multi-resistant germs. Qualitative phytochemical analysis of powder from the leafy stems of *M. charantia* revealed the presence of chemical compounds such as flavonoids, alkaloids, stetol-terpenes and saponosides. These results confirm the results of the work of Villarreal-La Torre and colleagues [22]. According to the work of Mada and colleagues, and Shoba and colleagues, the extract of this plant contains tannins [9; 23]. In addition, thin layer chromatography confirms that the powder of the leafy stems of *M. charantia* does indeed contain flavonoids and alkaloids and that tannins and anthocyanins are not represented. These data are consistent with the results of the phytochemical screening. It should be noted that spot 8 appeared at the deposition point of the *M. charantia* powder extract. This suggests that it would certainly correspond to another chemical group (or even several) which could not be displaced in the methanol-chloroform (50:50) solvent system used. This difference with the present study could be explained by the geographical location of the places of collection of the plant, the season, the period of harvesting of leafy stems, the physiological stage of the plant, the handling processes and the composition of the soil of the plants. collection locations could also justify these dissimilarities between the results published by the authors. Cytotoxicity research revealed that the extract does not induce any cytotoxic activity in *Artemia salina* larvae at the semi-lethal concentration of 6.25 mg/ml, greater than 0.1 mg/ml. According to the Mousseux scale [22], subject to further investigations, the aqueous extract of the leafy stems of *M. charantia* by extrapolation would be non-cytotoxic for human cells. These data reinforce the conclusions of a previous study carried out in Benin by [17]. The present study took into account; 24 microbial strains from the hospital environment. These strains come from fecal sludge from the septic tanks of the Ouémé Plateau Departmental University Hospital Center. Of the 24 strains selected, 10 strains were resistant to

all families of antibiotics tested and each had at least 1 resistance gene. These were two bacterial genera composed of 4 species of *K. pneumoniae*, 5 species of *K. oxytoca* and one species of *E. coli*. A study carried out in Benin by Ekhaise and colleagues revealed the presence of these two bacterial genera in hospital wastewater [26].

This proves that septic tanks in hospitals constitute a nest of bacteria. Antibiotic susceptibility testing showed resistance to ampicillin (100%), aztreonam (100%), amoxicillin combined with clavulanic acid (100%), ertapenem (90%), to ceftriaxone (90%), and to ciprofloxacin (60%) on the one hand and on the other hand that these germs harbor genes involved in the production of β lactamase such as SHV, TEM, CTX-M1, CTX- M15. These observed multi-resistances clearly reflect the presence of multi-resistant bacteria in hospital effluents. The work of Debabza and Hammami confirms this situation [27; 28]. This resistance may be linked to the fact that antibiotics are one of the most prescribed drugs in hospitals, among which β-lactams rank first. It could also be linked to the selective pressure exerted by practitioners and the presence of low concentration, non-metabolized antibiotics rejected by hospitals. Extracts from the leaves and stems of *M. charantia* exhibit antimicrobial activity compared to the different strains studied. The aqueous and ethanolic extracts were found to be active on the *E. coli* strain ATCC25922 with a MIC of 50 mg/ml and 25 mg/ml respectively for the aqueous and ethanolic extract, coupled with a bactericidal effect. In addition, only the ethanolic extract was active on the multi-resistant *E. coli* strain possessing 3 resistance genes. Similar proportions were observed by other authors in clinical isolates [22; 29]. Furthermore, the strains of the Klebsiella genus were sensitive to both extracts with the aqueous extract which was more active on 6 out of 9 strains compared to 4 out of 9 strains for the ethanolic extract. These observed inhibitions are followed by a MIC of between 50 and 100 mg/ml for the aqueous extract and by 25 and 100 mg/ml for the ethanolic extract with a bactericidal effect for each of the two extracts. These variations in activities would be linked to the presence of resistance genes identified in these strains. Indeed, *M. charantia* is a plant with multiple active compounds [23]. These compounds may be responsible for the antimicrobial activities of extracts of this plant. Other studies carried out in this direction confirm the antimicrobial activity observed in our study [22; 30-33]. The antibacterial activity observed in the extract is believed to be due to the presence of flavonoids, and alkaloids two phytochemical groups known for their antibacterial properties. It is therefore easy to see that extracts of leaves and stems of *M. charantia* constitute an opportunity, a serious avenue in the search for new molecules against bacterial resistance to antibiotics.

5. Conclusion

At the end of this study, phytochemical screening of the powder from the leafy stems of *M. charantia* revealed the presence of a diversity of chemical groups: flavonoids, alkaloids, stetol-terpenes and saponosides. The toxicity study confirmed an absence of cytotoxicity in *Artemia salina* larvae at therapeutic doses. In addition, the study of antibacterial activity revealed that the different aqueous and ethanolic extracts from this plant have an inhibitory effect on the development of the different microbial strains tested. Extracts from the leafy stems of *M. charantia* would therefore constitute a promising avenue in the research and development of new molecules that are more effective in the treatment of infections due to multi-resistant strains.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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