Investigational
Medicinal Chemistry & Pharmacology

Research Article Open Access

Methanol Extracts from *Manilkara zapota* with Moderate Antibacterial Activity Displayed Strong Antibiotic-Modulating Effects against Multidrug-Resistant Phenotypes

Franklin C. M. Ngongang[1,2], Aimé G. Fankam[1], Armelle T. Mbaveng[1*], Brice E. N. Wamba[1], Paul Nayim[1], Veronique P. Beng[2], and Victor Kuete[1**]

Abstract

Background: The emergence and persistence of multidrug-resistant (MDR) bacteria appears today as a serious threat of growing concern to human health. The aim of this present study was to investigate the antibacterial and antibiotic-modulating activity of the methanol extracts of *Manilkara zapota* (L.) P. Royen (Sapotaceae) against pathogenic strains belonging to Gram-positive bacterium, *Staphylococcus aureus*, and Gram-negative bacteria.

Methods: The antibacterial activity as well as the interactions between the plant extracts and the antibiotics was determined based on the minimum inhibitory concentration (MIC) using the microdilution method. The phytochemical screening of the various extracts was carried out according to the standard qualitative methods.

Results: Phytochemical analysis of the extracts revealed the presence of steroids and the absence of saponins in all the extracts. The other phytochemical classes were selectively distributed in the extracts. The extracts showed significant to moderate antibacterial activities (256 µg/mL ≤ MIC ≤ 1024 µg/mL) against the tested bacteria strains. Therefore, the leaves extract was more active. Furthermore, the leaves and seeds extract of *M. zapota* (at their MIC/2 and MIC/4) strongly potentiated, 2 to 16 folds the activity of tetracycline (TET), kanamycin (KAN), ciprofloxacin (CIP), and chloramphenicol (CHL) on 70% (7/10) to 80% (8/10) of the tested MDR bacteria. They can be sources of products with antibiotic modifying activity.

Conclusions: This study demonstrates that, the leaves extract of *Manilkara zapota* has moderate antibacterial and antibiotic modulatory activities, and therefore could be an interesting weapon against MDR bacteria.

Keywords: *Manilkara zapota;* multidrug-resistant; antibiotic; antibacterial.

*Correspondence: Tel.: +237 675468927; E-mail address: armbatsa@yahoo.fr; ORCID: https://orcid.org/0000-0003-4178-4967 (Prof. Dr A.T. Mbaveng); Tel.: +237 677355927; E-mail address: kuetevictor@yahoo.fr; ORCID: http://orcid.org/0000-0002-1070-1236 (Prof. Dr Victor Kuete)

Other authors emails: Franklin C. M. Ngongang: franklinngongang@yahoo.fr ; Aimé G. Fankam: agfankam@yahoo.fr : Paul Nayim: nayimpaul@yahoo.fr ; Brice E. N. Wamba: wambaelvis@yahoo.fr ; Veronique P. Beng: v.penlap@yahoo.fr

[1]Department of Biochemistry, Faculty of Science, University of Dschang, Dschang, Cameroon; [2]Department of Biochemistry, Faculty of Science, University of Yaoundé I, Yaoundé, Cameroon

Citation on this article: Ngongang FCM, Fankam AG, Mbaveng AT, Wamba BEN, Nayim P, Beng VP and Kuete V. Methanol Extracts from Manilkara zapota with Moderate Antibacterial Activity Displayed Strong Antibiotic-Modulating Effects against Multidrug-Resistant Phenotypes. Investigational Medicinal Chemistry and Pharmacology (2020) 3(1):37; Doi: https://dx.doi.org/10.31183/imcp.2020.00037

Background

Antibiotic treatment is one of the main approaches of modern medicine which is used to combat infections. Therefore, their non-judicious use has led to the emergence, spread, and persistence of multidrug-resistant (MDR) bacteria which appear today as a serious threat of growing concern to human health [1-3]. That is why, numerous important organizations, like the Centers for Disease Control and Prevention (CDC), and the World Health Organization (WHO) have declared antibiotic resistance to be a "global public health concern [4, 5]. The development of new treatments of bacterial infections appears amongst the strategies to address the antibiotic resistance of clinically important pathogens. In this direction, substances from plants could be an alternative because it has been shown that they have antimicrobial substances as well as those which may affect the activity of antibiotics by enhancing or reducing it [8 -10].

Manilkara zapota (L.) P. Royen, (synonyms: *Manilkara zapotilla, Manilkara achras, Mimusopus manilkara, Achras zapota*, and *Achras sapota*), commonly known as "sapodilla" in English [11], is a native to Mexico and Central America. It is the most known fruit tree species of Sapotaceae familly [12]. *Manilkara zapota* is mainly distributed in pantropical regions and cultivated for its fruit, timber and latex [13, 14]. Various parts of that plant are used in folk medicine in the management of inflammation, pain, fevers, coughs, diarrhea, dysentery, because they present diuretic and tonic properties and prevent formation of kidney and bladder stones; in addition, the fruit is edible due to its high nutritional content [11, 14]. Scientifically, *M. zapota* has demonstrated several biological activities amongst which anti-inflammatory and anti-pyretic [15, 16]; antidiarrheal [17]; analgesic [18]; antimicrobial [19, 20]; and antitumor [21, 22].

The aim of this study was to investigate the antibacterial and antibiotic-modulating activities of leaves, pericarps and seeds extracts of *M. zapota* against a panel of reference and multidrug resistant (MDR) strains belonging to Gram-positive bacterium, *Staphylococcus aureus*, and Gram-negative bacteria.

Methods

Plant materials and extraction

Manilkara zapota (L.) P. Royen (Sapotaceae) was collected in Souza-Moungo, Littoral region of Cameroon, and identified at the National Herbarium (Yaoundé, Cameroun) where the voucher specimen was deposited under the registration number 67008/HNC.

Preparation of Plant Extract.

Leaves, pericarps and seeds of *M. zapota* collected were cleaned, air-dried, and the powder of each sample (150 g) was soaked in methanol (500 mL) for 48 h at room temperature and then filtered using Whatman filter paper number 1. Next, each filtrate was collected and concentrated under reduced pressure using a rotary evaporator to yield a residue which constituted the crude methanol extract. All extracts were then kept at 4 ºC until further use.

Chemicals for Antibacterial Assays

Eight reference antibiotics (RA) were used in this study were purchased from Sigma-Aldrich (St Quentin Fallavier, France) They included: ampicillin (AMP), cefepime (CEF); chloramphenicol (CHL), ciprofloxacin (CIP), erythromycin (ERY), kanamycin (KAN), streptomycin (STP) and tetracycline (TET). *p*-Iodonitrotetrazolium (INT) (Sigma-Aldrich) chloride was used as microbial growth indicator. Dimethyl-sulfoxide (DMSO) was used to dissolve the plant extracts.

Bacteria Strains and Culture Media, and Growth Conditions.

A panel of 47 strains belonging to Gram-positive bacterium, *Staphylococcus aureus*, and Gram-negative bacteria were used in the study. Gram-negative bacteria included MDR isolates (laboratory collection) and reference strains of *Escherichia coli* (ATCC8739, ATCC10536, AG100, AG100ATet, AG102, MC4100, W3110), *Enterobacter aerogenes* (ATCC13048, EA27, EA289, EA298, EA294), *Klebsiella pneumoniae* (ATCC11296, KP55, KP63, K24), *Providencia stuartii* (NEA16, PS2636), *Enterobacter cloacae* (ECCI69), and *Pseudomonas aeruginosa* (PA01, PA124). The clinical strains were the laboratory collection from UMR-MD1, University of Marseille, France. The strains of *Staphylococcus aureus* used were as follows: a reference strain obtained from American Type Culture Collection (ATCC) (ATCC 25923), 1 methicillin-sensitive *S. aureus* (MSSA1), 7 methicillin-resistant *S. aureus* (MRSA) strains (MRSA3, MRSA4, MRSA6, MRSA8, MRSA9, MRSA11, MRSA12) (obtained from the culture collection of the Laboratory of Microbiology, Graduate School of Pharmaceutical Sciences, The University of Tokyo, Japan, and provided by Dr. Dzoyem of the University of Dschang [23, 24], and 17 resistant clinical laboratory strains of *S. aureus* (SA01, SA07, SA18, SA23, SA36, SA39, SA56, SA64, SA68, SA88, SA114, SA116, SA124, SA126, SA127, SA135, SA139) available in our Laboratory collection and previously isolated from patients in Ad-Lucem Hospital in Banka-Bafang (West Region of Cameroon) [25, 26]. The bacterial features are reported in Tables S1 and S2 (Supplementary Materials). These bacteria were maintained at 4 °C and sub-cultured overnight on a fresh Mueller Hinton Agar (MHA) before any antibacterial assay. Mueller Hinton broth (MHB) was used as liquid culture medium for antibacterial assays.

Preliminary Phytochemical Investigations.

The major classes of potential antibacterial phytochemicals such as triterpenes (Liebermann-Burchard's test), sterols (Salkowski's test), alkaloids (Mayer's test), polyphenols (ferric chloride test), flavonoids (aluminum chloride test), anthraquinones (Borntrager's test), saponins (foam test), and tannins (gelatin test) were investigated as previously described [27, 28].

Antibacterial Assays

The antibacterial activity of the different samples was determined by micro-dilution using INT colorimetric assay [29] with some modifications as previously described [30]. Briefly, the samples were dissolved in 10% dimethyl-sulfoxide (DMSO) /Mueller Hinton Broth (MHB) and serially diluted two-fold (in a 96-well microplate). Then, 100 µL of inoculum (2×10^6 CFU/mL) prepared in MHB was added in each well. Chloramphenicol or ciprofloxacin were used as reference drugs and the well containing the vehicle (DMSO 2.5%) as control. The plates were then covered with a sterile plate sealer and gently-shaken to mix the contents of the wells. The microplates were incubated at 37◦C for 18 h. MIC value of each sample, defined as the lowest sample concentration that inhibited complete bacteria growth was detected following addition of 40 µL INT (0.2 mg/mL) and incubation at 37 °C for 30 min. Each assay was performed in three independent tests in triplicate.

Antibiotic-Resistance Modifying Assay

The antibiotic-modifying effect of the extracts was evaluated by determining the MICs of antibiotics in the presence or absence of the plant extracts in the 96-wells plate as previously described. Briefly, after serial dilutions of antibiotics (256–0.5 µg/mL), the plant extracts at their sub-inhibitory concentrations (MIC/2 and MIC/4; selected after preliminary study assessed against *P. aeruginosa* PA124) (Table S1, Supplementary file) were added. The MIC of each treatment was determined as described above. Each assay was performed in three independent tests in duplicate. Modulation factors (MF), calculated as MIC of antibiotic alone/MIC of antibiotic alone + extract; were used to express the antibiotic-modulating effects of the plant extracts [8, 31].

Results

Qualitative Phytochemical Composition of the Extracts

The major classes of phytochemicals of the extracts of *M. zapota* were assessed and the results are summarized in Table 1. Steroids were present in all the extracts whilst alkaloids, anthraquinones, anthocyanin and saponins were absent. In addition, triterpenes, tannins, and polyphenols were found both in the leaves and seeds extracts of *M. zapota*.

Antibacterial Activity of the Extracts

The antibacterial activity of leaves, pericarps and seeds extracts, and CHL against 21 Gram-negative bacteria (Table 2) or CIP against 27 strains of *S. aureus* (Table 3) was determined. Results showed that those extracts presented selective antibacterial activity against all the strains of *S. aureus* and Gram-negative bacteria within the MIC range of 256-512 μg/mL. Therefore, the leaves extract was the most active, being active against 15/21 (71.43%) strains of Gram-negative bacteria (Table 2), and 15/26 (57,69 %) strains of *S. aureus* (Table 3). Extract of seeds was also active against 11/26 (42.31%) *S. aureus* strains (Table 3). The lowest MIC value of 256 μg/mL was noted only against *S. aureus* MRSA9 and MRSA4 strains, respectively with leaves and seeds extracts of *M. zapota*. The MICs of CHL were between 4 and 128 μg/mL against Gram-negative bacteria (Table 2) whilst those of CIP were below 4 μg/mL against *S. aureus* strains (Table 3).

Antibiotic-Resistance Modulation Activity of the Extracts

The leaves, pericarps and seeds extracts of *M. zapota* at MIC/2, MIC/4, MIC/8, and MIC/16 were first tested in combination with 8 antibiotics (CHL, TET, CIP, AMP, CEF, ERY, STR, and KAN) against *P. aeruginosa* PA124 (Table 4). It appeared that the best antibiotic-modulating effects were obtained with the extracts at MIC/2 and MIC/4. Globaly, extracts of *M. zapota* at MIC/2 and MIC/4, had increased 2-fold or more the activities of 4/8 antibiotics. Consequently, they were further tested in combination with six antibiotics (CHL, TET, CIP, ERY, STR, and KAN) against 10 Gram-negative bacteria, at MIC/2 and MIC/4 (Tables 5–7). Results showed that 2-fold or more increase of the antibiotics activities were observed against 30 to 80% of the tested resistant bacteria, mainly with the of leaves and seeds extracts. Leaves extract potentiated the activities of CIP (70%, at MIC/2 and MIC/4), ERY and TET (80%, at MIC/2) (Tables 5). In the case of the seeds extract, modulating effect was observed with CHL (70%, at MIC/2) and with TET (80% and 70%, at MIC/2 and MIC/4, respectively) (Table 7).

Discussion

Phytochemical Composition of Extracts.

Triterpenes, steroids and polyphenols like tannins were detected in the leaves and seeds extracts of *M. zapota* (Table 1). Their presence in these two extracts could explain in part their antibacterial activities observed [32, 33]. Previous chemical study of that plant resulted in isolation of flavonoids [34], tannins (mainly from unripe fruits) [34, 35] and triterpenes [36, 37]. This consolidates the presence of the above-mentioned metabolites in both extracts of *M. zapota*.

Antibacterial activity

Screening in order to search for new therapeutic solutions based on active compounds known in plants. This is especially important due to the observed increasing resistance of bacteria to antibiotics [38]. In this study, clinical strains of *S. aureus* as well as several Gram-negative bacteria tested were previously reported as resistant to at least one commonly used antibiotic (Tables S1 and S1, Supplementary file). According to the cut-off values indicating the antibacterial activity of an edible plant extract or its part proposed by Tamokou *et al*. [39], the leaves and seeds extracts of *Manilkara zapota* have presented significant (100 < MIC ≤ 512 µg/mL) to moderate (512 < MIC ≤ 2048 µg/mL) against the tested bacteria (Table 2-3), although the leaves extract was more active. It had significant activity against 29.62% (8/27) *S. aureus* strains tested, among which some MDR bacteria (SA01, SA39, SA114, MRSA3, MRSA6, MRSA9, MRSA11, MRSA112). It also displayed significant activity against 33,33% (7/21) of Gram-negative bacteria tested (*E. Coli*: ATCC 8739, AG100; *E. aerogenes*: ATCC 13048, EA27; *Klebsiella pneumoniae*: ATCC11296, KP55; *P. stuartii*: PS2636, NEA16). Several other studies have shown the *in vitro* antibacterial activities of at least one part of *Manilkara zapota*. It is the case of the work carried out by Banerjee *et al.* [40], which showed the antibacterial activity of the methanol and ethanol extracts of the leaves of *M. zapota* against Gram-negative bacteria. Ethyl acetate extract of leaves of *M. zapota* has also shown to be active against some bacteria strains including *Bacillus subtilis*, *Bacillus megaterium*, *Escherichia coli* and *Salmonella typhi* [41]. Furthermore, Priya *et al.* [20] have shown that aqueous and methanol extracts of flowers of this plant are active against *S. aureus*, *B. subtilis*, *P. aeruginosa*, and *S. typhi*. Being in agreement with the results of the previous works, this study also shows that the leaves extracts of *M. zapota* could be used to fight infections involving MDR bacteria.

Antibiotic-Modulation Effects of Extracts

Multidrug resistance (MDR) is a serious threat to human health and constitutes a growing challenge in medicine. The literature review has showed that extracts of medicinal or edible plants can be an alternative source of resistance modifying substances [38, 42, 43]. Tables 5–7 present the antibiotic-modulating activity of the leaves, pericarps and seeds extracts of *M. zapota* at MIC/2, MIC/4, in combination with 6 antibiotics (CHL, TET, CIP, ERY, STR, and KAN) against selected MDR bacteria. Extracts of leaves and seeds of *M. zapota* have improved the activity of some of the

tested antibiotics on more than 70% of the MDR bacteria used. It was the case of the leaves extract in combination with CIP (at MIC/2 and MIC/4), ERY and TET (at MIC/2) (Table 5), and that of the seeds extract in combination with CHL (at MIC/2), TET (at MIC/2 and MIC/4) (Table 7). It is known that natural products able to potentiate the activity of antibiotics on more than 70% of bacteria could be suggested as potential efflux pumps inhibitors [44]. Moreover, bacteria used in this part of our study express efflux pumps as one the resistance mechanism. This suggests that the leaves and seeds extract of *M. zapota* could contain the efflux pumps inhibitors, thus leading to an increase in the effectiveness of antibiotics [45]. According to Okusa and Duez [46], such effects may be due to the presence of alkaloids, flavonoids, terpenoids and tannins in those extracts. This study presents for the first time the potential of the tested plant extracts mainly that of leaves of *M. zapota* to reverse antibiotic resistance.

Table 1. Phytochemical composition of the extracts of *Manilkara zapota.*

Plant	Parts used	Phytochemical composition								
		Triterpenes	Flavonoids	Alkaloids	Anthraquinones	polyphenols	Anthocyanines	Saponins	Tannins	Steroids
M. zapota	Leave	+	+	-	-	+	-	-	+	+
	Pericarps	-	-	-	-	-	-	-	-	+
	Seeds	+	-	-	-	+	-	-	+	+

+ : present ; - : absent.

Table 2. Minimal inhibitory concentration (MIC) of the extracts of *M. zapota against* Gram-negative bacteria.

Gram-negative bacteria	Tested samples and MIC in μg/mL			
	Leaves	**Pericaps**	**Seeds**	**CHL**
E.coli				
ATCC8739	-	-	-	8
ATCC10536	-	-	-	4
AG100	1024	-	-	32
AG102	**512**	-	-	32
AG100ATet	1024	-	-	4
MC4100	1024	-	-	128
W3110	-	-	-	8
E. aerogenes				
ATCC 13048	**512**	1024	1024	8
EA27	**512**	-	-	128
EA289	1024	1024	-	4
EA294	-	-	-	2
EA298	1024	-	-	8
K. pneumoniae				
ATCC11296	**512**	-	-	8
K24	1024	-	-	16
KP55	**512**	-	**512**	64
KP63	-	-	-	16
P. stuartii				
NEA16	**512**	-	1024	64
PS2636	**512**	-	-	64
E. Cloacae				
ECCI69	1024	-	-	128
P. aeruginosa				
PA01	1024	-	-	128
PA124	-	-	-	32

MIC: Minimal inhibitory concentration; **CHL**: Chloramphenicol; **-**: > 1024.

Table 3. Minimal inhibitory concentration (MIC) of the extracts of *M. zapota* against *Staphylococcus aureus strains.*

Staphylococcus aureus **strain**	**Tested samples and MIC (µg/mL)**			
	Leaves	**Pericaps**	**Seeds**	**CIP**
ATCC25923	-	-	-	<0.5
SA01	**512**	-	-	<0.5
SA07	-	-	-	<0.5
SA18	-	-	-	<0.5
SA23	1024	-	**512**	<0.5
SA36	-	-	-	1
SA39	**512**	-	1024	<0.5
SA56	-	-	-	<0.5
SA64	1024	-	-	4
SA68	-	-	-	<0.5
SA88	1024	-	-	<0.5
SA114	**512**	1024	1024	<0.5
SA116	-	-	-	<0.5
SA124	-	-	1024	<0.5
SA126	-	-	-	<0.5
SA127	-	-	-	<0.5
SA135	-	-	-	<0.5
SA139	1024	-	-	<0.5
MSSA1	1024	-	**512**	2
MRSA3	**512**	-	1024	2
MRSA4	1024	-	**256**	1
MRSA6	**512**	-	-	2
MRSA8	1024	-	1024	2
MRSA9	**256**	-	1024	2
MRSA11	**512**	-	1024	2
MRSA12	**512**	-	1024	2

MIC: Minimal inhibitory concentration; CIP: ciprofloxacin; -: > 1024.

Table 4. MIC of antibiotics in combination with extracts of *M. zapota* at sub-inhibitory concentrations against *P. aeruginosa* PA124.

Plant Extracts [a]	Extract concentrations	Antibiotics [b] and minimal inhibitory concentration (µg/mL) and fold increase (in brackets)							
		CHL	**AMP**	**ERY**	**STP**	**KAN**	**TET**	**CIP**	**CEF**
	0	32	-	32	64	64	16	16	-
	MIC/2	128 (0.25)	-	16 (**2**)	128 (0.5)	32 (**2**)	8 (**2**)	2 (**8**)	-
MZL	MIC/4	128 (0.25)	-	32 (1)	256 (0.25)	32 (**2**)	16 (1)	8 (**2**)	-
	MIC/8	64 (0.5)	-	32 (1)	256 (0.25)	32 (**2**)	16 (1)	16 (1)	-
	MIC/16	64 (0.5)	-	32 (1)	256 (0.25)	32 (**2**)	16 (1)	16 (1)	-
	MIC/2	32 (1)	-	32 (1)	32 (**2**)	32 (**2**)	8 (**2**)	16 (2)	-
MZP	MIC/4	32 (1)	-	32 (1)	32 (**2**)	32 (**2**)	8 (**2**)	16 (1)	-
	MIC/8	32 (1)	-	32 (1)	64 (0.5)	32 (**2**)	8 (**2**)	16 (1)	-
	MIC/16	32 (1)	-	32 (1)	64 (0.5)	32 (**2**)	8 (**2**)	16 (1)	-
	MIC/2	16 (**2**)	-	32 (1)	32 (**2**)	16 (**4**)	4 (**4**)	16 (1)	-
MZS	MIC/4	32 (1)	-	32 (1)	32 (**2**)	32 (**2**)	8 (**2**)	16 (1)	-
	MIC/8	32 (1)	-	32 (1)	32 (**2**)	32 (**2**)	8 (**2**)	16 (1)	-
	MIC/16	32 (1)	-	32 (1)	32 (**2**)	32 (**2**)	8 (**2**)	16 (1)	-

[a]: *Manilkara zapota* Leave (MZL); *Manilkara zapota,* Pericarps (MZP); *Manilkara zapota,* Seeds (MZS). [b]: TET: tetracycline, KAN: kanamycin, STR: streptomycin, ERY: erythromycin, CHL: chloramphenicol; NOR: norfloxacin, CIP: ciprofloxacin, AMP: ampicillin; CEF: cefepime. **-**: MIC not detected at up to 256 µg/mL; Values in bold indicate antibiotic-modulating effect ≥2.

Table 5. Antibiotics modulating effect of the extract leaves of *M. zapota* at MIC/2 and MIC/4 on selected MDR bacteria.

Antibiotics	Extract concentration	Bacteria, MIC (*µ*g/mL), and modulating factors (in bracket)										Antibiotic modulating effect (%)
		E. coli		*E. aerogenes*		*K. pneumoniae*		*P. stuartii*		*P. aeruginosa*		
		AG102	**AG100$_{Tet}$**	**EA27**	**EA289**	**KP55**	**KP63**	**PS2636**	**NEA16**	**PA124**	**PA01**	
	0	64	8	64	64	64	64	32	64	32	64	
CHL	MIC/2	16 (**4**)	2 (**4**)	16 (**4**)	64 (1)	64 (1)	32 (**2**)	16 (**2**)	16 (**4**)	128 (0.25)	64 (1)	60.00
	MIC/4	16 (**4**)	2 (**4**)	32 (**2**)	64 (1)	64 (1)	64 (1)	32 (1)	32 (**2**)	128 (0.25)	64 (1)	40.00
	0	8	1	1	1	8	1	4	2	16	2	
CIP	MIC/2	2 (**4**)	≤ 0.5 (≥**2**)	1 (1)	≤ 0.5 (≥**2**)	8 (1)	≤ 0.5 (≥**2**)	0.5 (**8**)	2 (1)	2 (**8**)	1 (**2**)	**70.00**
	MIC/4	4 (**2**)	≤ 0.5 (≥**2**)	1 (1)	≤ 0.5 (≥**2**)	16 (0.5)	≤ 0.5 (≥**2**)	0.5 (**8**)	2 (1)	8 (**2**)	1 (**2**)	**70.00**
	0	32	4	16	32	64	64	8	32	64	16	
KAN	MIC/2	16 (**2**)	4 (1)	4 (**4**)	64 (0.5)	64 (1)	128 (0.5)	4 (**2**)	16 (**2**)	32 (**2**)	8 (**2**)	60.00
	MIC/4	16 (**2**)	4 (1)	4 (**4**)	64 (0.5)	64 (1)	128 (0.5)	4 (**2**)	16 (**2**)	32 (**2**)	8 (**2**)	60.00
	0	64	8	16	64	64	32	16	32	32	16	
ERY	MIC/2	32 (**2**)	8 (1)	4 (**4**)	32 (**2**)	16 (**4**)	16 (**2**)	2 (**8**)	8 (**4**)	16 (**2**)	16 (1)	**80.00**
	MIC/4	32 (**2**)	8 (1)	4 (**4**)	64 (1)	16 (**4**)	32 (1)	8 (**2**)	16 (**2**)	32 (1)	16 (1)	50.00
	0	128	256	256	64	64	256	-	16	64	256	
STP	MIC/2	64 (**2**)	256 (1)	64 (**4**)	128 (0.5)	32 (**2**)	64 (**4**)	128(≥**2**)	16 (1)	128 (0.5)	256 (1)	50.00
	MIC/4	64 (**2**)	256 (1)	64 (**4**)	128 (0.5)	32 (**2**)	256 (1)	256 (nd)	32(0.5)	128 (0.5)	256 (1)	30.00
TET	0	8	≤ 0.5	64	32	16	32	4	32	16	16	
	MIC/2	4 (**2**)	≤ 0.5 (n.a)	32 (**2**)	16 (**2**)	16 (1)	8 (**4**)	≤ 0.5 (≥**8**)	4 (**8**)	8 (**2**)	8 (**2**)	**80.00**
	MIC/4	4 (**2**)	≤ 0.5 (n.a)	64 (1)	16 (**2**)	16 (1)	16 (**2**)	≤ 0.5 (≥**8**)	4 (**8**)	16 (1)	8 (**2**)	60.00

[a]: TET: tetracycline, KAN: kanamycin, ERY: erythromycin, CHL: chloramphenicol, CIP: ciprofloxacin, AMP: ampicillin; **- :** MIC not detected at up to 256 µg/mL**; ( ):** Modulation factor or gain of activity; NA: Not applicable, Values in bold indicate antibiotic-modulating effect ≥2; **(%):** Percentage of Antibiotic's modulation Activity by the plant extracts.

Table 6. Antibiotics modulating effect of the pericarps extract of *M. zapota* at MIC/2 and MIC/4 on selected MDR bacteria.

Antibio-tics	Extract concentration	Bacteria, MIC (*µ*g/mL), and modulating factors (in bracket)										Antibiotic modulating effect (%)
		E. coli		*E. aerogenes*		*K. pneumoniae*		*P. stuartii*		*P. aeruginosa*		
		AG102	**AG100$_{Tet}$**	**EA27**	**EA289**	**KP55**	**KP63**	**PS2636**	**NEA16**	**PA124**	**PA01**	
	0	64	8	64	64	64	64	32	64	32	64	
CHL	MIC/2	32 (**2**)	8 (1)	32 (**2**)	64 (1)	32 (**2**)	32 (**2**)	128 (0.25)	32 (**2**)	32 (1)	64 (1)	50.00
	MIC/4	32 (**2**)	8 (1)	32 (**2**)	64 (1)	32 (**2**)	32 (**2**)	128 (0.25)	32 (**2**)	32 (1)	64 (1)	50.00
	0	8	1	1	1	8	1	4	2	16	2	
CIP	MIC/2	2 (**4**)	≤ 0.5 (≥**2**)	1 (1)	0.5 (**2**)	8 (1)	≤ 0.5 (≥**2**)	1 (**4**)	2 (1)	16 (1)	≤ 0.5 (≥**4**)	40.00
	MIC/4	2 (**4**)	≤ 0.5 (≥**2**)	1 (1)	1 (1)	8 (1)	≤ 0.5 (≥**2**)	2 (**2**)	2 (1)	16 (1)	1 (**2**)	40.00
	0	32	4	16	32	64	64	8	32	64	16	
KAN	MIC/2	32 (1)	4 (1)	8 (**2**)	64 (0.5)	128 (0.5)	16 (**4**)	2 (**4**)	2 **(16)**	32 (**2**)	8 (**2**)	60.00
	MIC/4	32 (1)	4 (1)	8 (**2**)	64 (0.5)	128 (0.5)	16 (**4**)	8 (1)	8 (**4**)	32 (**2**)	16 (1)	40.00
	0	64	8	16	64	64	32	16	32	32	16	
ERY	MIC/2	32 (**2**)	8 (1)	16 (1)	32 (**2**)	16 (**4**)	8 (**4**)	16 (1)	8 (**4**)	32 (1)	16 (1)	50.00
	MIC/4	32 (**2**)	8 (1)	16 (1)	64 (1)	16 (**4**)	32 (1)	16 (1)	8 (**4**)	32 (1)	16 (1)	30.00
	0	128	256	256	64	64	256	-	16	64	256	
STP	MIC/2	64 (**2**)	256 (1)	128 (**2**)	128 (0.5)	32 (**2**)	- (<0.5)	256 (≥**2**)	16 (1)	32 (**2**)	64 (**4**)	60.00
	MIC/4	128 (1)	256 (1)	256 (1)	128 (0.5)	32 (**2**)	- (<0.5)	-	16(1)	32 (**2**)	64 (**4**)	30.00
TET	0	8	≤ 0.5	64	32	16	32	4	32	16	16	
	MIC/2	8 (1)	≤ 0.5 (n.a)	16 (1)	16 (**2**)	16 (1)	16 (**2**)	16 (0.25)	16 (**2**)	8 (**2**)	8 (**2**)	50.00
	MIC/4	8 (1)	≤ 0.5 (n.a)	16 (1)	16 (**2**)	16 (1)	16 (**2**)	16 (0.25)	32 (1)	8 (**2**)	8 (**2**)	40.00

[a]: TET: tetracycline, KAN: kanamycin, ERY: erythromycin, CHL: chloramphenicol, CIP: ciprofloxacin, AMP: ampicillin; **- :** MIC not detected at up to 256 µg/mL**; ( ):** Modulation factor or gain of activity; n.a: Not applicable, Values in bold indicate antibiotic-modulating effect ≥2; **(%):** Percentage of Antibiotic's modulation Activity by the plant extracts.

Table 7. Antibiotics modulating effect of the seeds extract of *M. zapota* at MIC/2 and MIC/4 on selected MDR bacteria.

Antibio-tics	Extract concentration	Bacteria, MIC (*µg*/mL), and modulating factors (in bracket)										Antibiotic modulating effect (%)
		E. coli		*E. aerogenes*		*K. pneumoniae*		*P. stuartii*		*P. aeruginosa*		
		AG102	**AG100$_{Tet}$**	**EA27**	**EA289**	**KP55**	**KP63**	**PS2636**	**NEA16**	**PA124**	**PA01**	
	0	64	8	64	64	64	64	32	64	32	64	
CHL	MIC/2	8 (**8**)	2 (**4**)	32 (**2**)	32 (**2**)	64 (1)	32 (**2**)	64 (0.5)	32 (**2**)	16 (**2**)	64 (1)	**70.00**
	MIC/4	16 (**4**)	2 (**4**)	32 (**2**)	32 (**2**)	64 (1)	32 (**2**)	64 (0.5)	32 (**2**)	32 (1)	64 (1)	60.00
	0	8	1	1	1	8	1	4	2	16	2	
CIP	MIC/2	4 (**2**)	≤ 0.5 (≥**2**)	1 (1)	1 (1)	8 (1)	≤ 0.5 (≥**2**)	2 (**2**)	2 (1)	16 (1)	0.5 (**4**)	50.00
	MIC/4	8 (1)	≤ 0.5 (≥**2**)	1 (1)	1 (1)	8 (1)	≤ 0.5 (≥**2**)	4 (1)	2 (1)	16 (1)	0.5 (**4**)	30.00
	0	32	4	16	32	64	64	8	32	64	16	
KAN	MIC/2	64 (0.5)	4 (1)	8 (**2**)	64 (0.5)	64 (1)	256 (0.25)	8 (1)	8 (**4**)	16 (**4**)	16 (1)	30.00
	MIC/4	64 (0.5)	4 (1)	16 (1)	64 (0.5)	64 (1)	256 (0.25)	8 (1)	8 (**4**)	32 (**2**)	16 (1)	20.00
	0	64	8	16	64	64	32	16	32	32	16	
ERY	MIC/2	16 (**4**)	8 (1)	4 (**4**)	128 (0.5)	32 (**2**)	16 (**2**)	4 (**4**)	16 (**2**)	32 (1)	16 (1)	60.00
	MIC/4	32 (**2**)	8 (1)	16 (1)	128 (0.5)	32 (**2**)	16 (**2**)	8 (**2**)	16 (**2**)	32 (1)	16 (1)	50.00
	0	128	256	256	64	64	256	-	16	64	256	
STP	MIC/2	64 (**2**)	- (<0.5)	256 (1)	64 (1)	64 (1)	128 (**2**)	64 (≥**4**)	128 (0.125)	32 (**2**)	128 (**2**)	50.00
	MIC/4	64 (**2**)	- (<0.5)	256 (1)	64 (1)	64 (1)	256 (1)	128 (≥**2**)	256 (0.062)	32 (**2**)	128 (**2**)	40.00
TET	0	8	≤ 0.5	64	32	16	32	4	32	16	16	
	MIC/2	2 (**4**)	≤ 0.5 (n.a)	32 (**2**)	16 (**2**)	16 (1)	16 (**2**)	2 (**2**)	4 (**8**)	4 (**4**)	4 (**4**)	**80.00**
	MIC/4	4 (**2**)	≤ 0.5 (n.a)	32 (**2**)	16 (**2**)	16 (1)	32 (1)	2 (**2**)	4 (**8**)	8 (**2**)	8 (**2**)	**70.00**

[a]**:** TET: tetracycline, KAN: kanamycin, ERY: erythromycin, CHL: chloramphenicol, CIP: ciprofloxacin, AMP: ampicillin; **- :** MIC not detected at up to 256 µg/mL**; ( ):** Modulation factor or gain of activity; Values in bold indicate antibiotic-modulating effect ≥2; **(%):** Percentage of Antibiotic's modulation Activity by the plant extracts.

Conclusions

Globally, the results obtained demonstrates that leave extract of *Manilkara zapota* has moderate antibacterial and antibiotic modulatory activities, and therefore could be an interesting weapon against MDR bacteria. However, more data mainly phytochemical isolation of the active ingredients as well as the toxicological assays must be performed before its use.

Additional file

Supplementary file.docx. Table S1. Gram-negative bacteria and their features; Table S2. Staphylococcus aureus strains and features. Available online at: https://www.investchempharma.com/imcp37-supplementary-file/

Abbreviations

CEF: cefepime
CHL: chloramphenicol
CIP: ciprofloxacin
DMSO: dimethylsulfoxide
ERY: erythromycin
HNC: Herbier National du Cameroun
INT: *p*-iodonitrotetrazolium chloride
KAN: kanamycin
MDR: Multi-drug resistant
MHA: *Mueller Hinton Agar*
MHB: *Mueller Hinton Broth*
MIC: Minimal inhibitory concentration
MZL: *Manilkara zapota* Leave
MZP: *Manilkara zapota,* Pericarps
MZS: *Manilkara zapota,* Seeds
RA: Reference antibiotics
TET: tetracycline

Authors' Contribution

FCMN, BENW, PN carried out the study; ATM, VPB and VK designed the experiments. AGF prepared the data and wrote the manuscript; VK supervised the work and provided the facilities for antibacterial assays; all authors read and approved the final manuscript.

Acknowledgments

Authors are thankful to the Cameroon National Herbarium for identification of plants.

Conflict of interest

The authors declare that there is no conflict of interest.

Article history:

Received: 12 March 2020
Received in revised form: 13 April 2020
Accepted: 14 April 2020
Available online: 14 April 2020

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