Identification of Peptides in the Leaves of *Bauhinia rufescens* Lam (Fabaceae) and Evaluation of Their Antimicrobial Activities Against Pathogens for Aquaculture

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Abstract: *Bauhinia rufescens* Lam is a Cameroonian medicinal plant belonging to the Fabaceae family. It is used by indigenous people in the treatment of gout, diarrhea, dysentery, diabetes, malaria. It has antibacterial, antiparasitic, antifungal and antioxidant potential. The aim of this study is to identify peptides from the leaves of *Bauhinia rufescens* and assess their antimicrobial activities against certain pathogens in aquaculture. Previous studies have showed the antimicrobial properties of liquid extract on water against human pathogenic bacteria and the influenza virus. These properties have been mainly attributed to phenolic compounds. Phenolic compounds play an important role in human health due to their various pharmacological activities as anti-inflammatory, anti-allergic, antimicrobial, antiviral, and vasodilatory. However, other plant defense molecules, such as antimicrobial peptides (AMPs), may be present. In this work, we studied peptide extracts from the leaves of *Bauhinia rufescens* Lam. Mass spectrometry and analysis of peptides gave 3 to 3.6 kDa, among them, peptides rich in cysteine were identified with antimicrobial activity against various Gram-negative bacteria, including recurrent pathogens of Cameroonian aquaculture. In addition, membrane bleeding on the bacterial surface after exposure to the cyclotide was visualized by SEM microscopy and the SYTOX Green permeabilization test showed the ability to disrupt the bacterial membrane. The results obtained show that the peptides exert their action by destroying the bacterial membrane. Based on the results obtained, the medicinal value of this plant could be attributed to the presence of secondary metabolites.

Keywords: *Bauhinia rufescens*, Peptides, Antimicrobial Activity, Fish Pathogens, Membrane Damage

1. Introduction

In many developing countries, access to conventional medicine remains limited to large cities. Difficulties in travel, insufficient qualified personnel, high cost of services, conventional medicines and socio-economic factors have left a large part of the population with no other choice than that of traditional medicine to treat common and infectious diseases [1]. Medicinal plants are used on all continents for their effectiveness, their accessibility and the low cost compared to drugs sold in pharmacies. A scientific light on this ancestral knowledge is important to validate the use of some plants. In addition, the active ingredients of many drugs sold in pharmacies have been discovered in plants. This is the case, for example, of aspirin isolated from the white willow
originally used for treatment of fever or taxol isolated from yew and used in the treatment of breast or ovarian cancer. This reflects the importance of plants in the discovery of new active molecules [2]. Based on all the above, we studied a plant of the genus Bauhinia, *Bauhinia rufescens* Lam.

*Bauhinia rufescens* Lam is a Cameroonian plant of the Fabaceae family [3]. It is a very branchy shrub, native to semi-arid regions of Africa like the Sahel, it grows to a height of 1 to 3 meters high but up to 8 meters [4]. The leaves have a deep shade of green colour. The seeds in the pods are dark brown. The branches are arranged in the same plane, the smallest are in the shape of thorns. The bark is gray and lenticellate. The leaves are small, strongly bilobed and have a dull persistent grayish-green color. The flowers are greenish yellow to pink white, type five, grouped in 5 cm racemes. Species recommended for the creation of defensive, fodder or ornamental hedges. It fixes nitrogen in the air. It is an important shrub fodder [5, 6]. In traditional medicine, the plant is used in the treatment of gout, diarrhea, dysentery, diabetes, malaria. It has antibacterial, antiparasitic, antifungal and antioxidant potential [4, 7]. Previous studies have shown that this plant contains various compounds with strong biological activity, such as flavonoids, alkaloids, carbohydrates, flavonoids, saponins, tannins, glycosides, terpenes, proanthocyanidins, anthocyanins and phenolic acids [7-9]. These compounds provide protection against oxidative stress caused by reactive oxygen species, which are known to be involved in disorders like cancer and hypertension [10]. In addition, the elderberry liquid extract shows activity against human pathogenic bacteria and influenza viruses [11]. In addition to these compounds, antimicrobial peptides (AMPs) are small peptides ranging from ten to a few tens of amino acids, they play a key role in the defense of plants against pathogens [12]. Plant AMPs are generally rich in cysteine residues that form multiple disulfides. Families of cystein-rich peptides (CRPs) include thionines, defensins, hevein-like peptides, cyclotides, lipid transfer proteins, and the α-hairpinin and snakins family [13–15]. In this regard, studies have focused on the use of plant seed and plant improvement (SAP) as substitutes for chemical preservatives and insecticides in agriculture applications [16, 17]. In addition, the characteristics of these antimicrobial molecules make them usable in breeding and in public health, thus reducing the use of antibiotics. Plant AMPs have been isolated and characterized in the roots, leaves and seeds, although they are also present in floral tissues [18]. Leaf AMPs were characterized on the basis of cationic charge, thus pointing to the permeabilization of lipid bilayers as a possible mechanism of action [18]. However, since flowers have received less attention than other tissues, they may contain a number of SAPs still unknown with biotechnological applications. Among the plants used for this purpose is *Bauhinia rufescens* Lam. (common name “Kharroud” in the Far North), which has berries that contain antioxidant phytonutrients similar to those found in other elderberries [5, 6]. However, the antimicrobial properties of this medicinal plant have not been established to date. In this study, we have developed an experimental procedure to obtain a peptide extract from the leaves of *Bauhinia rufescens* Lam. Cysteine-rich peptides (CRPs) were identified by mass spectrometry, and peptide extracts were analyzed for antimicrobial properties, including activity against pathogenic bacteria that affect Cameroonian aquaculture. In addition, scanning electron microscopy (SEM) tests were performed in order to directly observe the response of bacterial cell morphology and membrane integrity to treatment with peptide extracts, thereby revealing the mechanism of cell destruction exercised. Therefore, the objective of this study is to identify peptides, the antimicrobial activity of the leaves of *Bauhinia rufescens* Lam in order to understand the nature of the main component responsible for its medicinal property.

2. Materials and Methods

2.1. Collection of Bauhinia rufescens Lam (Fabaceae)

The leaves of *Bauhinia rufescens* Lam were collected on January 14, 2018 in Kakatare, a District of the city of Maroua in the Far North Region between 5.30 a.m. and 6.30 a.m. The choice of this time interval was based on the principle that the morning harvest corresponds to the most favorable moment when the active ingredients of the plant are generally preserved [19, 20]. The plant material was identified by Dr Froumsia Moskia Botanist of the Department of Biological Sciences of the University of Maroua.

2.2. Extraction of Peptides

The leaves of *Bauhinia rufescens* Lam (Far North Region) were dried and crushed in a blender. They were then weighed (80 g) and homogenized in a mixture of dichloromethane (DCM)/methanol (MeOH) (1:1) (2 mL per g) [16]. The extract was filtered and transferred to a separating funnel. After that, pure UPW-Ultra water was added (2 mL per10 mL of extract) and the solution was mixed. The organic layer (bottom) was discarded and the aqueous layer was collected and placed on a rotary evaporator to remove MeOH. The extract was lyophilized and weighed. The dried extract was then reconstituted with water and was passed through an SPE polyamide cartridge DPA-6S (Sigma-Aldrich, St. Louis, MO, USA) to remove the polyphenols and other compounds. The aqueous elution was applied to a Sep-pak C18 Vac cartridge (Waters Associates, Milford, MA, USA) and equilibrated in acidified water (0.05% trifluoroacetic acid (TFA) in UPW-Ultra Pure Water). After washing with acidified water, the peptides were eluted at a flow-rate of 1 mL/min with 5%, 10%, 20%, 30%, 40%, 60% and 80% acetonitrile (ACN). The appropriate fractions were collected and the ACN was evaporated on a speedvac centrifuge. The fractions were then analyzed by reversed-phase (RP)-HPLC (Waters Associates, Milford, MA, USA) on a Water Corp XBridge™ BEH C18 column (100 × 4.6 mm, 3.5 µm) Waters Associates, Milford, MA, USA) using an ACN gradient of 0 to 70%, water containing 0.05% TFA as solvent A and ACN containing 0.05%
TFA as solvent B, at a flow rate of 1 mL/min for 8 min.

**2.3. Mass Spectrometry Analysis for Peptide Identification**

The mass spectra of each ACN fraction were acquired in a Microflex with a matrix-assisted laser desorption ionization mass spectrometer (MALDI-TOF) (Bruker Daltonics Inc., Billerica, MA, USA). The 40% fraction of ACN was then prepared for ESI MS/MS sequencing as described previously [21, 22]. Briefly, the extract was reduced (dithiothreitol), alkylated with iodoacetamide and digested enzymatically using trypsin or endo-GluC (Sigma-Aldrich and Promega Corp., Madison, WI, USA, respectively) [22]. The proteolyzed samples were examined in an LC-MS-MS system consisting of an Agilent 1100 HPLC (Agilent Technologies Inc., Santa Clara, CA, USA) coupled to an ESI-TRAP Esquire 4000 ion-trap mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany). For analysis of chromatograms and LC-ESI-MS-MS, Data Analysis version 3.2 (Bruker Daltonik GmbH, Bremen, Germany) was used. Mascot Server version 2.0 (Matrix Science, London, United Kingdom), PEAKS Studio version 8.0 (Bioinformatics Solutions Inc., Waterloo, ON, Canada) and CyBase have been used for the identification of peptides [23, 24].

**2.4. Antibacterial Dosage**

Antibacterial activity was determined using the microplate test, as previously described [25–28]. Three concentrations of the peptide extract were tested to evaluated antibacterial activity. 10 µg/mL, 50 µg/mL and 100 µg/mL of peptide extract (ACN fraction at 40%) were mixed with 100 µL of an exponential bacterial culture of *Escherichia coli*, *Vibrio anguillarum*, *Vibrio ordalii*, *Flavobacterium psychrophilum* and *Aeromonas salmonicida*. In addition, a synthetic peptide variant derived from phospholipase-A2 was used as a positive control [29]. The test was carried out at an initial OD of 0.001 at 620 nm in tryptic soy broth (TSB) for *Escherichia coli* and *Aeromonas salmonicida*, TSB containing 1.5% NaCl for *V. anguillarum* or and the liquid medium Anacker and Ordal’s (AOAE) for *F. psychrophilum*. After 16 h of incubation (37 °C for *Escherichia coli*; 24°C for *Aeromonas salmonicida subs salmonicida* and *Flavobacterium psychrophilum*; 25°C for *Vibrio anguillarum* and *Vibrio ordalii*), absorbance values were measured [30, 31].

**2.5. Phytochemical Screening of the Extract CH$_2$Cl$_2$/MeOH from Bauhinia rufescens Lam**

Phytochemistry is defined as the study of the chemical composition of medicinal plants or a phyto-drug via qualitative analysis, coloring/precipitation reactions and the use of chromatographic or spectrometric technics [32, 33]. In this work, a qualitative test was carried out to highlight the phenolic, flavonoid and alkaloid compounds in *Bauhinia rufescens* Lam, compounds with antibacterial properties [34].
The quantitative assessment of these compounds was subjective and based on the intensity of the coloring.

2.5.1. Highlighting Phenolic Compounds
A qualitative test was carried out according to the protocol described by Bekro et al. [35]. 2 mL of each solution was added to a test tube and 5 drops of 10% FeCl₃ were added. The appearance of the purple color has been interpreted as the abundance of phenolic compounds [36]. The decrease in intensity has been interpreted as the average presence of these compounds. The lack of coloring has been translated as the absence of the compound.

2.5.2. Detection of Flavonoids
The method described by Quettier-Deleu et al. [37] was used to detect flavonoids. This method uses aluminum trichloride as a reagent. It is based on the oxidation of flavonoids by this reagent, resulting in the formation of a yellow complex by the introduction of 1 mL of a solution of AlCl₃ in 1 mL of each extract (prepared in 80% methanol). The intensity of the coloration was interpreted as a quantitative marker of the presence of flavonoids.

2.5.3. Alkaloids
The Wagner's Test described by Shah et al. [36] was used to highlight the alkaloids. 5 drops of de Wagner's reagent (diluted iodine solution) are added to a test tube containing one milliliter (1 mL) of extract. The presence of the alkaloids is marked by the formation of a reddish-brown precipitate. The quantitative interpretation was based on the color and density of the precipitate. An intense precipitate refers to the abundance of alkaloids and vice versa.

2.6. SYTOX Green Bacteria Permeabilization Test
The SYTOX Green absorption test was carried out according to a procedure described above [25]. Cultures of *Escherichia coli* and *Aeromonas salmonicida* growing exponentially were diluted in 10 mM sodium phosphate buffer pH 7.2 to a cell density of 1 × 10⁸ CFU/mL. Then, 90 µL aliquots of these cell cultures were deposited in optical tubes of peptides rich cysteine (PRC) in real time and 5 µL of the solution of peptide extract (50 and 100 µg/mL) and 5 µL of peptide extract or a synthetic peptide derived from phospholipase-A2 at 20 µM. After incubation, the cells were washed three times with PBS. The bacterial pellets were then fixed overnight in 500 µL of 2.5% (v/v) glutaraldehyde in PBS at 4°C. Subsequently, the bacterial samples were dried with sets of graduated ethanol and then dried. A small amount of platinum was sputtered on the samples to avoid loading them into the microscope. The cells were examined with a scanning electron microscope (Hitachi SU 3500, Tokyo, Japan).

2.8. Polyphenol Quantification
The content of total phenols in the extracts of *Bauhinia rufescens* Lam was measured by Folin-Ciocalteu spectrophotometry test, according to the technique of Odeh et al. [38]. Methanol solutions of gallic acid at concentrations between 100 and 1000 µg/mL were used as the calibration curve. 5 µL of each gallic acid solution and extracts of *Bauhinia rufescens* Lam were used for the quantification of phenols. 75 µL of distilled water and 20 µL of Folin-Ciocalteu reagent and 1 N were added to each. After 3 min, 30 µL of Na₂CO₃ solution (10% w/v) were added, then 120 µL with distilled water were added. After 2 h of dark incubation, the absorbance at 760 nm was measured in a microplate reader. The total phenol content was expressed in mg/mL.

3. Results and Discussion

3.1. Identification of Peptides from *Bauhinia rufescens* Leaves
It is estimated that around 80% of the world's population uses natural products for primary health care purposes [39]. Scientific research supports the biological activity of many natural phytochemicals; in fact, several plant substances of natural origin have a wide spectrum of biological properties, including antioxidant, antibacterial, antiviral and anti-inflammatory activity, among others [40]. Plants are thus a source of biotechnological products. AMPs are a component of the defense system against phyto-pathogens. However, these peptides also show antimicrobial activity against various human pathogens, and therefore emerge as promising antibiotic compounds with important biotechnological applications [9]. In this study, we demonstrate the presence of AMP in the leaves of *Bauhinia rufescens* Lam, which is a medicinal plant used in the Far North regions. The experimental procedure for extracting peptides is illustrated in (Figure 2). The isolation and purification of peptides from plants can be complicated by their propensity to degrade when exposed to solvents [15]. However, plant CRPs show exceptional resistance to thermal/chemical denaturation [13]. In this study, we immersed the plant material in a DCM-MeOH mixture (1: 1, v/v) and leave overnight at room temperature, a procedure widely used for the extraction of...
peptides [15]. However, after the addition of water, the aqueous layer contained large amounts of polyphenols (0.987 mg/mL). In fact, older trees contain high amounts of polyphenolic compounds [41-44]. Thus, polyamide resin must be used for their elimination because a strong hydrogen bond occurs between polyphenols and polyamide, but the peptides are not retained on this column support [26]. The reverse phase analytical chromatogram (RP-HPLC) of a peptide extract of the leaves of Bauhinia rufescens Lam after extraction with solvent and purification C18 in batch shows several peptide peaks (Figure 3A), and molecular weights between 3.1 and 3.6 kDa were determined with them by matrix-assisted laser desorption ionization mass spectrometry (MALDI-TOF) (Figure 3B).

Figure 2. Schematic representation of the major steps for the peptide extraction procedure.

Figure 3. Characterization of peptide extract from leaves of Bauhinia rufescens Lam. (A) HPLC spectra of 40% acetonitrile (ACN) fraction of leaves peptide extract; (B) Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometer (MALDI-TOF) MS spectra of the 40% ACN fraction of leaves peptide extract.
Cyclotides are head-to-tail cysteine-rich peptides (CRPs) with typical masses of 2–4 kDa [15, 45–47], thus suggesting the presence of cyclotide peptides in the leaves of *Bauhinia rufescens* Lam. However, the structure adopted by the cyclotides prohibits the analysis of direct fragmentation. Thus, a partial identification was completed with MALDI-MS data where the primary structure of peptides from the leaves of *Bauhinia rufescens* Lam, was determined by means of an enzymatic fragmentation of reduced and alkylated peptides because these chemical modifications are necessary to produce precursor ions which lend themselves to MS/MS sequencing [30-31, 38]. The alkylated peptides were then cleaved with trypsin or Glu-C endoproteinase, and the resulting peptide fragments were identified by ESI-MS-MS mass spectrometry using Mascot and PEAKS servers. The partial characterization of the SAPs identified from the leaves of *Bauhinia rufescens* Lam, are presented in (Table 1). From the analysis of MS/MS data, the identification of a peptide as chassatide C10 is based on a correspondence with a small part of the sequence described for it in the literature (5/29=17% coverage of sequence), but the identification was completed with MALDI-MS data where the signal m/z 3212.107 was detected showing an error of-55.7 ppm compared to the expected m/z for this cyclotide. Likewise, from MS/MS data, a peptide was identified as glopa E on the basis of coincidence with part of the sequence described for it (6/30=20% sequence coverage); moreover, the MALDI-MS data suggest the presence of a peptide with an m/z of 3228.618 close to the expected value for glopa E of m/z 3227.398. In both cases, it would be necessary to detect the missing tryptic peptide to confirm the complete sequence. The identification of tryptic peptides such as cyclotides caripe 4 and vaby C is based on the sequence correspondence of 59 and 45% respectively, as determined from the analysis of MS/MS data. Finally, identification as phyb A is based on the 83% coincidence of the sequence. In summary, the MALDI-MS data show that the detected peptides are in the m/z range described for the cyclotides while the MS/MS data have shown a partial coincidence with known cyclotide sequences. However, more studies are necessary for the characterization the primary and secondary structures of these molecules identified in the leaves of *Bauhinia rufescens* Lam.

### Table 1. Peptides present in the leaves of *Bauhinia rufescens* Lam.

| Sequence detecteda | Peptides rich on Cysteine | Family |
|--------------------|--------------------------|--------|
| GEYGCGESYLIPFGPCYCVRQCVNKNb | Chassatide, CV (Chassalia chartacea) | Cyclotide |
| GIPCAECSCVWPCTKMLGSCXDKCVYNb | Glopa E (Gloeospermum pasciflorum hekking) | Cyclotide |
| LICSSCTLRPSPRCTVRHHICYLNa | Caripe 4 (Carapichea Ipecacuana) | Cyclotide |
| GLPVCGETACCRNTPGCSCSWPVCTRNa | Vaby C (Viola abyssinica) | Cyclotide |
| GIGCGESCVWPCSVAAIICSNKICYRNa | Phyb A (Petunia hybrida) | Cyclotide |

* Identified pep tidic fragments are showed in red; *b* Identified in trypsin digest. *c* Identified in endoproteinase GluC digest.

### 3.2. Antimicrobial Activity of Peptides from the Leaves of *Bauhinia rufescens* Lam

Plant antimicrobial peptides, including cyclotides, were initially studied because their main function is the control of opportunistic pathogens [49]. Given their broad antimicrobial spectrum, these molecules appear to be interesting targets to be exploited for improving animal health. In this work, we focused on the study of the antimicrobial activity of the peptide extract of the leaves of *Bauhinia rufescens* Lam against various Gram-negative bacterial pathogens that affect fish aquaculture. Antimicrobial resistance in traditional fish farming has been widely studied [50–53]. Fish farms are an environmental reservoir of antibiotic resistance genes, since an excess of food containing antibiotics is deposited on the seabed. In this regard, antimicrobial residues have been found in the sediments of marine fish farms [54]. In addition, 80 gram-negative strains that have been isolated from salmonid water samples have been studied [55]. For this reason, some plant extracts have been tested against a number of bacterial fish pathogens [56–58]. Here, the activity of the peptide extract of the leaves of *Bauhinia rufescens* Lam was evaluated against *A. salmonicida*, *F. psychrophilum*, *V. anguillarum* and *V. ordalii*, all of the Gram-negative bacterial pathogens found in aquaculture for salmon in Cameroon. Analyses on microplates have shown the ability of the extract to reduce the growth of all pathogens (Figure 4). The strongest antimicrobial effect is observed at a concentration of 100 µg/mL of the peptide extract.

### 3.3. Phytochemical Tests

Table 2 shows the observations relating to the detection of phenolic, flavonoid and alkaloid compounds in the extracts of the leaves of *Bauhinia rufescens* Lam.

### Table 2. Phytochemical composition of extracts of *Bauhinia rufescens* Lam.

| Plants | Compounds | Relative Quantity | Extracts |
|--------|-----------|------------------|----------|
| Phenolic compounds | +++ | Leaf methanol |
| Flavonoids | +++ | Aqueous methanol of the leaves |
| *Bauhinia rufescens* Lam | Alkaloids | ++ | Leaf decoction |
| & | & | & | Leaf infusion |
| & | & | & | Leaf methanol |
| & | & | Aqueous methanol of the leaves |

+++: Very abundant; ++: Medium; +: Low; 0: Absence.
Figure 4. Antimicrobial activity of the leaves de Bauhinia rufescens Lam peptide extract. Microplate antimicrobial assay of peptide extract against E. coli, A. salmonicida sp. salmonicida, F psychrophilum, V anguillarum and V. ordalii. Antibacterial activity was evaluated with 10 µg/mL, 50 µg/mL, or 100 µg/mL of the leaves peptide extract (n=6) in two independent experiment. Phospholipase-A2-derived synthetic peptide at 55.7 µg/mL was used as a positive control. Negative controls were performed under the same conditions without the addition of peptide.

In the table above, a varied distribution of the phenolic, flavonoid and alkaloid compounds is observed in the leaves of Bauhinia rufescens Lam. Indeed, the results obtained show that in the leaves of Bauhinia rufescens Lam, phenolic compounds are in abundance in the aqueous methanol extracts, obtained by decoction and infusion of the leaves. These compounds are moderately present in the methanol extract. As for the flavonoids, they are very abundant in the methanol and methanol-aqueous extracts of the leaves but moderately present in the extract obtained by decoction. Their quantity in the extract obtained by infusion is low. For alkaloids, they are moderately present in the leaves regardless of the extraction method used. This strong presence of these compounds (phenolic, alkaloid and flavonoid compounds) which was observed in (Table 2) above is in agreement with the results obtained by Muhammad et al. [7-8, 59].

3.4. Damage to the Bacterial Membrane Induced by the Peptides of the Leaves of Bauhinia rufescens Lam

One of the rare preserved characteristics of antimicrobial peptides (AMPs) is their cationic and hydrophobic composition [60]. This makes them well suited to interact with the anionic surfaces of microbial membranes, which generally have a high content of lipids, such as phosphatidylglycerol, cardiolipin, lypopolysaccharides and teichoic acids [61–63]. Several models of interaction of AMPs with the membranes, such as "barrel stave", "toroidal pore" or "carpet model" have been studied [64]. In the carpet model, the peptides form a layer of "carpet" which induces a weakness of the membrane, which finally ends in a collapse of the membrane by a detergent action. According to this mechanism, the peptides affect the local curvature of the bilayer cooperatively, so that a torus of high curvature is formed [60, 65]. The effect of the peptide extract from the leaves of Bauhinia rufescens Lam on the integrity of the bacterial membrane of E. coli and A. salmonicida were analyzed. This is generally studied using a fluorescent nucleic acid dye, such as SYTOX Green, which is impervious to living cells [66]. The synthetic peptide derived from phospholipase-A2 has been used as a positive control, since its action on bacterial membranes has been described previously [67]. As expected, a large increase in fluorescence occurred after treatment with the control peptide for 2 min (Figure 5). It is interesting to note that treatment with 50 µg/mL or 100 µg/mL of peptide extract has shown similar results, with an early increase in fluorescence (Figure 5).

In addition, direct visualization of damage to the bacterial membrane after treatment with the peptide extract was obtained by scanning electron microscopy (SEM) (Figure 6). SEM images of A. Salmonicida (mid-log growth phase) who were treated with the peptide extract showed damage to the membrane with multiple blisters. In addition, the intracellular
content was released into bacteria, accompanied by membrane bleeding, suggesting a disruption of the membrane as the mechanism of action of these peptides (Figure 6B). Thus, SEM images suggest that peptides from the leaves of Bauhinia rufescens use a carpet mechanism to kill bacteria, since the presence of membrane blebbing is associated with this model [29, 68, 69]. Nevertheless, future studies are necessary to identify the interactions of AMP of the leaves of Bauhinia rufescens Lam with the components of the bacterial membrane to understand the antimicrobial mechanisms of these AMPs [70].

Figure 6. Bacterial membrane damage induced by Bauhinia rufescens Lam peptide extract. Scanning electron microscopy (SEM) micrographs of A. salmonicida sp. salmonicida without peptide (A) and with the presence of 50 µg/mL of the leaves peptide extract (B). Segmented yellow quadrate shows a zoom of representative bacteria.

Figure 5. Membrane permeabilization influx of SYTOX Green in E. coli and A. salmonicida cells. The bacteria were exposed with 50 µg/mL or 100 µg/mL of the flowers peptide extract for 20 mins in the presence of 5 µM SYTOX Green. Phospholipase-A2-derived synthetic peptide at 55.7 µg/mL was used as a positive control. Negative controls were performed under the same conditions without the addition of peptide. The increase in fluorescence was recorded at 30 s intervals with SYBR green filter.

4. Conclusion

Seeds and plant breeding (SPB) appear as therapeutic alternatives to conventional antibiotics. Extracts from medicinal plants are an abundant source of new agents and could be exploited in various fields, such as the fight against infections in livestock. The presence of peptides rich in cysteine in the leaves of Bauhinia rufescens has been determined and has activity against various pathogens of fish of interest for Cameroonian aquaculture. Their antimicrobial activity has been shown to be linked to disruption of the bacterial cell membrane. However, more studies are needed to characterize the structure and activity of each peptide present in Bauhinia rufescens leaves.

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Conflicts of Interest

The authors declare no conflict of interest.

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