Research Article

Antimicrobial activity of *Luma apiculata* against resistant strains of *Staphylococcus aureus* and *Klebsiella pneumoniae*

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Abstract

Bacterial resistance is the mechanism through which the bacteria can decrease the action of antimicrobial agents. Studies have reported an antimicrobial action of *Luma apiculata*, however, there are no *in vitro* studies demonstrating the effect of this plant against multidrug-resistant bacteria. The objective of this study was to explore the antimicrobial properties of alcoholic extract of *Luma apiculata* against standard multi-resistant bacterial pathogens. Multidrug-resistant strains of *Staphylococcus aureus* ATCC 43300 (MRSA) and *Klebsiella pneumoniae* ATCC BAA 1705 (CRKP) were incubated independently for 72 h at 37°C with different concentrations (1-10,000 µg/mL) of alcoholic extract of *Luma apiculata*. The growth curve was studied up to 72 hours. The minimum inhibitory concentration (MIC) was determined by broth macrodilution method. The agar diffusion method was used to examine bacteriostatic activity and bactericidal activity was evaluated by a commercial kit and analyzed by flow cytometry. Analyses of chromatographic data, as well as mass spectrometry, were performed to determine the main compounds present in the extracts. *Luma apiculata* leaves extracts showed bactericidal activity and was effective attenuating growth in both bacterial strains. Thus, *Luma apiculata* could represent a natural alternative against multidrug-resistant bacterial strains. However, further studies should be undertaken to test their clinical utility.

Keywords: Antimicrobial activity; Arrayan; Flavonoids; Medicinal plants; Multiresistant bacteria

Introduction

Antimicrobial resistance (AR) represents an important health issue that requires worldwide attention. The importance of AR has grown gradually becoming a global issue

mainly because it can be transmitted between different hosts and rapidly spreads between continents [1, 2]. In recent years, antibiotic-resistant pathogens from the ESKAPE group (*Enterococcus faecium*, *Staphylococcus*

aureus, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.) have been frequently detected in patients, representing an emerging health threat [3]. The emergence of antibiotic-resistant forms of pathogenic *S. aureus* is a worldwide problem in clinical medicine [4]. Furthermore, methicillin-resistant *Staphylococcus aureus* (MRSA) remains as a major global cause of health-care-associated infections [5]. *Klebsiella pneumoniae* is an opportunistic pathogen in immunocompromised individuals that causes a wide range of infections, including pneumonia, urinary tract infection, bacteremia, and meningitis [6]. In the last two decades, strains confirmed to produce *Klebsiella pneumoniae* carbapenemase (KPC) have been attributed to numerous nosocomial infections worldwide.

Despite the fact that *Klebsiella pneumoniae* is the main producer of β -lactamase type KPC, other species such as Enterobacteriaceae (*Klebsiella oxytoca*, *Enterobacter* spp., *Escherichia coli*, *Salmonella* spp., *Citrobacter freundii*, and *Serratia* spp.) and *Pseudomonas aeruginosa* also produce enzymes that confer resistance to β -lactamic agents [7, 8].

In recent years, there has been an increased interest in studying the antibacterial properties of natural sources such as plants. Antimicrobial compounds from these natural sources, including essential oils or plant extracts, are currently available for their use in alternative therapies [9].

A number of studies from different plant species have shown antibacterial properties [10–12]. The arrayan (*Luma apiculata* (DC.) Burret) is a Chilean myrtle that has been used in traditional medicine [13]. The medicinal properties of Myrtle leaves include aromatic, astringent, balsamic and anti-inflammatory actions [14]. Previous reports have shown an inhibitory activity of the xanthine oxidase

enzyme by Paraguayan and Chilean Myrtaceae species commonly used in folk medicine to treat gout [15] and also as astringent [16]. Leaves of *Luma apiculata* are used to treat diarrhea, dysentery, and ingestion [17]. In addition, Simirgiotis *et al.* [18] described the phenolic compounds pattern, highlighting its high polyphenolic contents and demonstrated the high antioxidant capacity in leaves of *Luma apiculata*.

Despite the traditional use of *Luma apiculata*, few investigations have been performed to prove its therapeutic effect as anti-infective agent to establish potential medicinal activity against persistent infections. The objective of this study was to explore the antimicrobial properties of alcoholic extracts of *Luma apiculata* leaves against standard multi-resistant bacterial pathogens.

Materials and methods

Chemicals and reagents

Bacteria were grown in Mueller-Hinton broth medium (Oxoid, Madrid, España) and Mueller-Hinton agar plate (Biomérieux, Marcy-l'orme, France). Antimicrobial disk reagents: vancomycin E-test, cefoxitin (30 μ g) (Biomérieux, Marcy-l'orme, France), gentamicin (10 μ g) and meropenem (10 μ g) were obtained from Mast Diagnostics (Merseyside, UK). LIVE/DEAD®BacLight bacterial viability Kit was purchased from Molecular Probes (Eugene, OR, USA) and used in accordance with the manufacturer's instructions.

Collection and preparation of plant samples

Fresh plants were collected from different geographical areas of the Araucania region (37°35'–39°37'S, 90°50'W) located in the Southern part of Chile during September 2017. *L. apiculata* was identified according to Herbal Medicines guide [14]. And confirmed by HERBARIO CONC (Botanical Department, University of Concepción, Chile). The voucher number of the sample is

CONC 186475. Fresh leaves were washed with sterilized water and allowed to dry in air. Dried leaves were homogenized to a fine powder using a pestle and mortar and stored in a sterile bottle at 4 °C for further analysis.

Sample preparation

Ten grams of dried leaves powder (*L. apiculata*) were extracted with 40 mL of 95% methanol and mixed on a rotary shaker to 170 rpm for 24 h at 30 °C. The extracts were filtered using Whatman filter paper No. 1 and then concentrated using a rotary vacuum evaporator (RE-52AA, Hitech-Lab) at 40 °C. The alcoholic extract had a concentration of 815.2 mg/mL dry material, which was dissolved with 1 mL DMSO 0.01% in distilled water. The extracts obtained were stored at -20 °C in an air tight bottle for further investigation.

Microorganisms

The following strains were obtained from the American Type Culture Collection (ATCC; Rockville, MD) and used in this study: Methicillin and oxacillin-resistant *Staphylococcus aureus* (ATCC® 43300™) and *Klebsiella pneumoniae* (ATCC® BAA-1705™) carbapenemase-positive. According to the American Type Culture Collection (ATCC) *Klebsiella pneumoniae* (ATCC® BAA-1705™) is resistant to: Amoxicillin/Clavulanic Acid, Piperacillin, Ampicillin, Ampicillin/Sulbactam, Cefazolin, Cefoxitin, Ceftazidime, Ceftriaxone, Cefepime, Meropenem, Ertapenem, Imipenem, Ciprofloxacin, Levofloxacin, Amikacin, Nitrofurantoin, Aztreonam, Trimethoprim/Sulfamethoxazole and sensitive to Gentamycin. Both strains were reconstituted according to the manufacturer's recommendations and incubated at 37 °C for 24 h. The purity of the cultures was confirmed by Gram staining.

Proof of resistance and sensitivity to antibiotics

Although ATCC has tested every one of the strains to verify species, KPC production,

and multidrug resistance, we confirmed the relative resistance of MRSA and CRKP strains using disk diffusion method with cefoxitin (30 µg) and meropenem (10 µg), and sensitivity with vancomycin and gentamicin (10 µg), respectively (Figure 1). The agar plates were incubated at 37 °C for 24 h.

Effect of plant extract on bacterial growth curve

To evaluate the effect of plant extracts on bacterial growth curve, different concentrations of extract were prepared (1, 10, 100, 1000 and 10000 µg/mL). All concentrations of both bacterial strains were incubated at an initial concentration of 0.5 McFarland (1.5×10^8 cells per cm³). Bacterial concentrations and bacterial growth rates were calculated by measuring the optical density (OD) at the wavelengths of 600 nm at 0, 3, 6, 24, 48 and 72 h (OD of 0.1 corresponds to a concentration of 1.5×10^8 cells per cm³). Bacterial strains without plant extracts were used as controls.

Determination of minimum inhibitory concentration (MIC) of plant extracts

For the determination of MIC, the broth macrodilution susceptibility assay was performed, according to standard M100-S9 of the National Committee for Clinical Laboratory Standards [19]. Six dilutions series of plant extracts were prepared from 1 to 10,000 µg/mL, into test tubes containing 10 mL Mueller Hinton broth medium. The bacterial broth cultures were adjusted to 0.5 McFarland turbidity standards and added into each of the test tubes. The medium containing a mixture of bacteria and extracts was incubated at 37 °C for 24 h in a shaker water bath. Bacterial strains without plant extracts were used as controls.

Antimicrobial susceptibility testing

The antibacterial activity assay of plant extracts was developed using the agar diffusion method. The MRSA and CRKP strains were performed by Kirby-Bauer

method on Muller Hinton agar plates. Whatman filter No.1 paper discs were soaked with 10 μ L of extract in concentrations of 1, 10, 100, 1000 and 10,000 μ g/mL and air dried before the assay [20]. Cultures were incubated at 37 °C for 24 h prior to testing, using the criteria of standard zone sizes of inhibition according to Clinical and Laboratory Standards Institute (CLSI) guidelines [19]. After the period of incubation, the antimicrobial activity was measured in millimeters of the zone of inhibition and categorized as sensitive or resistant.

Bacteriostatic activity of plant extracts

To estimate the bacteriostatic activity of alcoholic extracts of *L. apiculata*, each Mueller Hinton broth tubes used to calculate the MIC was transferred onto the surface of Muller Hinton agar plates and incubated at 37 °C for 24 h. Inhibition of bacterial growth was considered as bacteriostatic activity of that extract.

Bactericidal activity of plant extracts

To assess the effect of plant extracts on bactericidal activity, concentrations of 100, 1,000 and 10,000 μ g/mL of each extract were prepared. Bacterial strains without plant extracts were used as controls. The bacterial strains were incubated at an initial concentration of 0.5 McFarland (1.5×10^8 cells per cm^3) at 37 °C for 72 h. Direct measurement of bactericidal activity was assessed by using LIVE/DEAD[®] BacLight bacterial viability Kit (Molecular Probes, Eugene, OR, USA). This assay uses two-color fluorescences, which differs in the ability to penetrate cell membranes. The green fluorescent SYTO[®] 9 stains the nucleic acids of both living and dead bacteria, while Propidium Iodide (red) labels the nucleus of bacteria with damaged and leaky membranes. The fluorescence analysis was performed using a FACSCanto[™] II flow cytometer (BD Biosciences, San Jose, CA, USA). Data were acquired and analysed with the software

FACSDiva version 6.1.3 (Becton, Dickinson and Company, BD Biosciences, San Jose, CA, USA). At least 10,000 events were acquired for each sample. Fluorochrome excitation was carried out using a 488 nm argon-ion laser. Green fluorescence was collected with a 530/30 nm bandpass filter and orange fluorescence through a 585/42 nm bandpass filter, both were analyzed on logarithmic scales.

HPLC-UV- ESI MS/MS analysis

The extracts were analyzed by using HPLC-UV- ESI/MS/MS system consisting of a Shimadzu Prominence LC-20AD liquid chromatograph (Shimadzu, Milan, Italy) with an UV/VIS detector coupled to a 3200 QTRAP Hybrid Quadrupole-Linear Ion Trap Mass Spectrometer (Applied Biosystems, AB Sciex, Foster City, CA, USA) equipped with an Electrospray ionization (ESI) Turbo ion-spray interface. The chromatographic separation was carried out using an Inertsil ODS-3 C18 column (2.1 x 150 mm, 3 μ m) with a sample injection volume of 10 μ L. The flow rate was set at 0.25 mL/min and the column was maintained at 25 °C. The working wavelength of the UV/VIS detector was performed at $\lambda = 280$ nm. The control of components of the HPLC system, data collection and analysis were carried out by Analyst[®] 1.5.1 software (Applied Biosystems/AB Sciex). The mobile phase consisted of 0.1% (v/v) formic acid in water (A) and 0.1% (v/v) formic acid in acetonitrile (B). The gradient program was used as follows with a total analysis time of 40 min: 80% A (0-1 min), 78% A (20 min), 50% A (30 min), 0% A (31-35min), and 80% B (36-40 min).

The analyses were performed using the Turbo V^(R) ion spray in positive and negative ionization mode by using the information dependent acquisition (IDA) method editor. Enhanced MS (EMS) spectra were acquired between m/z 100-1,000 at scan speed of 1,000 Da/s. Enhanced production ion (EPI) spectra

were acquired between m/z 50-1,000 at scan speed of 4,000 Da/s which provided a fragment footprint of compounds of interest.

Statistical analysis

All trials were repeated at least five times. The experiments were carried out in duplicate and results were expressed as mean $\pm$ S.D. Statistical evaluation was performed with the Prism 6 software package (GraphPad, La Jolla, CA, USA), applying D'Agostino test to assess Gaussian distribution. Analysis of variance (ANOVA) was applied to determine the effect produced by different concentrations of the plant extracts and Bonferroni post hoc test was used to perform multiple comparisons. Statistical significance was established for $P < 0.05$.

Results

Proof of resistance and sensitivity to antibiotics

As shown in (Figure 1), inhibition area of MRSA-isolates was approximately 17 mm for 30 μ g cefoxitin disk (resistant $\leq$ 21 mm) (Figure 1Ai). An elliptical zone was obtained against the MRSA strain with a vancomycin-MIC of 0.75 μ g/mL (sensitive MIC $\leq$ 2 μ g/mL) (Figure 1Aii). Furthermore, isolates of CRKP had an inhibition area of 14 mm for 10 μ g meropenem disk (resistant $\leq$ 19 mm) (Figure 1Biii) and 22 mm for 10 μ g gentamicin disk (sensitive $\geq$ 15 mm) (Figure 1Biv).

Effect of plant extracts on bacterial growth curve

Description of the bacterial growth curves is shown in (Figure 2). Exponential growth phase and stationary phase were observed early at 24 h, and 72 h, for *S. aureus* and *K. pneumoniae* respectively. The bacterial growth of *S. aureus* and *K. pneumoniae* was diminished 6-fold by the addition of alcoholic extract of *L. apiculata*, compared to the profiles without any plant extract ($P < 0.0001$). *L. apiculata* did not alter the growth curve of *S. aureus* between concentrations of

1 μ g/mL to 100 μ g/mL dry material, compared with the control group ($P > 0.05$). Contrary, we observed that growth curves of *S. aureus* were reduced significantly with concentrations of 1,000 μ g/mL and 10,000 μ g/mL dry material after 6 h and 48 h of incubation respectively ($P < 0.001$), compared with the control group (Figure 2A). Treatment with an alcoholic extract of *L. apiculata* against *K. pneumoniae* revealed no significant difference among dry material concentrations of 1 μ g/mL to 100 μ g/mL compared to the untreated control ($P > 0.05$). However, significant differences were found after 6 h with 10,000 μ g/mL and after 24 h with 1,000 μ g/mL of dry material compared to the untreated control ($P < 0.001$; Figure 2B).

Determination of minimum inhibitory concentrations

Determination of MIC was not possible due to the appearance of intense turbidity during the test conditions for all plant extracts. The same growth inhibitory activity can be observed with the agar diffusion method, which is adequate to assess the possible antimicrobial activity of natural extracts [21].

Sensitivity analysis to extracts by the agar diffusion method

A clear circular area of inhibition was obtained from the agar diffusion test. The bacterial growth inhibition for *S. aureus* using 30 μ g cefoxitin disc (Figure 3F) as resistant control, was approximately 16 mm (specific breakpoint $\leq$ 21 mm for resistant and $\geq$ 22 mm for susceptible) and using vancomycin-MIC (Figure 3G) as sensitive control, it was 0.75 μ g/mL (specific breakpoint $\leq$ 2 μ g/mL sensitive). When we evaluated growth inhibitory activity of plant extract against *S. aureus*, only concentrations of 1,000 μ g/mL and 10,000 μ g/mL showed a significative inhibitory activity (Figures 3A, B, C respectively). At the concentration of 1,000 μ g/mL an inhibition zone of 9 mm was observed (Figure 3D). The maximum

inhibition zone (14 mm) was detected at the concentration of 10,000 $\mu\text{g/mL}$ (Figure 3E). The bacterial growth inhibition for *K. pneumoniae* using 10 μg meropenem disc (Figure 4F) as resistant control, was approximately 15 mm (specific breakpoint ≤ 19 mm for resistant and ≥ 22 mm for susceptible) and using 10 μg gentamicin disc (Figure 4G) as sensitive control, it was approximately 21 mm (specific breakpoint ≥ 15 mm sensitive). Discs with concentrations of 1, 10 and 100 $\mu\text{g/mL}$ were found to be resistant, showing no signs of inhibition (Figure 4A, B, C respectively). At the higher concentrations of 1,000 and 10,000 $\mu\text{g/mL}$ a small inhibition zone (7 mm) was observed (Figures 4D and E, respectively).

Bacteriostatic activity of plant extracts

Bacteriostatic activity of *L. apiculata* was only observed against MRSA. When concentrations of 10,000 $\mu\text{g/mL}$ were challenged against MRSA an inhibition of the development of bacterial colonies was observed (Figure 5F).

Bactericidal activity of plant extract

The bactericidal activity of plant extracts against *S. aureus* ATCC 43300 (Figure 6A) and *K. pneumoniae* ATCC BAA 1705 (Figure 6B) was statistically significant at the concentrations of 1,000 and 10,000 $\mu\text{g/mL}$ compared to control. The bactericidal activity detected in the alcoholic extract of *L. apiculata* against *S. aureus* ATCC 43300 was significantly more effective than for *K. pneumoniae* ATCC BAA 1705 at the concentration of 1,000 and 10,000 $\mu\text{g/mL}$.

HPLC-UV- ESI MS/MS analysis

Nine flavonoids, including (+)-epicatechin, myricetin, myricetin-3-glucoside, quercetin, Quercetin-3-glucoside, isorhamnetin, Quercetin-3-Rhamnoside (Quercitrin), Isorhamnetin-3-Rutinoside, Isorhamnetin-3-Glucoside were identified from the ethanolic extract of *L. apiculata*. All of them classified as flavonoid compounds. The profile of the principal compounds detected in the ethanolic extract of *L. apiculata* is shown in (Table 1).

Figure 1. Disc agar-diffusion method (A) Culture of MRSA; (B) Culture of CRKP. Discs: i) Resistance test of *S. aureus* to cefoxitin (FOX disc contain 30 μg); ii) Sensitivity test of *S. aureus* to vancomycin E-test MICs (VA); iii) Resistance test of *K. pneumoniae* to meropenem (MEM disc contain 10 μg); iv) Sensitivity test of *K. pneumoniae* to gentamycin (GE disc contain 10 μg)

Figure 2. Bacterial growth showing the mean $\pm$ SD on the different concentrations of plant extracts versus time curve. (A) Growth profiles of *S. aureus* in alcoholic extracts of *L. apiculata*. (B) Growth profiles of *K. pneumoniae* in alcoholic extracts of *L. apiculata*. Two-way ANOVA test was used to examine any statistically significant differences. Levels of statistical significance *P<0.05, **P<0.01, *P<0.001**

Figure 3. Agar diffusion assay using *S. aureus* ATCC 43300. Cefoxitin disk (FOX) and vancomycin E-test were used for detection of resistance and sensitivity, respectively. *L. apiculata* extracts at concentrations of 1 (A), 10 (B), 100 (C), 1,000 (D), 10,000 (E) µg/mL, (F) FOX and (G) vancomycin E-test

Figure 4. Agar diffusion assay using *K. pneumoniae* ATCC BAA 1705. Meropenem disk (MEM) and gentamicin disk (GE) were used for detection of resistance and sensitivity, respectively. *L. apiculata* extracts at concentrations of 1 (A), 10 (B), 100 (C), 1,000 (D), 10,000 (E) $\mu\text{g/mL}$, meropenem disk (F) and gentamicin disk (G)

Figure 5. Bacteriostatic activity of different concentrations of vegetable extracts against *S. aureus*: (A), 0 $\mu\text{g/mL}$; (B) 1 $\mu\text{g/mL}$; (C) 10 $\mu\text{g/mL}$; (D) 100 $\mu\text{g/mL}$; (E) 1,000 $\mu\text{g/mL}$; (F) 10,000 $\mu\text{g/mL}$ and *K. pneumoniae*: (G) 0 $\mu\text{g/mL}$; (H) 1 $\mu\text{g/mL}$; (I) 10 $\mu\text{g/mL}$; (J) 100 $\mu\text{g/mL}$; (K) 1,000 $\mu\text{g/mL}$; (L) 10,000 $\mu\text{g/mL}$

Figure 6. Bactericidal effect with different plant extract concentrations on viability of MRSA and CRKD. The experiment was repeated five times in parallel to different control antibiotics. Bars represent mean $\pm$ SD. One-way ANOVA test was used to examine any statistically significant differences. Levels of statistical significance * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. (A) Alcoholic extract of *Luma apiculata* against MRSA strain. (B) Alcoholic extract of *L. apiculata* against CRKD strain

Discussion

Antimicrobial resistance has turned into a severe health threat concern worldwide promoting scientific interventions and encourages to find new therapeutic alternatives. In March of 2015, 36 novel broad-spectrum antimicrobials that can treat serious bacterial infections are under clinical trials [22]. Plant species have recently received attention for their antimicrobial compounds usually stored as secondary metabolites [11]. In our study, an alcoholic extract from leaves of *Luma apiculata* was used. The alcoholic extraction is more effective because the concentration required to inhibit the bacteria growth is lower, and the bacterial inhibitory effect is stronger than the aqueous extract [23]. Although flavonoids are soluble in water, better recovery is achieved by using polar solvents such as ethanol, obtaining a greater amount of phenolic and flavonoid compounds which are attributed with antibacterial activity [24].

Luma apiculata showed antimicrobial activity only with the highest concentrations

used in this study. We did not test for cytotoxicity at these concentrations but there are no reports of cytotoxicity of these concentrations in somatic cells. In terms of selectivity, a number of studies have shown that non-cancer cells are less sensitive to plant extracts than tumor cell lines, which can be explained by the growth rate of tumor cells compared with non-cancer cells [25]. In addition, a toxicity study suggested that myricetin, a compound present in *L. apiculata* extract, does not exert cytotoxicity towards normal breast epithelial MCF10A cells, hence, researchers concluded that the compound is safe for further *in vivo* studies [26]. This strongly suggests that selective toxicity of *L. apiculata* is an important factor that should be investigated.

The results of this study show that plant extract inhibited bacterial growth with the highest concentrations used. The inhibitory action of bacterial growth can be due to the presence of monoterpenes in the plant [27]. These organic compounds exert a protective function against biotic attacks such as

infections caused by viral, bacterial and fungal pathogens. Possible mechanisms of

action may be due to the lipid bilayer disruption by the terpenes [12, 28].

Table 1. Identification of phenolic compounds in *Luma apiculata* leaves by an HPLC/UV-ESI MS MS system. The analyses were performed using the Turbo V(R) ion spray in positive (A) and negative (B) ionization mode

A

Compounds	Retention Time (min)	[M+H] ⁺ (m/z)	Fragments (MS2)								Class
Procyanidin B1		579	579	561	427	409	291	247			
(+)-Epicatechin	50,7	291	291	273	249	207	179	161	147		Flavonoid
Myricetin-3-glucoside	56,2	481	481	319	273	245	153				Flavonoid
Myricetin	56,8	319	319	301	273	245	217	179	165	153	Flavonoid
Quercetin	57,1	303	303	285	257	229	201	165	153	137	Flavonoid
Quercetin-3-glucoside	57,5	465	303								Flavonoid
Isorhamnetin	58,4	317	302	285	274	257	245	229	153		Flavonoid
Quercetin-3-Rhamnoside (Quercitrin)	59,8	449	303	287							Flavonoid

B

Compounds	Retention Time (min)	[M-H] ⁻ (m/z)	Fragments (MS2)											Class
Gallocatechin	48,9	305	305	219	167	125								Flavonoid
(+)-Epicatechin	51,1	289	289	271	245	221	203	188	175	151	137	125		Flavonoid
Myricetin-3-Galactoside	56,2	479	479	316	287	271								Flavonoid
Quercetin-3-glucoside	57,6	463	463	300										Flavonoid
Quercetin-3-Rhamnoside (Quercitrin)	58,8	447	447	300	284									Flavonoid
Isorhamnetin-3-Rutinoside	59,1	623	623	315										Flavonoid
Isorhamnetin-3-Glucoside	59,5	477	477	315										Flavonoid

The bactericidal activity detected in the alcoholic extract of *L. apiculata* against *S. aureus* was significantly more effective than for *K. pneumoniae*. This is probably due to the composition of the outer membrane of both bacteria. According to Pitout and Laupland [29] Gram-negative bacteria are more resistant to the extracts than Gram-positive bacteria, because they have hydrophilic polysaccharide chains, which act as a barrier against extracts or essential oils.

Several studies suggest that the mechanism of action of some antimicrobial compounds, such as phenolic compounds, involves damage to both, wall and bacterial cell membrane, which facilitates the leaking of intracellular constituents like proteins, inorganic ions and several enzymes from the cells, triggering a permanent cell damage [30].

The phenolic compounds such as Epicatechin, Myricetin, Quercetin,

Isorhamnetin and Gallocatechin detected in *L. apiculata* are widely accepted as medicinally important compounds [31]. Flavonoids interfere with specific intracellular or surface enzymes and many bacterial virulence factors such as toxins, enzymes and signal receptors [32], which could explain the bacteriostatic activity of the extract against MRSA. Previous studies have reported that many medicinal plants containing flavonoids are widely used in traditional medicine for the treatment of human diseases. Flavonoids have been reported to exhibit a variety of important biological activities, including antioxidant properties, inhibition of histamine release and activities of several enzymes [33–35]. More importantly, it has also been reported antimicrobial activity; for example, flavonoids have been found to be effective against Gram-positive bacteria [36]. Another study revealed bacteriostatic activity in several flavonoids tested separately, confirming that quercetin was the most effective inhibitor [37]. In addition, when flavonoids are used in combination with antibiotics show synergistic effects against strain bacterial [30].

This study may not be adequate to suggest a potential new antibiotic agent, considering that antibacterial activity was observed at higher concentrations than those reported in other plant extracts. However, this approach should be considered as a preliminary step to find out new promising molecules with antibacterial activity and to unravel their possible mechanism of microorganism inhibition. Further studies are still needed to assess the influence of different portions of the plant extracts including flowers, leaves or stems, on the bacterial growth rate and as well the evaluation of possible synergistic effects with antibiotics or other medicinal plant extracts.

Conclusion

Luma apiculata has antibacterial activity against standard multidrug-resistance strains of *S. aureus* and *K. pneumoniae*. The bactericidal activity detected in the alcoholic extract of *L. apiculata* against *S. aureus* was more effective than for *K. pneumoniae*. Flavonoids were the principal compounds detected in the ethanolic extract of *L. apiculata*.

Authors' contributions

Conceived and designed the experiments: FF Treulen, GE Salcedo & EA Paz, Performed the experiments: GE Salcedo, FI Garrido & CE Morales, Analyzed the data: FF Treulen, FI Garrido & CE Morales, Contributed reagents/ materials/ analysis tools: RN Felmer, Wrote the paper: FF Treulen, EA Paz & RN Felmer.

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