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Research Article

Antimicrobial activity of extracts from *Ziziphus mauritiana* (Rhamnaceae) bark, leaf and fruit against *Staphylococcus* isolated from fish

Mohamadou Salamatou[1] and **James Ronald Bayoï**[2*]

1. Department of Agriculture, Livestock and Postharvest Technology, National Advanced School of Engineering Maroua, P.O Box 46, Maroua, Cameroon.
2. Department of Biological Sciences, Faculty of Science, University of Maroua, PO Box 814, Maroua, Cameroon.

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Corresponding Author
Prof. Dr. James Ronald Bayoï
E-mail:
jabar982002@gmail.com
Tel: +237699458665

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Abstract

The antimicrobial activity of some extracts from *Ziziphus mauritiana* bark, leaf, and fruit was tested on two *Staphylococcus* species including *S. aureus* and *S. epidermidis*. For this purpose, plant material was harvested in Mokolo (Far North Region, Cameroon), and the microbiological material was isolated from smoked fish sold in a market located in Maroua (Far North Region, Cameroon). Increasing polarity solvent-based extraction technique was conducted using single solvents in the following order: hexane, acetone, ethanol, and methanol. Furthermore, ethanol-water (70%, v/v) system solvent was used according to the hot-maceration technique. The antimicrobial activity of the extracts was evaluated by measuring inhibition diameter and the determination of the minimum inhibitory and bactericidal concentrations (MICs and MBCs**).** The highest extraction yields (32.60% and 38%) displayed with the methanol and ethanol-water bark extracts, respectively. The extracts showed mutable antimicrobial activity on both *Staphylococcus* species, with the strongest antimicrobial activity obtained with ethanol-water bark extract displaying inhibition clear rings of 38.66 ± 3.51 and 43.00 ± 2.64 mm on *Staphylococcus aureus* and *Staphylococcus epidermidis,* respectively. Since most of the MIC/MBC ratios ranged between 2 and 4, it could be suggested that *Z. mauritiana* extracts exerted a bactericidal action on both isolated *Staphylococcus* species. Given the above and based on the results obtained in this study, the ethanol-water extracts from *Z. mauritiana* barks, leaves and fruits could be considered as potent biological alternatives to chemicals used during the storage of fish. These *Ziziphus* extracts could also be an encouraging way to improve fish safety and valuable food preservatives.

1. Introduction

Fish is a popular global food and a low-cost source of protein in many nations consumed by about a billion people around the world. It is one of the most relevant protein sources, contributing about 13% of the animal protein consumed globally. The nutritional properties of fish include minimal saturated fat, good supply of essential fatty acids, and low cholesterol making it excellent for young children, adults, and the elderly [1]. Therefore, fish and sea products represent valuable source of nutrients; and contribute significantly to nutrition and food security [2]. Its nutritional value is particularly important in under developed sub-Saharan countries where more than 34 % of the population is affected by chronic malnutrition.

Traditional preservation techniques such as drying and smoking, salting and cold preservation are observed. However, lack of hygiene or inadequate storage cause losses of fish through the development of microbiological contaminants that cause qualitative

losses [3], infectious diseases, and collective food poisoning [4]. Currently, foodborne illnesses are a public health problem and represent a significant cause of mortality in under developed countries [5]. Several microbial agents including some species of *Staphylococcus* genus such *S. aureus* are responsible for this health issue. *S. aureus* is one of the three bacteria incriminated in more than 90% of collective food poisoning cases [5].

Ziziphus genus belongs to the Rhamnaceae family comprising 40 species of thorny shrubs and small trees [6]. In Cameroon, four species have been identified including *Ziziphus mauritiana, Ziziphus mucronata, Ziziphus spina-christi,* and *Ziziphus abyssinica,* with *Ziziphus mauritiana* and *Ziziphus mucronata* recognized as the most widespread in the country. The leaves of *Ziziphus mauritiana* are commonly used in traditional medicine for the treatment of whooping cough, while the crushed or pressed fruits are used to treat earache [6]. Previous studies showed that the methanol extract of *Ziziphus* species was active on numerous microorganisms such as *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Aspergillus niger* and *Fusarium solani* [7]. It is believed that this antimicrobial activity relies on bioactive compounds such as polyphenols, saponins, tannins previously evidenced in *Ziziphus mauritiana* [8].

To date, no study has been conducted on the food preservation properties of *Ziziphus mauritiana* encountered in Cameroon. Therefore, the current work is in line for contributing to the search for new natural preservatives to alleviate the food losses and safety issues caused by fish consumption. The interest of this work is to identify the *Ziziphus mauritiana* extracts displaying the highest antimicrobial potential.

2. Materials and methods

2.1. Plant material

Ziziphus mauritiana (leaves and barks) was harvested in the field in January, 2018 in the locality of Mokolo in Mayo-Tsanaga Division (Far North Region, Cameroon), while its fruits were purchased from the local market. The parts of the plant selected for the current study including leaves, barks and fruits (Fig. 1) were authenticated at the Institute of Agricultural Research for Development (IRAD) (Far North Region, Cameroon).

Figure 1. Pictures of some *Z. mauritiana* parts. (a) Leaves, (b) Fruits, (c) Barks.

2.2. Isolation and identification of microorganisms

The samples of smoked fish purchased at the local market in the city of Maroua were used for germ isolation. The samples were previously powdered using an electrical grinder and diluted in sterile saline water (0.85%, w/v). The suspensions (1 mL) were added to test tubes containing enriched nutrient broth, and they were incubated for a night at room

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temperature. After that, quadrant or streak plating using a sterile Pasteur pipette was made in petri dishes containing Chapman agar. The plates were inverted and incubated for 24 h at 37° C for the isolation of *Staphylococcus* colonies [9]. For identification, three colonies of identical colour on the surface of the media were taken at random. The isolated colonies were purified by re-plating on fresh Chapman agar until a pure culture was obtained. The purity was estimated by observing their macroscopic appearance on the surface of Chapman agar culture medium [9]. The colonies were then plated in screw tubes and stored at 4° C for further use.

2.3. Preparation of extracts

The method described by Bourgou *et al.* [10] was adopted with some slight modifications. The extracting solvents were hexane, acetone, ethanol, methanol and ethanol-water. It is important to note that among the solvents used in this study, some of them were not recognized as safe (GRAS). These solvents including hexane, acetone and methanol are used in food with many limitations. However, solvents such as ethanol and water are commonly used in the food industry, and they are generally recognized as safe (GRAS). The first four solvents (hexane, acetone, ethanol, methanol) were used according to the increasing polarity technique, while the last ethanol-water solvent system was employed according to the hot-maceration technique. The various selected parts (fruits, leaves and barks) of *Z. mauritiana* were washed using clean tap water, oven dried and powdered. Primarily, 50 grams of powder from each plant part were mixed with 500 mL of hexane into an Erlenmeyer flask, left under stirring for 4 hours and allowed to stand at room temperature for 24 hours. The mixtures were filtered through Whatman no.1 filter paper and the filtrates were kept, and evaporated under vacuum until dry extracts were obtained. The residues were dried and resuspended into 500 mL of acetone, the next solvent. The preparations were left to macerate for 24 hours and the dried extracts were collected after filtration and evaporation of the various filtrates. The same procedure was repeated with ethanol and methanol solvents using the residues previously collected after acetone extraction. Furthermore, each powder was macerated for 24 hours at 60° C using ethanol-water (70%, v/v). After cooling, the macerating mixture was kept at room temperature for 2 weeks and then the co-solvent was evaporated under vacuum until dry extract was collected. All dried extracts were stored at 4° C until use. The extraction yields were calculated using the following formula:

$$\boldsymbol{R = (\frac{mE}{mP}) \times 100}$$

Where,

R: extraction yield (%);

mE: mass of the extract;

mP: mass of the plant powder

2.4. Preparation of the inoculum

Streak plating on Muller Hinton agar was performed to obtain pure colonies. After 24 h-incubation, 5 to 10 well isolated colonies were selected with a platinum loop and transferred to a tube containing 10 mL of sterile saline water (0.85%). The obtained suspension considered as the stock microbial solution was serially 10-fold diluted using 9 mL of sterile saline water and turbidity was measured at a wavelength of 625 nm. The stock or diluted tubes displaying values between 0.08 and 0.1 were considered as 0.5 Mc Farland corresponding to 10^6 CFU.

2.5. Qualitative assay

The disc method described by Mighri *et al.* [11] was used. The 0.5 Mc Farland bacterial suspensions (100 µL) were surface plated in Petri dishes containing Mueller Hington (MH) agar and allowed to cool to room temperature. Paper discs (6 mm diameter) were placed on the surface of the agar medium. They were impregnated with 5 µL of the different *Z. mauritiana* extracts. After incubation for 24 hours at 37° C, the inhibition diameters were measured. The antimicrobial activity of each extract was presumed using the scale previously reported by Bouyahya [12] as follows: inhibition clear zone lower than 12 mm for low antimicrobial activity; inhibition clear zone ranged between 12 and 20 mm for medium antimicrobial activity; inhibition clear zone higher than 20 mm for strong antimicrobial activity.

2.6. Quantitative assays

2.6.1. Determination of the minimum inhibitory concentration (MIC)

Each of the extracts was diluted in ethanol (diluent) and different concentrations (200 mg/mL, 100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL, 6.25 mg/mL, 3.12

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Table 1. Extraction yields of different parts of *Ziziphus mauritiana*

Plant part	Solvent used	Yields (%)	Aspect, colour
Barks	Hexane	4	Pasty, yellow
	Acetone	8.16	Powdery, brown
	Ethanol	29.54	Dough, brown
	Methanol	32.60	Dough, brown
	Ethanol-water	38	Powdery, brown
Leaves	Hexane	4	Viscous paste, green
	Acetone	6.66	Viscous paste, green
	Ethanol	10.86	Viscous paste, green
	Methanol	12.24	Pasty, green
	Ethanol-water	28	Pasty, green
Fruits	Hexane	2	Powdery, yellow
	Acetone	2.12	Pasty, yellow-orange
	Ethanol	19.14	Pasty, yellow-orange
	Methanol	24.65	Pasty, yellow-orange
	Ethanol-water	18	Pasty, yellow-orange

12 mg/mL, 1.56 mg/mL, 0.78 mg/mL, 0.39 mg/mL) were prepared for the determination of Minimum Inhibitory Concentration (MIC) using the broth micro-dilution method as described by Mamadou *et al.* [13]. In microplates, a culture broth inoculated with the microorganisms to be tested was introduced into each well. These wells were supplemented with the different extracts having a corresponding concentration. Control wells containing either the broth without inoculum (negative control) or the broth with inoculum without extracts or antibiotic (positive control) were made. Erythromycin (2.5 mg/mL) was used as a reference antibiotic. The microplates were then carefully covered with film paper. After incubation for 24 hours at room temperature, a solution of blue thiazolyl blue tetrazolium was added to be instantly metabolized by viable microorganisms into a blue formazan derivative indicating a positive result whereas the color remained unchanged for a negative result. All the concentrations cited above that prevented the formation of blue colour were considered as inhibitory concentrations and the lowest one was equivalent to the MIC.

2.6.2. Minimum bactericidal concentration (MBC)

The minimum bactericidal concentration (MBC) was determined using the method previously described by Guinoiseau [14]. A drop of solution was taken from the wells not showing blue staining and was streaked on the surface of MH agar previously poured into Petri dishes. After incubation, the concentrations of wells with no colonies were considered as bactericidal, and the smallest of them were noted as MBC. The MBC/MIC ratios were determined and the effect of various extracts was deducted as follows: MBC/MIC ≤ 4, bactericidal effect; 4 < MBC/MIC < 32, bacteriostatic effect; MBC/MIC > 32, no effect.

2.7. Statistical analysis

All measurements were done in triplicate and the values were presented as mean ± standard deviation. Raw data from qualitative and quantitative tests were analysed using one-way ANOVA and conducted by Xlstat 2007.8.04 software. The mean values were separated by pair thanks to HSD Tukey post hoc test at a significance level of $p < 0.05$.

3. Results and discussion

3.1. Extraction yields

According to the results recorded in Table 1, it can be noted that the highest extraction yields were obtained with the barks (38%) and leaves (28%) macerated in 70%, ethanol-water for 2 weeks. Regarding pure solvents, methanol provided the extracts with the highest yields, 32.60% for the bark extract, 12.24% for the leaf extract and 24.65% for the fruit extract. While hexane gave the lowest yields of 4% for the bark and leaf extracts and 2% for the fruit extract. Abalaka *et al.* [15] reported lower results with stem and leaf of *Ziziphus mauritiana* macerated for 48 hours with various solvents. They obtained 0.47% and 0.46% with hexane, 0.73% and 3.71% with chloroform, 1.55% and 1.69% with methanol for stem and leaf extracts, respectively. Similarly, to our results, these authors noted that hexane provide the lowest extraction yields. The differences in extraction yield can be explained by the solvent used. Each solvent has a solubilizing power related to its physicochemical properties, defining its polarity and hydrophilicity. Through its disruption properties, solvent has the capability of ensuring its diffusion in the plant tissues. An indicator of affinity towards water, a high polarity results either in the direct action of the solvent on the polar constituents of the cell content, or in the solubilization of some of these constituents or in a disruption of interactions [16]. This difference in yield can also be explained by the difference in the powder particles.

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Table 2. Diameters of inhibition and presumed antimicrobial activity of different extracts of *Ziziphus mauritiana*

Plant part used	Solvent used	Inhibition diameter (mm)		Antimicrobial activity*	
		S. aureus	*S. epidermidis*	*S. aureus*	*S. epidermidis*
Bark	Hexane	$13.66 \pm 1.52^{ABCD,a}$	$15.66 \pm 1.52^{AB,a}$	Medium	Medium
	Acetone	$19.33 \pm 1.52^{DEF,a}$	$20.00 \pm 6.08^{BC,a}$	Medium	Medium
	Ethanol	$17.66 \pm 3.78^{CDEF,a}$	$17.00 \pm 1.00^{AB,a}$	Medium	Medium
	Methanol	$17.66 \pm 2.51^{CDEF,a}$	$17.00 \pm 3.60^{AB,a}$	Medium	Medium
	Ethanol-water	$38.66 \pm 3.51^{H,a}$	$43.00 \pm 2.64^{E,a}$	Strong	Strong
Leaf	Hexane	$12.00 \pm 1.00^{ABC,a}$	$12.33 \pm 1.52^{A,a}$	Medium	Medium
	Acetone	$15.66 \pm 2.08^{BCDE,a}$	$17.00 \pm 2.00^{AB,a}$	Medium	Medium
	Ethanol	$15.00 \pm 2.00^{BCD,a}$	$15.00 \pm 1.00^{AB,a}$	Medium	Medium
	Methanol	$14.33 \pm 1.52^{ABCD,a}$	$15.00 \pm 2.00^{AB,a}$	Medium	Medium
	Ethanol-water	$35.33 \pm 8.38^{GH,a}$	$39.66 \pm 0.57^{E,a}$	Strong	Strong
Fruit	Hexane	$7.66 \pm 1.52^{A,a}$	$11.33 \pm 1.15^{A,b}$	Low	Low
	Acetone	$12.00 \pm 1.00^{ABC,a}$	$15.66 \pm 1.52^{AB,b}$	Medium	Medium
	Ethanol	$10.33 \pm 1.52^{AB,a}$	$12.66 \pm 1.52^{A,a}$	Low	Medium
	Methanol	$11.00 \pm 2.00^{ABC,a}$	$12.66 \pm 1.15^{A,a}$	Low	Medium
	Ethanol-water	$24.33 \pm 6.08^{F,a}$	$25.00 \pm 0.00^{C,a}$	Strong	Strong
Erythromycin (2.5 mg/mL)		$33.33 \pm 11.54^{GH,a}$	$32.33 \pm 3.78^{D,a}$	Strong	Strong

For each column, the values assigned to the same capital letter (A, B, C, D, F) in superscript are not significantly different ($p<0.05$). For each line, the values assigned to the same lower-case letter (a, b) in superscript are not significantly different ($p<0.05$). (*): The antimicrobial activity of each extract was presumed using the scale reported by Bouyahya [12]. *S. aureus* : *Staphylococcus aureus*, *S. epidermidis* : *Staphylococcus epidermidis*.

Indeed, the barks have the highest extraction yields because their particles were found finer than those of leaves and fruits. All authors agreed on the positive effect of grinding on extraction operations. The grinding of the solid allows to intensify the phenomena of transfer of the solvent through the increase of the specific surface, but also by the reduction of the penetration distance in the material. However, a certain limit should not be exceeded regarding the fineness of the particles. The presence of fine particles induces an exaggeration in this sense and implies a notable reduction in the permeability of the bed of solids to the solvent, which leads to the establishment of preferential currents thus blocking the extraction process in some places where the solvent no longer flows [17]. Time also influences the yield. The extraction time has a very important role in the extraction process. The longer the extraction time, the higher the extraction yield. The quantities of extracted substances depend on the residence time of the material within the solvent. In the current study, the residence time of *Z. mauritiana* in ethanol-water was higher than that of single solvents (hexane, acetone, ethanol and methanol). The various parts (bark, leaf and fruit) were left to macerate for 2 weeks in ethanol-water, while the same parts were macerated only for 24 hours in single solvents. Therefore, this differential extraction time could explain why co-solvent ethanol-water extracted in most of cases more substances than pure and single solvents.

3.2. Inhibition diameter

According to the results (Table 2), it can be seen that the extracts of *Ziziphus mauritiana* have an activity on both microorganisms tested. This antimicrobial activity changes from low to strong depending on the plant part and solvent used. The lowest antimicrobial activity (7.66 ± 1.52 and 11.33 ± 1.15 mm) was obtained by the hexane fruits extract on *S. aureus* and *S. epidermidis*, respectively, whereas the strongest antimicrobial activity was recorded by the ethanol-water extract from *Z. mauritiana* bark on *S. aureus* (38.66 ± 3.51) and *S. epidermidis* (43.00 ± 2.64 mm). Therefore, it could be suggested that the extracts from barks appear more active than those of fruits and leaves of *Z. mauritiana*. Furthermore, the ethanol-water (70%, v/v) extracts recovered after a maceration at room temperature for 2 weeks were found more effective compared to those obtained with unique solvents (hexane, acetone, ethanol and methanol). This finding shows that mixing water with some organic solvents was more effective than unique organic solvents. This might be explained by the fact that adding of a small amount of water to an organic

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Table 3. Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and MBC/MIC ratio of *Ziziphus mauritiana* extracts.

Plant part used	Solvent used	MIC (mg/mL)		MBC (mg/mL)		MBC/MIC ratios	
		S. aureus	*S. epi.*	*S. aureus*	*S. epi.*	*S. aureus*	*S. epi*
Bark	Hexane	50	12.5	200	25	4	2
	Acetone	6.25	6.25	25	12.5	4	2
	Ethanol	25	6.25	50	25	2	4
	Methanol	25	6.25	100	50	4	8
	Ethanol-water	50	12.5	200	50	4	4
Leaf	Hexane	100	25	>200	>200	ND	ND
	Acetone	12.5	6.25	50	25	4	4
	Ethanol	50	6.25	100	25	2	4
	Methanol	50	25	100	50	2	2
	Ethanol-water	50	25	>200	200	ND	8
Fruit	Hexane	200	50	>200	>200	ND	ND
	Acetone	25	25	100	50	4	2
	Ethanol	100	25	>200	200	ND	8
	Methanol	100	25	>200	100	ND	4
	Ethanol-water	100	100	>200	>200	ND	ND

S. aureus: Staphylococcus aureus, *S. epi: Staphylococcus epidermidis*, ND: Not determined

solvent such as ethanol or methanol increases the mass transfer process as well as the solubility of bioactive phenolic compounds from the plant matrix to the extraction solvent. This was confirmed by a previous study conducted by Dezoumbe *et al.* [18] on quantitative screening of *Jatropha gossypifolia* leaf extracts. The authors reported that ethanol-water extracts contain more total phenolics (21.23 mgGAE/g), flavonoids (12.53 mgQE/g) and condensed tannins (7.13 mgCE/g) than acetone (14.76 mgGAE/g, 7.51 mgQE/g and 5.51 mgCE/g, respectively), hexane (17.04 mgGAE/g, 8.96 mgQE/g and 5.96 mgCE/g, respectively) and ethanol (12.92 mgGAE/g, 7.72 mgQE/g and 4.72 mgCE/g, respectively) extracts. Moreover, erythromycin (2.5 mg/mL) used as reference recorded an antimicrobial activity lower than that obtained with the ethanol-water extracts from *Z. mauritiana* barks and fruits. Regarding single and pure solvent, the acetone provided the most active extracts on both microorganisms tested. Similar observations were reported by Bayoï *et al.* [19] on a study conducted on the antimicrobial activity of tamarind leaf extracts against four bacterial species including *Staphylococcus aureus* and *Staphylococcus epidermidis*.

3.3. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The results reported in Table 3 shows that the MICs and MBCs are variable from one extract to another. MIC values ranged from 6.25 mg/mL to 200 mg/mL and more. The acetone bark extract showed the lowest MIC values with *Staphylococcus aureus* (6.25 mg/mL). Furthermore, acetone, ethanol and methanol bark extracts as well as acetone and ethanol leaf extracts recorded similar MIC values with *Staphylococcus epidermidis* (6.25 mg/mL). The highest MIC values were noted with the ethanol-water extract on *S. epidermidis* (100 mg/mL), and the hexane fruit extract on *S. aureus* (200 mg/mL). The current results are higher than those reported by Ashraf *et al.* [7]. The authors obtained MICs values of 0.08 mg/mL and 1.62 mg/mL with methanol and hexane *Z. mauritiana* leaf extracts, respectively. Abakala *et al.* [15] reported similar results than that of recorded in the current work with ethanol extracts from the leaves of *Z. mauritiana* and *Z. spina-christi*.

MBC values changed between 12.5 mg/mL and 200 mg/mL (or more than 200 mg/mL). The lowest value was obtained with acetone bark extract on *Staphylococcus epidermidis* (12.5 mg/mL) and the highest values were recorded with the fruit extracts on the same Gram-positive bacteria (> 200 mg/mL).

The values of MBC/MIC ratio change between 2 and 8 suggesting that *Z. mauritiana* extracts exerted either a bactericidal effect or a bacteriostatic effect. The lowest values with *S. aureus* (MBC/MIC = 2) were computed

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by ethanol bark extract as well as ethanol and methanol leaf extracts. Regarding *S. epidermidis*, hexane and acetone bark extracts, methanol leaf extract, and acetone fruit extract recorded the lowest MBC/MIC ratios equal to 2. According to these values, it can be suggested that ethanol bark extract, and ethanol and methanol leaf extracts exerted a bactericidal effect on *S. aureus*, similarly, hexane and acetone bark extracts, methanol leaf extract, and acetone fruit extract exhibited a bactericidal action on *S. epidermidis*. Acetone leaf and fruit extracts and the majority of the bark extracts showed the highest MBC/MIC ratios (4) with *S. aureus* suggesting they had a bacteriostatic activity. Furthermore, the highest MBC/MIC values (8) were recorded with methanol bark, ethanol-water leaf and ethanol fruit *Z. mauritiana* extracts on *S. epidermidis*. The aforementioned MBC/MIC ratios suggest that these three extracts also exerted a bacteriostatic activity on *S. epidermidis*. Globally, the extracts used in the current study showed stronger activity on *Staphylococcus epidermidis* compared to *Staphylococcus aureus*. This confirmed that the antimicrobial activity of the plant extracts changes from one microorganism to another.

The antimicrobial activity of fifteen extracts studied in the current research could be explained by the presence of polyphenols. Mohamadou *et al.* [20] reported average polyphenol contents of 58.25 ± 4.17 and 61.46 ± 1.59 mgGAE/g in 80% ethanol extracts from *Z. mauritiana* leaf and bark, respectively. Indeed, the antimicrobial activity of polyphenols is recognized. Many works support that the preferential site of action of phenolic compounds is the bacterial cytoplasmic membrane [21]. These bioactive compounds are able to disorganize the cell membrane which becomes more permeable to water and ions. The biological activity of the polyphenols leads to potassium leakage which is the first evidence of irreversible damage to the microbial membrane [22]. Mohamadou *et al.* [20] also reported flavonoids in ethanol-water (80%, v/v) extracts from *Z. mauritiana* bark and leaf. Flavonoids considered as one of the main polyphenols group are responsible for scavenging or chelating processes and can also disrupt the microbial membranes [23]. Tannins, another polyphenolic compounds group, possess a high affinity for metal ions and especially iron, some microorganisms require these ions to execute functions such as the reduction of precursor ribonucleotides in DNA formation [24].

4. Conclusions

In order to contribute in improving of storage and safety of foods, the antimicrobial activity of some parts (bark, leaf and fruit) of *Z. mauritiana* were tested against two *Staphylococcus* species isolated from local fish. The hot-maceration extraction was found to be more efficient than the increasing polarity solvent-based extraction. The ethanol-water extracts and especially those from *Z. mauritiana* barks displayed the best antimicrobial activity. Therefore, the ethanol-water bark extract could be suggested as a serious alternative to enhance the safety of fish and a promising tool contributing to the fight against food illnesses induced by Staphylococci pathogens.

Authors' contributions

Literature review, plant collection, experiments and data analyses, manuscript drafting, M.S.; Conception, supervision, revision of statistical analysis and manuscript, J.R.B.

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Availability of data and materials

All data will be made available on request according to the journal policy.

Conflicts of interest

The authors declare no conflict of interest.

References

1. Tacon, A.G.; Metian, M. Fish matters: importance of aquatic foods in human nutrition and global food supply. Rev. Fish. Sci. 2013, 21(1), 22-38. https://doi.org/10.1080/10641262.2012.753405.

https://doi.org/10.58985/jafsb.2024.v02i03.56

2. Béné, C.; Arthur, R.; Norbury, H.; Edwar, H.; Allison, M. Contribution of fisheries and aquaculture to food security and poverty reduction; assessing the current evidence. World Dev. 2016, 79, 177-196. https://doi.org/10.1016/j.worlddev.2015.11.007
3. Anihouvi, V.H.; Huonhouigan, J.P.; Ayernor, G.S. La production et la commercialisation du landouin un condiment à base de poisson fermenté du Golfe du Benin. Cahiers Agric. 2005, 4 (3), 323-330.
4. Anani, K.; Hudson, J.B.; de Souza L.C.; Akpagana, K.; Towe, G.H.N.; Amason, J.T.; Gbeassor, M. Investigation of medicinal plants of Togo for antiviral and antimicrobial activities. Pharm. Biol. 2000, 38(1), 40-45. https://doi.org/ 10.1076/1388-0209(200001)3811-BFT040.
5. Delmas, G.; Le Querrec, F.; Weill, F-X.; Gallay, A.; Espié, E.; Haeghebaert, S.; Vaillant, V. Les toxi-infections alimentaires collectives en France en 2001-2003. Institut de veille sanitaire, Direction générale de l'alimentation, Centre national de référence des Salmonella. 2003.
6. Sawadogo, S.Y. Phytochemical studies and biological activities of *Zizyphus mauritiana* Lam (Rhamnaceae) root barks and *Ziziphus mucronata* Willd (Rhamnaceae) leaves. PhD thesis in Pharmacy, University of Bamako, Bamako, Mali. 2012, 130 pages.
7. Ashraf, A.; Raja, A. S.; Farooq, A.; Shaukat, A. S.; Khalid, M.A. Chemical composition and biological activities of leaves of Ziziphus mauritiana L. native to Pakistan. Pak. J. Bot. 2015, 47 (1), 367-376.
8. Abalaka, M.E.; Mann, A.; Adeyemo S.O. Studies on in-vitro antioxidant and free radical scavenging potential and phytochemical screening of leaves of *Ziziphus mauritiana* L. and *Ziziphus spina-christi* L. compared with Ascorbic acid. J. Med. Genet. Genom. 2011, 3 (2), 28 - 34.
9. Hayette, M.P.; Huynen, P.; Meex, C. Practical work in general microbiology. Support de travaux pratiques, Université de Lièges, Lièges, Belgique. 2010, 62 pages
10. Bourgou, S.; Serairi, B.R.; Medini, F.; Ksouri, R. Effect of solvent and extraction method on phenolic compounds content and antioxidant potentialities of Euphorbia helioscopia. J. Sci. Agric. Biotechnol. 2016, 28(12), 1649-1655.
11. Mighri, H.; Hadjlaoui, H.; Akrout, A.; Najjaa, H.; Neffati, M. Antimicrobial and antioxidant activities of Artemesia herba-alba essential oil cultivated in Tunisian arid zone. Comptes Rendus Chimie. 2010, 13, 380-386.
12. Bouyahya, A.; Abrini, J.; Bakri, Y.; Dakka, N. Phytochemical screening and evaluation of antioxidant and antibacterial activity of *Origanum compactum* extracts. Phytother. 2017, 5. https://doi.org/10.1007/s10298-017-1101-8.
13. Mamadou, R.S.; Moussa, I.; Sessou, P.; Yehouenou, B.; Agbangnan, P.D.C.; Illagouma, A.T.; Abdoulaye, A.; Sohounhloue, D.C.K; Ikhiri, K. Phytochemical study, antiradical, antibacterial and antifungal activities of extracts of *Sebastiania chamaelea* (L.) Müll. Arg. J. Soc. Ouest-Afr. Chem. 2014, 037, 10-17.
14. Guinoiseau, E. Antibacterial molecules from essential oils: separation, identification and mode of action. PhD thesis, University of Corsica-Pasquale, Corte, France. 2010, 149 pages.
15. Abakala, M.E.; Daniyan, S.Y.; Mann, A. Evaluation of the antimicrobial activities of two Ziziphus species (*Ziziphus mauritiana* L. and *Ziziphus spina-christi* L.) on some microbial pathogens. Afr. J. Pharm. Pharmacol. 2010, 4 (4), 135-139.
16. Pradal, D. Eco-processes for the extraction of antioxidant polyphenols from an agro-food co-product. PhD thesis, Université Lille 1, Lille, France. 2016, 266 pages.
17. Nguyen, V.C. Control of oilseed technological aptitude by structural modification: applications to in-situ extraction and transesterification operations. Doctorate thesis, University of La Rochelle, La Rochelle, France. 2010, 191 pages.
18. Dezoumbe, D.H.; Bayoï, J.R.; Djoulde, D.R.; Etoa, F.X. Quantitative phytochemical screening and in-vitro antioxidant activities of *Jatropha gossypifolia* L. leaf extracts aiming antimicrobial and therapeutical applications. J. Nat. Prod. Ressour. 2022, 8 (1), 284-288. https://doi.org/10.30799/jnpr.106.22080102.
19. Bayoï, J.R.; Foundikou, B.Y.; Etoa, F.-X. In vitro bioactive properties of the tamarind (*Tamarindus indica*) leaf extracts and its application for preservation at room temperature of an indigenous roselle (*Hibiscus sabdariffa*)-based drink. J. Agric. Food Res. 2021, 6, 1-12, 100241. https://doi.org/10.1016/j.jafr.2021.100241.
20. Mohamadou, S.; Bayoï, J.R.; Roger, D.D.; Francky Steve, N.S.; Leopold, T.N. Phytochemical screening and antimicrobial activity of *Ziziphus mauritiana* Lam. and *Ziziphus mucronata* Lam. extracts. J. Adv. Med. Pharm. Sci. 2021, 23(8), 25–37. https://doi.org/10.9734/JAMPS/2021/v23i830252.
21. Kahlouche-Riachi, F. Chemical evaluation and antibacterial activity of some medicinal plants from Algeria. Thesis for the doctorate in science, University of Constantine 1, Constantine, Algeria. 2014, 148 pages.
22. Djahra, B.A.; Bordjiba, O.; Benkherara, S. Antibacterial activity of flavonoids from a spontaneous medicinal plant *Marrubium vulgare* L. from the region of El Tarf (North-East Algeria). Sci. Technol. Rev. Synth. 2012, 24, 29-37.
23. Boutalbi, S. Chemical screening and biological activity

https://doi.org/10.58985/jafsb.2024.v02i03.56

of spirulina (*Arthrospira platensis*). Master thesis, Kasdi Merbah University Ouargla, Algeria. 2014, 82 pages.

24. Kimenyi, P.; Kabakura, M.G.; Bajyana, S.E. In vitro study of antibacterial and antifungal activity of hydroethanolic extract of Maytenus undata barks. J. Etud. Rwandaises, Series C: Life Science and Natural Sciences. 2009.

https://doi.org/10.58985/jafsb.2024.v02i03.56