Journal of Advances in Medical and Pharmaceutical Sciences

23(8): 25-37, 2021; Article no.JAMPS.76766
ISSN: 2394-1111

Phytochemical Screening and Antimicrobial Activity of *Ziziphus mauritiana* Lam. And *Ziziphus mucronate* Lam. Extracts

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Authors' contributions

This work was carried out in collaboration among all authors. Author SM collected the samples and carried out experiments. Author BJ review and editing the paper and authors DDR and TNL supervise the work. All the authors have read and approved the manuscript.

Article Information

DOI: 10.9734/JAMPS/2021/v23i830252
Editor(s):
(1) Prof. Hamdy A, Sliem, Suez Canal University, Egypt and Qassim university and EL-Jouf university , Saudi Arabia.
Reviewers:
(1) Gabriela Tataringa, Universitatea de Medicină şi Farmacie "Grigore T. Popa", Romania.
(2) Patricia Durán Ospina, Universidad Técnica de Manabí, Ecuador.
Complete Peer review History: https://www.sdiarticle4.com/review-history/76766

Original Research Article

Received 30 August 2021
Accepted 08 November 2021
Published 10 November 2021

ABSTRACT

The objective of this study was to determine the phytochemical profile and antimicrobial activity of leaf and bark extracts of *Ziziphus mauritiana* and *Ziziphus mucronata*. For this purpose, an extraction by maceration using ethanol was carried out and the extracts were subjected to antibacterial activity assessment through qualitative and quantitative approaches. The qualitative tests were performed using agar well diffusion method while for quantitative tests minimal inhibitory, bactericidal and fungicidal concentration (MIC, MBC or MFC) were used determined through microdilution in microplates method. The antimicrobial mechanisms such as cell lysis and action on the proton pump of the extracts were also studied. The highest extraction yield was obtained with

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the *Z. mauritiana* leaf extract (28.8%). In addition, the highest contents of polyphenols (256.6 mg GEA/g DM) and flavonoids (165.2 mg CE/g DM) were obtained with the bark of *Z. mucronata*. The bark extracts of *Z. mauritiana* exhibited highest antibacterial activity (36.7 mm on *Staphylococcus aureus*) while, *Z. mucronata* extracts are more active on fungi, with the highest activity on *Candida albicans* (26.7 mm). The antimicrobial activity of the extract increases with the concentration and, regarding the MBC/MIC and MFC/MIC ratio, the extracts showed bacteriostatic activity on the different bacteria and fungi and the possible mechanism included an activity on the proton pump. The bark and leaf extracts of *Z. mauritiana* and *Z. mucronata* could be used in traditional medicine to treat infections due to these multiple pathogens.

Keywords: Ziziphus mauritiana; Ziziphus mucronata; phytochemical composition; antimicrobial activity.

1. INTRODUCTION

It is well documented that plants are being used as the main source of therapeutics for the human beings for centuries to this date. Medicinal plants have a good importance in the field of research because they are safe to use for the rural communities. They are constituted by wide complex chemical called secondary metabolites, which make them very interested in the field of therapeutics [1]. *Ziziphus* Mill. is one of the most important genera of the family Rhamnaceae, which is widely distributed all over the world and is used for centuries in locale medicine systems [2]. Nowadays, there are about 100 species distributed in tropical and subtropical regions worldwide [1]. *Ziziphus* plants are traditionally used as medicine for the treatment of various diseases such as digestive disorders, urinary troubles, diabetes, skin infections, diarrhea, fever, bronchitis, liver complaints, anaemia, etc [1].

In Cameroon, four species are mostly found such as *Z. mauritiana, Z. mucronata*, *Z. spina-christi* and *Z.abyssinica* whereas the most widespread are *Z. mauritiana and Z. mucronata*. The fruits of *Z. mauritiana* and *Z. mucronate* are consumed in Cameroon as snacks. Carbohydrates, starch, proteins, sugar, and vitamins are abundantly found in *Ziziphus* species. Concerning *Z. mauritiana,* all the parts of this plant are very effective against the different types of diseases. Its leaves are useful in the treatment of diarrhea, wounds, abscesses, swelling and gonorrhoea. Authors reported the presence of bioactive compound including polyphenols, saponins, tannins on *Z. mauritiana* [1]. In addition, Koita *et al.* [2] identified the catechin, rutin (quercetin 3-O-rutinoside), delphinidin-3-glucoside, isoquercetin (hyperoside), quercitrin (quercetin 3,7-O-L-dirhanmopyranoside) in the aqueous extract of the fruits. The methanolic extract exhibited great activity against *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Aspergillus niger*, *Fusarium solani* [3]. On the other hand, *Z. mucronata* fruits are used to treat neurological diseases in South Africa [4] and physicochemical analysis revealed that *Z. mauritiana* possesses many nutrients such as protein, lipids, fiber, phosphorus, iron, sodium, magnesium, potassium, zinc, manganese [5]. Moreover, literature mentioned the interesting antioxidant, choline esterase inhibitory and antimicrobial activities [5,6].

Thus, the aim of the current study was to evaluate the antimicrobial activity of *Z.mucronata* and *Z. mauritiana* against some selected strains and the possible mechanism implicated.

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials

Leaves and barks of the two plants (*Z. mauritiana* and *Z. mucronata*) were collected in polythene from Mokolo (Mayo-Tsanaga Department) and Kaélé (Mayo-Kani Department) localities, Far North region, Cameroon in January 2020. The collection was under specialist supervision and these plants are commonly known to everyone. These plants were authenticated by at IRAD (specialized organisms) in Maroua. The various plant parts were thoroughly washed, sun-dried for 10–14 days and ground into powder using a laboratory mill prior to analysis and packed in polythene bags for further use.

2.2 Biological Material

Antimicrobial activity of *Z. mauritiana* and Z. *mucronate* extracts was determined against Gram-positive microorganisms using

Staphylococcus aureus (UBOCC-A-316003), Gram-negative microorganisms as *Pseudomonas aeruginosa* (UBOCC-A-316004) and fungi as *Candida albicans* (UBOCC-A-211002), *Fusarium moniliforme* (UBOCC-A-101149), procured from the Food Microbiology Laboratory, Ngaoundere, Cameroon.

2.3 Preparation of Extracts

The maceration method was followed for the extraction. Hundred grams powder of dried leaves and bark was added into 400 mL of 80 % ethanol in an Erlenmeyer flask (1000 mL capacity) and resulting mixture was vortexed well. The Erlenmeyer will be subjected to magnetic stirring (750 rpm) for 4 h and left to stand at room temperature for 24 h. The mixture will be filtered through 0.45 µm Whatman N°1 filter paper. The operation will be repeated three times. After 72 h, the filtrate will be evaporated under vacuum using a rotavapor until the dry extract is obtained, then the extract obtained will be recovered in a tube and stored at 4 °C until use. The extraction yields will then be calculated using the following formula:

$$Y\ (\%) = \left(\frac{mE}{mV}\right) \times 100$$

Where: *mE* represents the mass of the extract, and *mV* is the mass of the plant material.
The dried ethanolic extract was used for phytochemical screening and antimicrobial activities.

2.4 Determination of total Polyphenols and Flavonoids

2.4.1Polyphenol assay

Polyphenols were estimated by the Folin–Ciocalteu method [7]. Fifty microliters of extract was diluted in 2.5 mL of distilled water and then 250 µL of Folin–Ciocalteu reagent added. The mixture was vortexed, allowed for 5 min at room temperature and after shaking, 500 µL of 20% sodium carbonate was added, vortexed and incubated at 40 °C for 30 min. The mixture was kept at room temperature and in the dark for 60 min, the absorbance was read by spectrophotometer at a wavelength (λ) of 765 nm. A standard range in aqueous medium was carried out with a control polyphenol of gallic acid. The polyphenol contents were deduced from the calibration ranges established with gallic acid and are expressed in milligrams of gallic acid equivalent per gram of dry matter (mg GAE/g dry matter).

2.4.2 Determination of flavonoids

The flavonoids determination was performed by aluminum trichloride method [7]. Briefly, 500 µL of ethanolic extracts was mixed with 1500 µL of distilled water and 150 µl of 5% sodium nitrate, then allowed for 5 min at room temperature and in the dark. To this mixture will then be added 150 µL of 10% aluminum trichloride; after a rest of 11 min in the dark; 500 µL of 1 M Shinoda reagent was added. The mixture subjected to vortex stirring, the optical density was read with a UV spectrophotometer at a wavelength (λ) of 510 nm. A calibration curve was performed with a standard (catechin) at different concentrations and under the same operating conditions as the samples. The total flavonoid contents of the plant extracts studied is expressed as milligram (mg) catechin equivalent per gram of dry matter (mg EC/g DM).

2.5 Antimicrobial Activity of Extracts

2.5.1 Agar-well diffusion method

The well-in-agar method described by Yala *et al.* [8] was used with some modifications.

Preparation of the microbial suspension: Streak plating on Muller Hinton agar was done to obtain pure colonies. After incubation, for 24 h, 5–10 isolated colonies were selected with a platinum loop and transferred to a tube containing 5 mL of physiological water which was considered as the stock solution. Then different dilutions in test tubes containing 9 mL of sterile saline water (NaCl, 0.8%, *m/v*) were prepared. The optical density was measured with a spectrometer at 625 nm. The tubes with values between 0.08 and 0.1 corresponding to 10^6 CFU, i.e., 0.5 Mc Farland, were used for the tests.

Test procedure: The microbial suspensions (50 µL) were surface plated in the 5 cm diameter Petri dishes containing the agar plates previously poured and left to cool at room temperature. Wells were made in the agar at a rate of one well per plate. These were impregnated with 25 µL (200 mg/ mL) of the different *Ziziphus* extracts. After incubation, the results were read by measuring the inhibition diameter in mm, which corresponds to the activity of the extracts on the strain. Activity scale was determined as described by Bouyahya *et al*. [9]: Low: diameter

≤ 12 mm; Average: 12 mm < diameter < 20 mm; Strong: diameter ≥ 20 mm

2.5.2 Minimum Inhibitory concentration determination

The minimum inhibitory concentrations (MIC) were determined by the liquid micro dilution method as described by Mishra and Padhy [10].

Preparation of extracts: Dilutions in 10% DMSO (dimethyl sulphoxide) solvent were made for each extract. The different concentrations were prepared: 200 mg; 100 mg; 50 mg; 25 mg; 12.5 mg; 6.25 mg; 3.12 mg; 1.56 mg.

Preparation of the medium: Mueller Hinton broth was prepared, the broth was supplemented with D-glucose (0.1%) and phenol red (0.0018%) was added as a developer.

Test procedure: In the microplates, a culture broth (150 µL) inoculated with the microorganism to be tested (50 µL) was introduced into each well. These wells were then supplemented with the different concentration of extracts (50 µL) and then incubated for 18 h at 37 °C. Control wells containing only the broth without inoculum (Negative Control) and those containing the broth with the inoculum without extracts (Positive Control) were used. After incubation, colour appearances were observed to determine the inhibition activity of extracts.

2.5.3 Minimum bactericidal concentration and minimum fungicidal concentration

The method described by Guinoiseau [11] was used. Briefly, a drop of the solution was taken from the wells not showing yellow staining and streaked on the surface of the MH agar previously poured into Petri dishes. After incubation, the concentrations of the wells showing no colonies were considered as minimum bactericidal or minimum fungicidal concentrations (MBC or MFC), the smaller of the latter are noted as CMB or CMF.

2.6 Bacterial and Fungal Growth Inhibition: Test in Liquid Medium

Bacterial and fungal growth within extract were determined by the method described by Babii *et al.* [12]. To do so, A volume of 500 µL of bacterial or fungal inoculum was added in 9 mL of MH broth and 500 µL of extracts at different concentrations (1/2MIC, MIC, 4MIC). The blank will consist of the broth only and the control consists of the broth and inoculum. The flasks were incubated at 37°C with agitation. Bacterial or fungal growth was measured by spectrophotometer reading at 600 nm at different intervals (0, 2, 4, 6, 8, 10, 12 and 14 h). Each experiment was performed twice.

2.7 Mode of Action of Extracts

2.7.1 Cell lysis experiment

Cell lysis was determined by the method described by Carson *et al.* [13]. This method assessed a possible bacteriolytic action of extracts by measuring the absorbance at 620 nm. Indeed, a non-lysed microorganism absorbs at 620 nm, so there will be bacteriolysis if the absorbance at 620 nm decreases over time. Briefly young cultured of bacteria or fungal strains were centrifuged at 10,000g for 12 minutes at 4 °C. The pellet was washed twice with sodium phosphate buffer (PBS) and resuspended in PBS-Tween 80 (0.01%, *v/v*). The bacterial and fungal suspensions obtained were standardized to 3.10^{10} CFU.mL^{-1}, deposited (1 mL) in a sterile tube in the absence (negative bacteriolysis control) or presence of extract (1 mL) at three concentrations, one corresponding to the ½MIC, one to the MIC (2MIC) and the other to four times the MIC (4MIC) in MH broth (9 mL). The resulting suspensions were incubated under agitation and at 0, 30, 60, 90, 120, 150 and 180 min, they were homogenized for 20 seconds and their absorbance was measured at 620 nm. Each experiment was performed twice. The results are expressed as the ratio of the absorbance measured at time T to the absorbance at 620 nm measured at time zero in percentages.

$$PC = \frac{A620\ (T)}{A620\ (T_0)} \times 100$$

Where PC: Cellular percentage; A620 (T): absorbance at 620 nm at a given time; A620 (T_0): absorbance at 620 nm at T = 0

2.7.2 Effect on the proton pump

Proton pump activity was determined as described by Monk and Perlin [14] and modified by Manavathu *et al*. [15]. Four hundred milliliter of bacterial and fungal cultured (24 h at 35 °C) were centrifuged at 10,000g for 12 minutes at 4°C. The pellet was washed twice, the first time with distilled water and a second time with

potassium chloride (KCl, 50 mM). The pellets (0.5 g) were resuspended in 40 mL of KCl and the suspensions were incubated at 4°C for 18 h for glucose depletion. Different concentrations (1/2MIC, MIC, 4MIC) of extracts were added (5 mL) to the different bacterial and fungal suspensions depleted in glucose, the negative control was DMSO. The bacterial and fungal suspensions were incubated for 10 minutes at room temperature with agitation. The pH of the suspensions was measured at 0, 15, 30, 45, 60, 75 and 90 minutes.

3. RESULTS AND DISCUSSION

3.1 Extraction Yield

The extraction yield of different plant was calculated, and data are illustrated on Fig. 1. We observed the highest extractions yields with *Z. mauritiana* extract (28.8%) followed by for *Z. mucronata* extract (26%). Concerning the part of the plant, the higher values of extraction were obtained using the leaves compared to barks.

This work revealed that extraction yield was species- and part- of plant dependent. Our results are similar to those obtained by Bouyahya *et al*. [9]. Indeed, the particle size may explain the difference obtained in this study. Moreover, several authors agree with the remarkable effect of grinding on the extraction process. Grinding should improve the phenomena of a solvent transfer through the increase of the specific surface (exchange surface between solvent and solid), but also through the reduction of the diffusion distance in the material [16].

3.2Phytochemical Characteristics of Extracts

Total polyphenols and flavonoids content of the barks and leaves extracts were evaluated and values are illustrated in Table 1. We noted that bark extracts contain more total polyphenols than leaf extracts with values of 61.5mgEAG/g and 59.7mgEAG/g respectively for *Z. mauritiana* and *Z. mucronata*. In addition, the bark extract of *Z. mauritiana* contained more polyphenols compared to *Z. mucronata* although the difference was not significant (P>0.05). The similar observations were carried out with the flavonoid content of the bark extracts. Nevertheless, the flavonoid content was significantly influenced by the part of plant (P<0.05) and not with the plant species. Specifically, we noted that the bark extract of *Z. mucronata* contains more flavonoids compared to extract of *Z. mauritiana*.

Fig. 1. Extraction yield (%) of different

Table 1. Total polyphenol and flavonoid contents of different extracts

Plant species	Part	Polyphenol (mg GAE/g)	Flavonoid (mg CE/g)
Ziziphus mauritiana	Bark	61.46 ± 1.59^{B}	146.56 ± 7.47^{B}
	Leaf	58.25 ± 4.17^{B}	29.66 ± 21.35^{A}
Ziziphus mucronata	Bark	59.65 ± 0.89^{B}	165.21 ± 11.08^{B}
	Leaf	52.16 ± 2.44^{A}	18.06 ± 6.26^{A}

A, B…: for each column, the values assigned to the same capital letter in superscript are not significantly different (p<0.05); mg GAE/g DM: mg gallic acid equivalent/g dry matter mg CE/g DM: mg catechin equivalent/g dry matter

Our results are in agreement with those obtained by Asharf *et al*. [3] on the leaves of *Z. mauritiana* from Pakistan. The values of flavonoids and polyphenols obtained in this study were higher compared to those related by Ibrahim *et al*. [17] with aqueous extracts the barks and leaves of *Z. mucronata* but less to results obtained with ethanolic extracts of barks and leaves. This variability could be due to numerous parameters including the geoclimatic characteristic of the sampling site, the harvested period and the age of the plant. The variations in total polyphenols and flavonoids in the different parts of *Ziziphus* can be explained by the chemical nature, solubility and availability of these compounds in the different parts [18]. In addition, other workers mentioned the occurrence of many other compounds in the leaves of *Z. mauritiana* such as glycosides, saponins, tannins, alkaloids, terpenoids and lignins responsible for interesting biological effects such as antimicrobial activity [1].

3.3 Antimicrobial Activity of Relevant Extracts

The antimicrobial activity was assessed using the agar-well method and data are illustrated in Table 2. It emerges that the activity of varies with the extracts used and the tested strains with inhibition diameter ranging from 36.7 mm (*Z. mauritiana* bark extract) on *S. aureus* to 5.6 mm (*Z. mucronata* leaf extract) on the same strain. We also noted that *Z. mucronata* leaf and barks extract has low activity on bacteria with diameters ranging from 5.7 to 7.3 mm while, *Z. mauritiana* leaf extract shows high activity on *S. aureus* and *P. aeruginosa*. On the other hand, *Z. mucronata* leaf extract exhibited strong activity on *C. albicans* with inhibition diameter of 21.7 mm while, *Z. mucronata* bark extract has strong activity on *C. albicans* and *F. moniliforme* with the inhibition diameters of 26.7 mm and 24.0 mm respectively. Concerning *Z. mauritiana,* the bark extract shows high activity on *Candida albicans* (30.0 mm), while leaves extract showed low activity on *Candida albicans* and no activity on *F. moniliforme*. The standard antimicrobial used as control showed higher activity on the organisms than the extracts. This is not surprising because standard antibiotics are well refined industrial products so there is no doubt their activity will be more compared to crude extracts [1]. Authors reported a great antifungal activity of ethanolic extract from *Z. mucronata* bark against several fungal strains [12].

Due to these observations, *Z. mauritiana* extract was selected for the minimal inhibitory and bactericidal concentration determination while, *Z. mucronata* was selected for minimal inhibitory and fungicidal concentrations and results are mentioned in Table 3. The MIC values for *Z. mauritiana* bark extracts were 6.25 mg/mL and the MBC were ranged from 25 to 50 mg/mL. Concerning *Z. mauritiana* leaf extract. the MICs vary from 6.25 and 200 mg/mL and the lowest value was obtained with *S. aureus* (6.25 mg/mL) while, the highest value with *P. aeruginosa* (200 mg/mL). Concerning *Z. mucronata* bark extract, the MIC values were ranged from 1.56 to 6.25 mg/mL with the lowest value obtained with *Candida albicans*. The lowest value MFC was obtained with *Candida albicans* (12.5 mg/mL) while the highest value with *Fusarium moniliforme* (100 mg/mL). As for the *Z. mucronata* leaf extract, the MIC value was ranged from 6.25 to 25 mg/mL and the MFC ranged from 50 and > 200 mg/mL. The values for the MBC/MIC ratio were between 4 and 16 synonyms of the bacteriostatic effect of tested extracts.

Table 2. Antimicrobial activity of different extracts using agar-well diffusion method

Species and Antimicrobial	Part	Inhibition diameter (mm)			
		S. aureus	***P. aeruginosa***	***C. albicans***	***F. moniliforme***
Z. mucronata	Leaf	$5.66\pm1.15^{A,a}$	$7.33\pm2.30^{A,a}$	$21.66\pm5.77^{B,abc}$	$16.66\pm2.88^{B,a}$
	Bark	$8.33\pm2.08^{A,a}$	$5.66\pm1.15^{A,a}$	$26.66\pm2.88^{B,bc}$	$24.00\pm6.55^{B,a}$
Z. mauritiana	Leaf	$24.00\pm3.60^{AB,ab}$	$27.00\pm6.08^{B,c}$	$10.00\pm10.00^{A,a}$	/
	Bark	$36.66\pm2.66^{A,bc}$	$13.66\pm2.51^{A,b}$	$30.00\pm5.00^{A,c}$	/
Ciprofloxacin (5 mg/mL)		$45.00\pm0.00^{A,c}$	$50.00\pm0.00B^{,d}$	ND	ND
Nystatin (500,000 IU)		/	/	$13.33\pm12.58^{A,a}$ b	$23.66\pm10.96^{A,a}$

S. aureus: Staphylococcus aureus, P. aeruginosa: Pseudomonas aeruginosa, C. albicans: Candida albicans, F. moniliforme: Fusarium moniliforme, Z.: Ziziphus; A, B...: *for each column, the values assigned to the same capital letter in superscript are not significantly different (p<0.05);* a, b, c...: *for each line, the values assigned to the same lower-case letter in superscript are not significantly different (p<0.05)*

Table 3. Minimal inhibitory, bactericidal and fungicidal concentration (MIC, MBC, MFC; in mg/mL)

Species	Part	Test	*S. aureus*	*P. aeruginosa*	*C. albicans*	*F. moniliforme*
Z. mauritiana	**Bark**	MIC	6.25	6.25	ND	ND
		MBC	25	50	ND	ND
		MBC/MIC	4	8	ND	ND
	Leaf	MIC	6.25	200	ND	ND
		MBC	100	> 200	ND	ND
		MBC/MIC	16	/	ND	ND
Z. mucronata	**Bark**	MIC	ND	ND	1.56	6.25
		MFC	ND	ND	12.5	100
		MFC/MIC	ND	ND	8	16
	Leaf	MIC	ND	ND	6.25	25
		MFC	ND	ND	50	>200
		MFC/MIC	ND	ND	8	/

ND : Not determined ; / : Not calculated; S. aureus: Staphylococcus aureus, P. aeruginosa: Pseudomonas aeruginosa, C. albicans: Candida albicans, F. moniliforme: Fusarium moniliforme, Z. mauritiana: Ziziphus mauritiana, Z. mucronata: Ziziphus mucronata

The antimicrobial activity of different extracts obtained in this study could be explained by the presence of polyphenols and flavonoids as mentioned early. The antibacterial activity of polyphenols is well established and several workers revealed that the main site of action of phenolic compounds is the bacterial cell membrane [19,20]. On the other hand, antimicrobial activity of flavonoids has also been documented. Flavonoids are responsible for scavenging or chelating processes and can also disrupt microbial membranes [21]. The astringent properties of tannins can also induce their complexation with enzymes or substrates. Many microbial enzymes in the form of crude or purified filtrates are inhibited when mixed with tannins. From this, enzymes involved in cellular metabolism such as peptidoglycan biosynthesis and other essential functions could be inhibited after permeabilization of the cell membrane by tannins also possessing protein binding properties [22]. In addition, other organic compounds not found in this study and detected by several researchers in the *Z. mauritiana* extracts including tannins, saponins, resins, and cardiac glycosides have been reported to exhibit great antimicrobial activity and could be the reason for the activities recorded against these test organisms. Several authors have also reported that the antimicrobial activity is related to the ability of terpenes to affect not only permeability but also other functions of cell membranes. These compounds might cross the cell membranes, thus penetrating into the interior of the cell and interacting with critical intracellular sites. Fundamentally, the cell wall components of bacteria are quite different from those of fungi. While the cell wall of bacteria is either made up of acetyl muramic acid (AMA) or acetyl glucose amine (AGA), fungal cell wall is made up of fungal cellulose, chitin, etc. This may explain the reason for the differences in their susceptibility to the plants extracts in this experiment.

3.4 Inhibition Kinetic of Bacterial and Fungal Strains using Plants Extracts

The microbial growth of different tested strains in liquid medium within plant extract compared to control (without extract) was assessed and the profiles are illustrated in Figs 2, 3, 4 and 5. In general, it emerges from these figures that the inhibitory capacity of each extract increases with the concentration regardless of the strain tested with maximum inhibition levels at the 4MIC concentrations (6.25; 25; 100 and 800 mg/mL) followed by MIC concentrations depending on the species studied and the part of the plant used. On the other hand, we also noted in this study an absence of inhibitory activity using the 1/2MIC concentrations with profiles similar to the control (in absence of extracts). Many authors mentioned that this could be due to the presence of many bioactive compounds in these extracts as mentioned above. For example, Abedini *et al*. [23] reported the antibacterial activity of rosmarinic acid and methyl rosmarinate against *Staphylococcus epidermidis* (5001) and Bocquet [24] xanthohumol, described the great effect of desmethylxanthohumol and lupulone on *Staphylococcus aureus* (T28.1). On the other hand, our results are slightly different with those obtained by Babii *et al*. [12] mentioning the total inhibition *Staphylococcus aureus* using MIC and 2MIC concentrations.

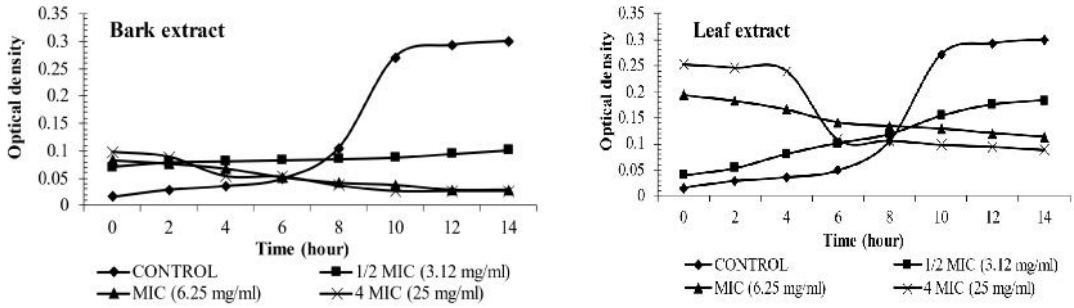

Fig. 2. Growth curve of *Staphylococcus aureus in the* presence of *Z. mauritiana* extracts

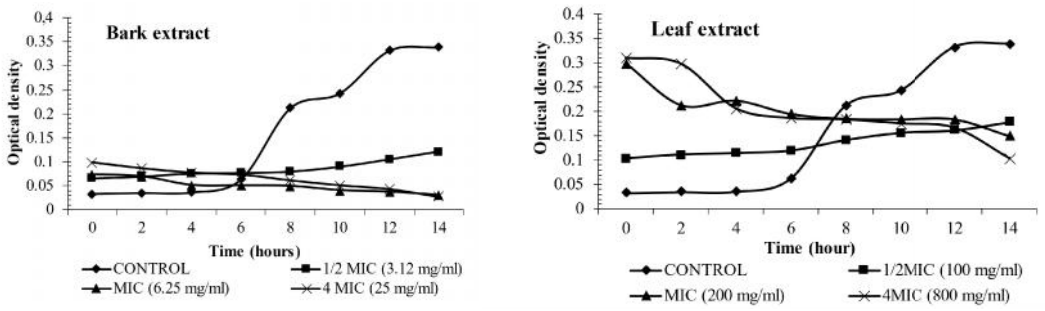

Fig. 3. Growth curve of *Pseudomonas aeruginosa* in the presence of *Z. mauritiana* extracts

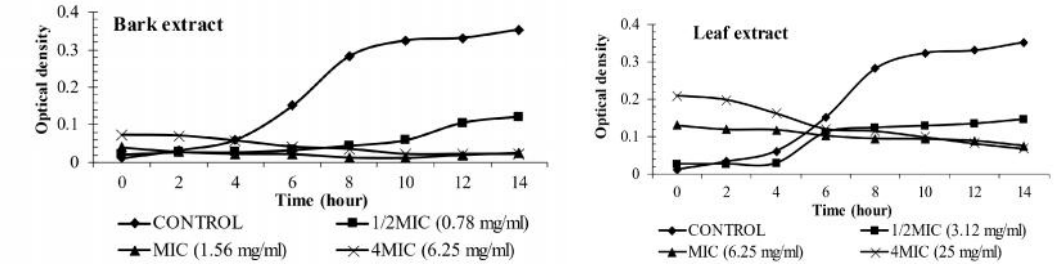

Fig. 4. Growth curve of *Candida albicans in the* presence of *Z. mucronata* extracts

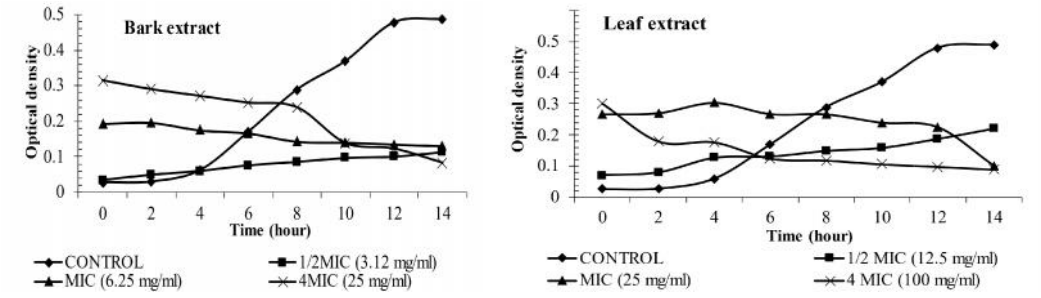

Fig. 5. Growth curve of *Fusarium moniliforme in the* presence of *Z. mucronata* extracts

3.5 Mechanisms of Action of Extracts

3.5.1 Cell lysis experiment

The survival rate of each tested strains in presence of extracts is illustrated in the Figs 6, 7, 8 and 9. We noted from these figures that the survival rate did not vary with the increase in the extract concentration (excepted control and 1/2MIC concentration), the incubation time and the strain tested. However, we observed maximum values of microbial survival in the control tubes (in the absence of extract) followed by the 1/2MIC concentration tubes and the less survival rates using the extracts at concentration MIC and 4MIC. This results once again demonstrates the inhibitory effect of the extracts used and also shows that the inhibition observed would be linked to cell lysis characterized by a gradual drop in optical density measured (to 620 nm).

For example, for the bacteriolytic effect of MIC concentration of *Z. mauritiana* bark extract on *Staphylococcus aureus* the percentage at 30 minutes is 99.1 % and after three hours it will be 96.90 %. For the concentration 4 MIC, the percentage after 30 minutes is 99.37 % and at the end the percentage is 90.41 %. Similarly, we noted the survival rate of 94.82% and 91.13% of *S. aureus* after 30 minutes and 3 h of incubation using MIC concentration of *Z. mauritiana* leaf extract. Concerning *Z. mucronata* leaf extract, we registered the survival rate of 99.9% and 96.2% of *F. moniliforme* after 30 minutes and 3 h of incubation using MIC concentration of *Z. mauritiana* leaf extract, while the survival rate at concentration 4MIC was 98.2% at the end the percentage is 93.1% after 30 minutes and 3 h of incubation. Globally, the cytolytic effect of extract was strain-dependent and we noted the absence of activity of *Z. mauritiana* leaves and bark extracts on *S. aureus*. The similar observation has been reported in the literature using different concentrations (MIC and 8MIC) of *Inula graveolens* and *Santolina corsica* essential oil [11].

3.5.2 Activity on the proton pump

Other reports suggested that the components of the essential oils and maybe solvent extract cross the cell membrane, interacting with the enzymes and proteins of the membrane, such as the H+-ATPase pumping membrane, so producing a flux of protons towards the cell exterior, which induces changes in the cells and, ultimately, their death. The Figs 10, 11, 12 and 13 show the results of the effects of the extracts on *Staphylococcus aureus, Pseudomonas aeruginosa, C. albicans, F. moniliforme*. We noted that all extracts at the concentration 4MIC exhibit interesting activity on the proton pump ATPase. Indeed, at this concentration the pH does not change during the experimentation, while for the MIC and 1/2MIC concentrations the pH decreases over time. Our results are in agreement with to those obtained by Ambadiang *et al*. [25] on *Escherichia coli* ATCC8739 using *Cassia sieberiana* DC bark reporting a great effect on the pump proton at the concentration equal to the MIC value. In addition, Seukep *et al*. [26] obtained an effect on the proton pump at concentration 2MIC (2-fold higher than the MIC) with n-hexane fraction of *Plukenetia huayllabambana* fruit against *Salmonella enterica.* In addition to the mechanism described above, some studies have been conducted to understand other mechanisms of action of plant extracts and suggest that they also react with active sites of enzymes or act as an H+ carrier, depleting adenosine triphosphate pool [25,26]. Literature mentioned that the proton-pumping ability of microorganisms mediated by the H+-ATPase at the expense of energy is crucial for the regulation of the internal pH of fungal cells.

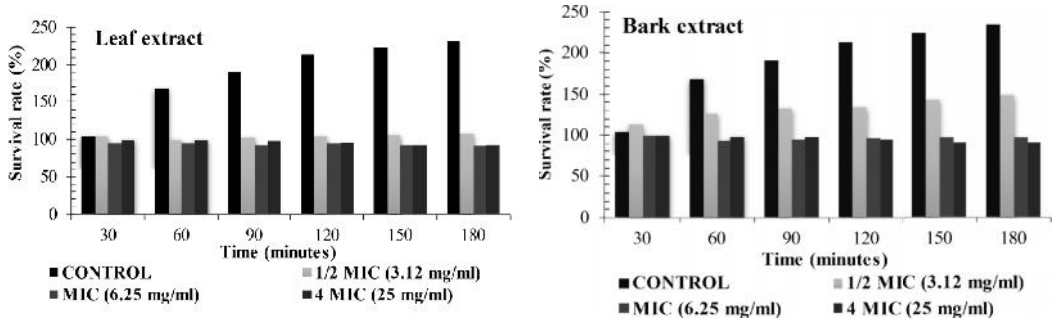

Fig. 6. *Staphylococcus aureus* cell lysis experiment in the presence of *Z. mauritiana* extracts

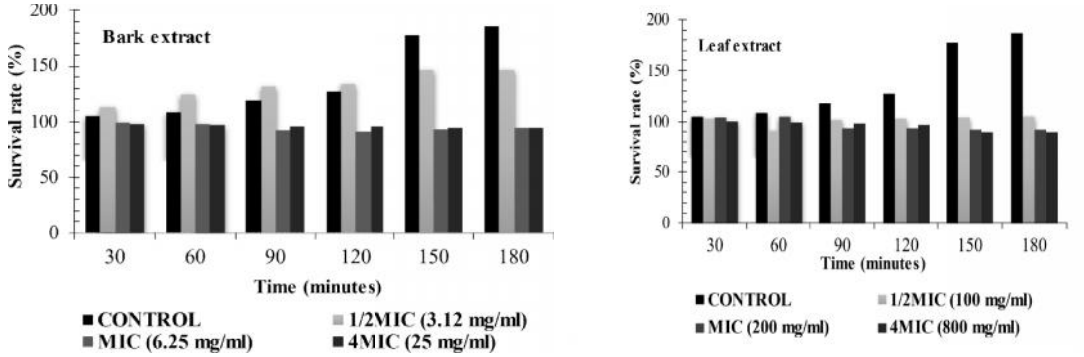

Fig. 7. *Pseudomonas aeruginosa* cell lysis experiment in the presence of *Z. mauritiana* extracts

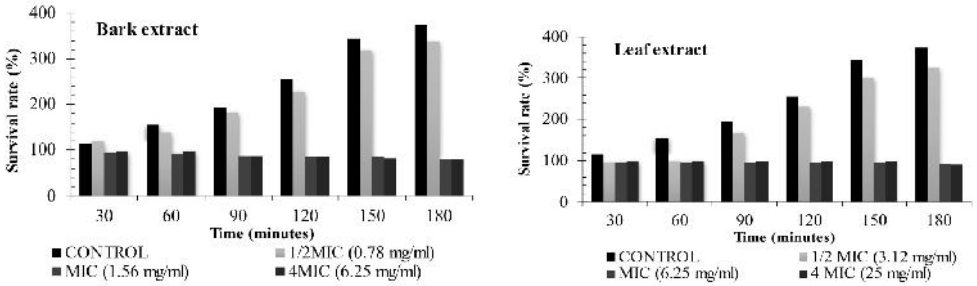

Fig. 8. *Candida albicans* cell lysis experiment in the presence of *Z. mucronata* extracts

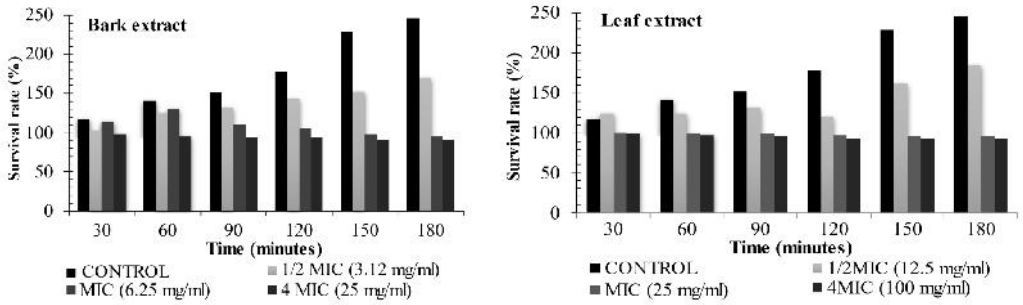

Fig. 9. *Fusarium moniliforme* cell lysis experiment in the presence of *Z. mucronata* extracts

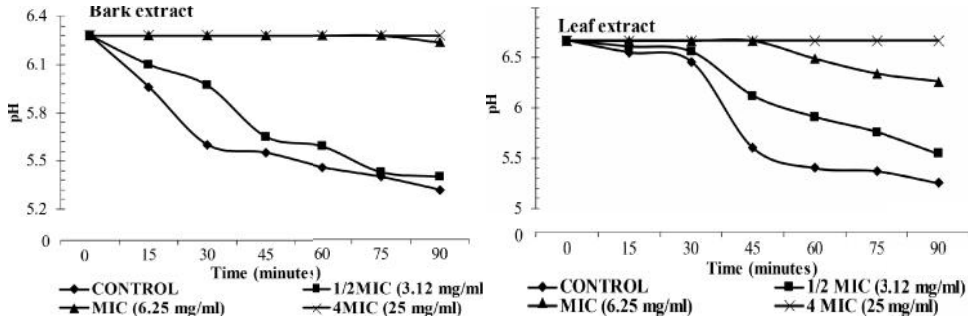

Fig. 10. Effect of different concentration of *Z. mauritiana* extracts on the proton pump activity of *Staphylococcus aureus*

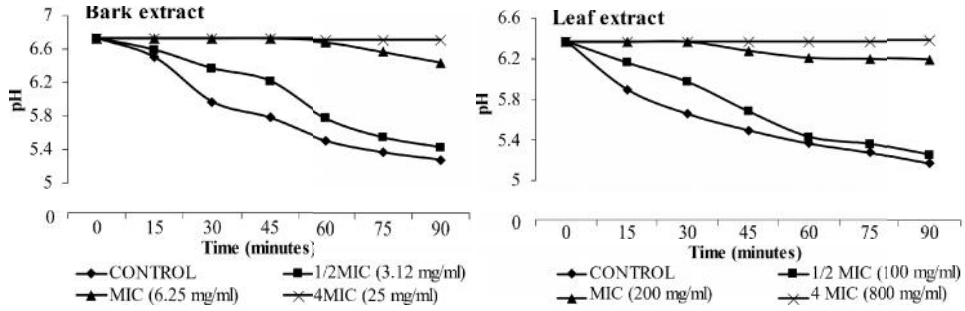

Fig. 11. Effect of different concentration of *Z. mauritiana* extracts on the proton pump activity of *Pseudomonas aeruginosa*

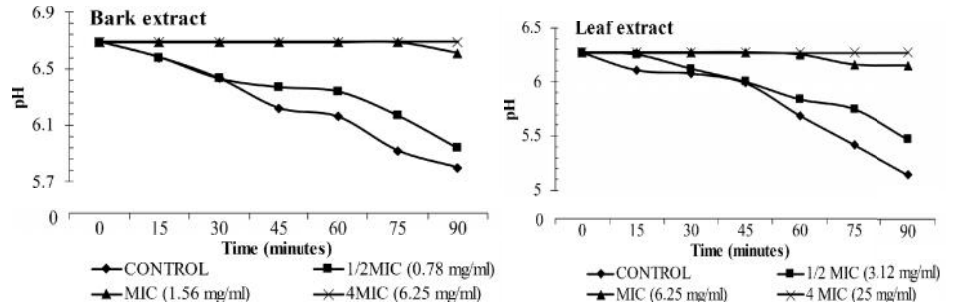

Fig. 12. Effect of different concentration of *Z. mucronata* extracts on the proton pump activity of *Candida albicans*

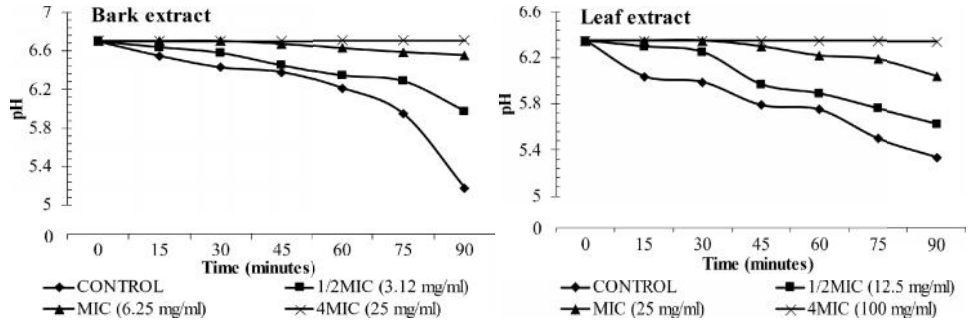

Fig. 13. Effect of different concentration of *Z. mucronata* extracts on the proton pump activity of *Fusarium moniliforme*

The ATPase enzyme maintains cell homeostasis and osmotic stability by regulating the concentrations of ions inside the cell. The efficiency of this enzyme can be estimated by measuring decrease in the pH of the cell suspension [27]. This is because when glucose is added to non-growing cells, it is rapidly taken into the cells by their inbuilt transport systems. To power this transport system, protons are pumped out of the cell by membrane ATPase, which results in a decrease in the pH of the external medium that the cells are suspended [27].

4. CONCLUSION

The objective of this study was to determine the phytochemical profile and antimicrobial activity of *Ziziphus mauritiana* and *Ziziphus mucronate* leaf and bark extracts. We can conclude that leaves and bark extracts of these plants contained secondary metabolites including phenolic compounds and flavonoids with interesting antimicrobial activities. They have an effective function on different types of infectious diseases from fungal and bacterial sources. Hence, they should be used in the preparation of medicinal

drugs for the treatment of several intestinal and skin diseases.

DATA AVAILABILITY

The data used to support the findings of this study are available from the corresponding authors on request.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENTS

We extend our sincere thanks to the Bioprocesses Laboratory of the University Institute of Technology, University of Ngaoundere, for providing us with the reagents and equipment necessary to carry out this work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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