



Revista Brasileira de Farmacognosia

BRAZILIAN JOURNAL OF PHARMACOGNOSY

www.journals.elsevier.com/revista-brasileira-de-farmacognosia



Original article

Study of the antimicrobial activity of metal complexes and their ligands through bioassays applied to plant extracts

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ARTICLE INFO

Article history:

Received 16 October 2013

Accepted 4 April 2014

Keywords:

Bacterial resistance

Antibiotics

Metal complex

Antibacterial activity

Disk diffusion

ABSTRACT

The appearance of resistant bacteria was found to reduce the efficiency of antimicrobial therapies with the current antibiotics, thereby increasing the need for more efficient drugs for the treatment of infections. Several studies have demonstrated an increase in antimicrobial activity following the interaction of several compounds with metal ions. The present study used a methodology adapted for antimicrobial bioassays using plant extracts, in compliance with the standards of the Clinical and Laboratory Standards Institute against Gram-positive and Gram-negative bacteria. The results obtained were considered appropriate for determining MIC, MBC as for performing antimicrobial sensitivity testing with good efficiency and reproducibility. The bacteria *Pseudomonas fluorescens* exhibited high sensitivity to the tested compounds, being efficient to evaluate the antibacterial activity. The bioassays with the metal complexes of flavonoid quercetin and Ga(III) ions, and synthetic ligand H2bbppd and Cu(II) ions showed a greater inhibitory effect than their individual ligands, thus, the addition indicated an increase in the antimicrobial activity after the coordination. Both metal complexes exhibit good antimicrobial performances, such as low minimum inhibitory concentration (MIC \leq 250 μ g/ml), bactericidal effect and a broad activity spectrum, which qualify these compounds as suitable candidates to the next step of drugs fabrication. Nevertheless, further studies on the mechanism of growth inhibition and toxicity are needed, in order to evaluate the potential of therapeutic application.

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Introduction

Annually, millions of people die due to infections caused by microorganisms resistant to current antibiotics (WHO, 2012). When an antibiotic is discovered and commercially available,

the appearance of resistant strains begins to reduce its clinical utility after a period of indiscriminate use, leading to future use restriction (Rocha et al., 2011). The use of antibiotics with broad spectrum of action and low toxicity can reduce the efficacy of future antimicrobial therapies, leading to the use of drugs

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with larger selective toxicity (Tortora et al., 2003; Willey et al., 2008). In addition, the use of restricted antibiotics can cause the failure of antimicrobial therapy, thereby increasing the rate of morbidity and mortality, along with the treatment costs (Garcia, 2011).

Microbiologists acknowledge that Gram-negative bacteria own mechanisms specialized in the extrusion of strange substances out of the cell (efflux bomb), limiting the access of antimicrobial agents to its active site. Consequently, it prevents the accumulation of antibiotics in the interior of the cell, and inhibits the action of antimicrobial agents (Reichling et al., 2005). Furthermore, the enzymes are known to be capable of rendering drugs inactive (β -lactamases), making the cell resistant to them in their periplasmic space (Silva, 2011).

In an analogous way, the Gram-positive bacteria protect their cytoplasmic membrane with a thick cell wall. The myriad layers of peptidoglycans hinder the passage of hydrophobic compounds owing to the presence of sugars and amino acids (Sartori, 2005). By virtue of that, there has been a rise in research studies aiming at producing compounds with a broad spectrum of activity, and with action mechanisms unknown to the pathogenic bacteria. In spite of its low virulence in the human, *Pseudomonas fluorescens* (a Gram-negative bacterium) is considered as a health problem, due to its resistance against antiseptics and antibiotics used in medical centers (Gershman et al., 2008). Given the scarcity of reports regarding its use in the study of antimicrobial activity, the strain was selected purposefully for its evaluation among other bacteria of clinical interest.

In the search for new antimicrobials, effective in the treatment of infections caused by multiresistant bacteria, due consideration ought to be given for the synthesis of drugs with new activation targets, as well as the potentialization of the activity of compounds with known antimicrobial activity (Schaechter et al., 2002; Masunari and Tavares, 2006). A new strategy in the production of drugs proposes the interaction of metal ions for antibiotics within three study fields: the first one aiming at creating a reversed mechanism of microbial resistance; the second one seeking to promote the development of new drugs with an action mechanism unknown to the pathogenic bacteria; and a third one aiming at reducing the toxicity of the metal ion in the form of a complex (Rocha et al., 2011).

The interaction of metal ions with organic ligands shows better antimicrobial activity compared to free ligands (not coordinated), and as such, it justifies the investigation of new drugs with unknown mechanism of action against pathogenic bacteria. The use of these new compounds is likely to have great potential against pathogenic bacteria, nonetheless, the need for new methodologies of evaluation of antimicrobial activity cannot be relegated to the background. Admittedly, owing to the innovative character of this approach, we have not found enough information in the literature regarding the use of specific bioassays involving metal complexes. Although, there is a great variety of laboratory methods that can be used to measure the susceptibility of bacteria to antimicrobial agents *in vitro*, these are applied, for example, to plant extracts in different fractions of solvent (Mamidala and Gujjeti, 2013; Soniya et al., 2013).

The above mentioned methods are effective for the determination of parameters such as the minimal inhibitory concentration (MIC), the minimal bactericidal concentration (MBC), as well as performing the susceptibility test to antimicrobials. These methodologies are known to have, undoubtedly, good efficiency and reproductivity, but in a mixture of compounds it turns too hard to establish which is the active substance acting as the antimicrobial agent (Gonçalves et al., 2011; Sá et al., 2011; Prasannabalaji et al., 2012). Therefore, the purpose of this research was to evaluate the antimicrobial activity of some metal complexes, and to compare their performance against free ligands. The response to standardized Gram-positive and Gram-negative bacteria was taken as a reference; including the applicability of the use of *Pseudomonas fluorescens* and the methodologies for bioassays, as a proper methodology for the evaluation of antimicrobial activity of plant extracts.

Materials and methods

Materials

The bacterial strains *Staphylococcus aureus* (ATCC SP 25923); *Enterococcus faecalis* (ATCC SP 19433); *Escherichia coli* (ATCC SP 11229), and *Pseudomonas fluorescens* (ATCC SP 13525), purchased for this work, were reconstituted in sterile distilled water, cultured in Muller-Hinton medium, and incubated at 37°C for 24 h. The standardized antimicrobial CEFAR discs, containing Aztreonam (AZT) μg ; Ceftazidime (CAZ) 30 μg ; Chloramphenicol (CLO) 10 μg ; Imipenem (IPM) 10 μg ; Tetracycline (TET) 30 μg ; Vancomycin (VAN) 10 μg .

Synthesis of the chemical compounds

Metal Complex 1 - Ga(III)-Quercetin

Ga(III) ions were complexed with quercetin ligand using the methodology proposed by Simões et al. (2013), which involves the reaction between the flavonoid quercetin with gallium(III) nitrate salt, in a 3:1 stoichiometric proportion. The results obtained through CNH and infrared spectroscopy indicate the formation of a mononuclear metal complex with adequate purity degree for the bioassays experiments. The elemental analysis of CNH for $\text{GaC}_{45}\text{H}_{39}\text{O}_{27}$: MM 1,081.51 g mol^{-1} ; calculated: C 49.98 % e H 3.63 %; found: C 49.41 % e H 3.51 %.

Synthetic ligand - H2bbppd

The synthetic ligand N,N',N,N'-bis[2-hydroxy-3,5-di-tert-butylbenzyl)(2-pyridylmethyl)]-1-3-diaminopropane, identified in this work as H2bbppd was synthesized in line with the methodology proposed by Cabeza et al. (2010). The CNH and infrared spectroscopy results indicate the formation of a mononuclear metal complex with a purity degree adequate for its use in bioassays and for the synthesis of the Cu(II) complex. The elemental analysis of CNH for $\text{C}_{45}\text{H}_{64}\text{N}_4\text{O}_2$: MM 693 g mol^{-1} ; C 77.99; H 9.31; N 8.08 %. Found: C 77.51; H 9.42; N 8.20 %. IR (KBr), in cm^{-1} : 3400-3300 ($\nu_{\text{O-H}}$); 2956 ($\nu_{\text{C-H}}$, tert-butyl); 1596, 1477 ($\nu_{\text{C=N, C=C}}$, aromatics); 1394 ($\delta_{\text{O-H}}$, phenol); 1362 ($\delta_{\text{C-H}}$, tert-butyl); 1237 ($\nu_{\text{C-O}}$, phenol); 879 ($\delta_{\text{C-H}}$, aromatics); 756 ($\delta_{\text{C-H}}$ pyridine).

Metal complex 2 - Cu(II)-H2bhppd

The metal complex was synthesized according to the methodology suggested by Favarin et al. (2010), using perchlorate as a counter-ion. The CNH and infrared spectroscopy results indicate the formation of a mononuclear metallic complex with a purity degree adequate for its use in bioassays. The elemental analysis of CHN for $\text{CuC}_{45}\text{H}_{63}\text{N}_4\text{O}_2 \cdot \text{ClO}_4 \cdot \text{CH}_3\text{OH}$: MM 887.03 g/mol⁻¹; C 62.29; H 7.61; N 6.32 %. Found: C 62.63; H 7.52; N 6.46%. IR (KBr), in cm⁻¹: 3400-3300 ($\nu_{\text{O-H}}$); 2956 ($\nu_{\text{C-H}}$, tert-butyl); 1610-1445 ($\nu_{\text{C=N,C=C}}$, aromatics); 1390 ($\delta_{\text{O-H}}$, phenol); 1362 ($\delta_{\text{C-H}}$, tert-butyl); 1234 ($\nu_{\text{C-O}}$, phenol); 1096 ($\nu_{\text{Cl-O}}$, ClO_4); 880 ($\delta_{\text{C-H}}$, aromatics); 763 ($\delta_{\text{C-H}}$, pyridine).

Biological assays

The methodologies applied in the bioassays were elaborated based on the standards of the manuals of Clinical and Laboratory Standards Institute (CLSI) used by the National Agency for Sanitary Surveillance (Anvisa) for Antimicrobial Agents Sensitivity Tests by Dilution and Disk-diffusion (M7-A7 and M2-A8, respectively) adapted in this study to use of organic solvents in the solubilization of metal complexes and their ligands.

Minimal inhibitory concentration

The minimal inhibitory concentration (MIC) was determined by broth macrodilution method. Initially, compound dilutions were performed (organic ligands and metal complexes) in the solvent with the best solubility, and nine concentrations were adjusted by serial dilution 2:1 (1000, 500, 250, 125, 62.5) and intermediary values obtained (600, 400, 300, 200).

The following inocula were produced using bacterial colonies with incubation times, not superior to 24 h, adjusted to the McFarland standard solution of 0.5 of bacterial turbidity, in a concentration of 1.5×10^8 CFU/ml (colonies forming unities for milliliter) and, following, the concentration of 5×10^5 CFU/ml with Luria-Bertani broth (Sambrook and Russell, 2001).

For the determination of the MIC, an aliquot of 1 ml of each test solution was poured in each test tube (in the specified concentrations) as well as 1 ml of the bacterial suspension, except in the negative control tube. The tubes were incubated at $35 \pm 2^\circ\text{C}$ for 20 h, and afterwards the turbidity (bacterial growth) was evaluated. The MIC was determined as the lowest concentration of the compound capable of inhibiting microbial growth.

Negative Control tubes were separated (lower concentration of the compound and LB broth without the inoculum); as well as Positive Control tubes (LB broth and adjusted bacterial growth). The bioassays were performed in duplicate with three repetitions for each bacterial strain; upon the detection of an error or contamination the result was discarded and the test redone.

Minimal bactericidal concentration

The minimal bactericidal concentration (MBC) was determined by the Petri dish sowing method. The procedures to determine the MBC were initiated concomitant with the procedures for the determination of the MIC. After the incubation period for the determination of the MIC, an

aliquot of 0.1 ml was retrieved from each test tube, including the positive and negative (without the inoculum) control. The inocula were distributed on the surface of the culture medium in Petri plates, with the support of a Drigalski spatula. The plates were incubated at a temperature of $35 \pm 2^\circ\text{C}$ for 20 h. After this period the presence of bacterial colonies was observed in each plate.

The MBC was defined as the lowest concentration of the compound capable of preventing the microbial growth in culture medium (formation of bacterial colonies). The bioassays were performed in duplicate with three repetitions for each bacterial strains; once an error or contamination was detected the result was discarded and the test was redone.

Antimicrobial sensibility testing by disk diffusion

The antimicrobial sensibility testing (antibiogram) was performed by the disk diffusion method (Kirby-Bauer method), using sterile filter paper discs saturated with solutions adjusted to the MIC obtained for each compound. Initially, 20 ml of culture medium was distributed on each Petri dish (90 mm). After the solidification of the medium, the plates were kept with their covers left ajar for 2 min in the incubator, and afterwards lidded and turned over and refrigerated until use. Before use, the plates were taken out from the refrigerator and put inside the incubator at 25°C for approximately 30 min. After that bacterial inocula were produced with an incubation time not over 24 h, and adjusted to the standard solution of the 0.5 McFarland scale. A sterile swab was introduced into the test tube, pressing it against the tube wall to remove the excess, and distributing the inoculum on the surface of the culture medium. Soon thereafter, the plates were put to dry in an incubator at 35°C for 10 min. Five antimicrobial plates impregnated with the compounds were, in turn, put in each Petri plate. A disk was set in the center of the plate and the other four around it, making sure that the distance from the center to another disc was no less than 20 mm, and that the disc was not close to the border.

The plates were then placed in an incubator at a temperature of $35 \pm 2^\circ\text{C}$ for 18 h. After this period, the inhibition halos produced around the disc (including the diameter of the disc) were measured, using a digital caliper rule. Inhibition zones superior to 7 mm in diameter were considered as positive results.

For the negative control, a Petri dish containing only the Miller-Hinton culture medium was included in each incubation. For the control of the bacterial inoculum, at each bioassay, two Petri dishes containing standard antimicrobial discs were incubated.

The bioassays were performed in triplicate with three repetitions, and when contamination was detected, the results were discarded and the test was redone. The measurements of the inhibition halos were evaluated statistically evaluated using an ANOVA followed by Tukey Test ($p < 0.05$) in each bioassay; and the averages of the halo measurements were compared among themselves using the Kruskal-Wallis test ($p < 0.05$), which is a non-parametric test appropriate to compare 3 or more independent samples (Ayres et al., 2007), using BioEstat 5.0 software.

Results and discussions

Table 1 illustrates the determination of the Minimal Inhibitory Concentration and Minimal Bactericidal Concentration for the compounds tested obtained from the bioassays. The ones that show susceptibility of the tested strains to the flavonoid quercetin presented different MIC values when ethanol (400 µg/ml) and methanol (500 µg/ml) were used as solvents. This outcome is probably due to the solubility of quercetin in different solvents, as previously described by Razmara et al. (2010), who determined the order of solubility as: ethanol > methanol > water. The effect of quercetin on bacterial growth was similar when the same solvent was used in dilutions, which can indicate the same action mechanism against Gram-positive and Gram-negative bacteria. A wide array of studies have reported that the antimicrobial activity of flavonoids is likely to be related to their ability to form complexes with soluble extracellular proteins and with the cell wall, for the lipophilic character of these compounds, which may cause the rupture of the cell membrane of microorganisms (Sartori, 2005). Besides, in bacteria, the permeability of the cell membrane is associated with the loss of ions as well as the reduction of its potential (Trombeta et al., 2005), causing damage that may lead to the extravasation of macromolecules, resulting in a collapse of the cellular functions and, consequently, the bacterial death (Tortora et al., 2003).

Many studies have reported that the antimicrobial activity of flavonoids is likely to be related to their ability to form complexes with soluble extracellular proteins and with the cell wall, or yet for the lipophilicity character of these compounds, which may be able to cause the rupture of the cell membrane of the microorganisms (Sartori, 2005). Besides, in the bacteria, the permeability of the cell membrane is associated with the loss of the ions and the reduction of its potential (Trombeta et al., 2005), causing damages that may lead to the extravasation of macromolecules, resulting in a collapse of the cellular functions and consequently the bacterial death (Tortora et

al., 2003). The MIC values for the metal complex 1 indicate an increase in the antimicrobial activity of quercetin following the interaction with Ga (III) ions, which may as well reinforce the idea that the compound shares the same mechanism as its ligand. The synthetic ligand H2bbppd presented a relatively good antimicrobial activity against the tested strains, being able to inhibit the microbial growth in low concentrations, showing MIC and MBC equal to 250 and 500 µg/ml, respectively.

The results obtained with metal complex 2 suggest an increase in the antimicrobial activity after the interaction of the synthetic ligand H2bbppd with Cu (II) ions. Studies show that metal complexes with copper ions penetrate more easily through the bacterial cell wall, due to the proteic denaturation of the sulphhydryl group (Goulart, 2011), destroying the bacterial cell wall.

The analysis of the results suggests two hypotheses for the action mechanism of the evaluated compounds: 1) Modification of the cell membrane which caused the loss of cell constituents; 2) Inhibition of the synthesis of the cell wall constituents. To verify any of these possible mechanisms, further specific studies are essential (identification of alterations in the cell wall and cell membrane constituents; and modifications in the bacterial DNA).

The criteria for categorizing the antimicrobial activity of phenolic compounds, synthetic ligands and metal complexes used in this work have not been previously found in the literature. However, several studies have used a classification based on the MIC results to evaluate the antimicrobial activity of plant extracts and their fractions, as: good, MIC inferior to 100 µg/ml; moderate: MIC between 100 and 500 µg/ml; weak: MIC between 500 and 1000 µg/ml; and inactive when the MIC is superior to 1000 µg/ml (Holetz et al., 2002; Dalmarco et al., 2010). This way, it was possible to evaluate the antimicrobial activity of the metal complexes 1 and 2 as good and their ligands' as moderate.

The inhibitory effect of the metal complexes 1 and 2 was found to be bactericidal, as suggested by Berche et al. (1988 *apud* Konaté et al., 2012; Abou et al., 2013), who proposed

Table 1

Results of the Minimal Inhibitory Concentration (MIC) and of the Minimal Bactericidal Concentration (MBC) for the compounds evaluated, expressed in µg/ml.

Compound	Solvent	Gram-negative bacteria				Gram-positive bacteria			
		<i>S. aureus</i>		<i>E. faecalis</i>		<i>E. coli</i>		<i>P. fluorescens</i>	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
EtOH	ethanol	R	R	R	R	R	R	R	R
MeOH	methanol	R	R	R	R	R	R	R	R
QEtOH	ethanol	400	1000	400	1000	400	1000	400	1000
QMeOH	methanol	500	>1000	500	>1000	500	>1000	500	>1000
MC1	methanol	250	500	250	500	250	500	250	500
H2bbppd	ethanol	250	500	250	500	250	500	250	500
MC2	ethanol	125	250	125	250	125	250	125	250

EtOH, pure ethanol solvent; MeOH, pure methanol solvent; QEtOH, quercetin ethanolic solution; QMeOH, quercetin methanolic solution; MC1, metal complex 1; H2bbppd, synthetic ligand H2bbppd; MC2, metal complex 2. R, No bacterial growth inhibition was observed.

that when the MBC/MIC ratio is less than or equal to 4.0, the agent will be considered bactericidal, and when this ratio is over 4.0 it should be considered bacteriostatic. The evaluated compounds inhibited the growth of all Gram-positive (e.g. *S. aureus* and *E. faecalis*) and Gram-negative bacteria (e.g. *E. coli* e *P. fluorescens*), indicating a broad spectrum of activity while opening perspectives for their use as future drugs.

In the antimicrobial sensibility test by disk diffusion (AST), the bacteria *E. faecalis* (Gram-positive) and *P. fluorescens* (Gram-negative) displayed higher susceptibility to quercetin when diluted in ethanol (250 µg/ml), showing average values of inhibition halo of 14.20 and 13.14 mm, respectively (Table 2). This result confirms the hypothesis that the solubility of the compound interferes with the antimicrobial activity, possibly by the reduction of the quercetin diffusion over the bacterial inoculum.

Table 3 and 4 show the results for the AST with the flavonoid quercetin, the synthetic ligand H2bbppd and the metal complexes 1 and 2. The metal complex 1 showed a better inhibitory effect than its ligand, with larger inhibition halos using half of the concentration utilized in the bioassay with quercetin (MIC of de 250 µg/ml).

The lowest susceptibility to the compound was observed in *Escherichia coli*, with relatively lower values, which does not discard the use of this compound in the treatment of infections caused by this strain. The results of the AST with the metal complex 2 showed better antimicrobial activity compared to the free ligand, with inhibition values relatively larger using/consuming half of the concentration used for the bioassay with H2bbppd (MIC of 125 µg/ml).

The higher susceptibility was observed for the *Enterococcus faecalis* bacterium, with an inhibition halo of 18.68 mm, which indicates a pharmacological potential in the growth control of this bacterium, cited as the first of the three main

Table 2
Results of the Minimal Inhibitory Concentration (MIC) and of the Minimal Bactericidal Concentration (MBC) for the compounds evaluated, expressed in µg/ml.

	Solvent	
	Ethanol	Methanol
Gram-positive bacteria		
<i>Staphylococcus aureus</i> (ATCC 25923)	11.43 ± 1.17 ^{Aa}	9.84 ± 1.24 ^{Bb}
<i>Enterococcus faecalis</i> (ATCC 19433)	14.20 ± 1.67 ^{Ba}	10.37 ± 0.57 ^{Ab}
Average	12.81	10,10
Gram-negative bacteria		
<i>Escherichia coli</i> (ATCC 11229)	11.80 ± 2.80 ^{Aa}	10.50 ± 1.70 ^{Ab}
<i>Pseudomonas fluorescens</i> (ATCC 13525)	13.14 ± 1.42 ^{Aa}	10.28 ± 0.66 ^{Ab}
Average	12.47	10.39

Averages followed by the same capital letter in the columns and lower case in the lines do not differ significantly among themselves, by the Kruskal-Wallis test with 95%.

Table 3

Diameter of the inhibition halo, average and standard deviation (mm), by the action of the metal complex 1 in methanol.

	Compound	
	QMeOH	MC1
Gram-positive bacteria		
<i>Staphylococcus aureus</i> (ATCC 25923)	9.84 ± 1.24 ^{Aa}	13.26 ± 1.16 ^{Ab}
<i>Enterococcus faecalis</i> (ATCC 19433)	10.37 ± 0.57 ^{Aa}	12.58 ± 1.53 ^{Ab}
Average	10.10	12.94
Gram-negative bacteria		
<i>Escherichia coli</i> (ATCC 11229)	10.50 ± 1.70 ^{Ba}	10.90 ± 1.82 ^{Ba}
<i>Pseudomonas fluorescens</i> (ATCC 13525)	10.28 ± 0.66 ^{Aa}	13.36 ± 1.38 ^{Ab}
Average	10.39	12.13

Averages followed by the same capital letter in the columns and lower case in the lines do not differ significantly among themselves, by the Kruskal-Wallis test with 95% of reliance. QMeOH, quercetin diluted in methanol; MC1, metal complex 1 diluted in methanol.

Table 4

Diameter of the inhibition halo, average and standard deviation (mm), by the action of the ligand H2bbppd and the metal complex 2 in methanol.

	Compound	
	H2bbpppd	MC2
Gram-positive bacteria		
<i>Staphylococcus aureus</i> (ATCC 25923)	10.63 ± 1.39 ^{Aa}	10.95 ± 0.74 ^{Aa}
<i>Enterococcus faecalis</i> (ATCC 19433)	11.15 ± 1.64 ^{Aa}	18.68 ± 2.68 ^{Bb}
Average	10.89	14,81
Gram-negative bacteria		
<i>Escherichia coli</i> (ATCC 11229)	12.56 ± 2.40 ^{Aa}	11.96 ± 2.54 ^{Ab}
<i>Pseudomonas fluorescens</i> (ATCC 13525)	12.74 ± 2.50 ^{Aa}	13.76 ± 0.95 ^{Cb}
Average	12.65	12.86

Averages followed by the same capital letter in the columns and lower case in the lines do not differ significantly among themselves, by the Kruskal-Wallis test with 95% of reliance. MC2: metal complex 2 diluted in methanol.

causes of hospital infections (Willey, 2008). Thus, it can be considered that metal complexes 1 and 2 show antimicrobial characteristics, such as low minimal inhibitory concentration (MIC ≤ 250 µg/ml), bactericidal effect and broad spectrum of activity. However, to confirm pharmacological potential of these compounds, it is essential to perform studies in order to evaluate their toxicity, since these studies assist in the screening of a large variety of compounds with biologic activity, to define their potential for therapeutic application (Carballo et al., 2002).

Pseudomonas fluorescens was found to be efficient to evaluate the antimicrobial activity of the metal complexes as well as their ligands, showing MIC and MBC values equal to those obtained by the other tested strains. The AST showed that the *P. fluorescens* bacterium is more sensitive to both the metal complex 1 (13.36 mm) and the synthetic ligand H2bbppd (12.74 mm), and when exposed to the metal complex 2 (13.76 mm), it was found to be the second most susceptible bacterium.

Conclusion

The studies performed showed the applicability of the methodologies used in antimicrobial assays with plant extracts for the complexes and their ligands. The results showed that the methodologies are appropriate for determining the MIC, the MBC, and to carry out the antimicrobial susceptibility test by disk diffusion with good efficiency and reproductivity. The data obtained in the bioassays are quantitative and/or qualitative in nature, and are related to the susceptibility of the bacterial strains to the tested compounds. The *Pseudomonas fluorescens* strain showed high susceptibility to the tested compounds, good growth and easily visualized colonies, which makes it useful for evaluation studies involving antibacterial activity of metal complexes as well as other compounds. Furthermore, the analysis of the results also showed a good antimicrobial activity of the metal complexes 1 and 2 relatively superior to their ligands namely quercetin and H2bbppd. Furthermore, the MIC values obtained for these metal complexes (I added) indicated their potential for pharmacological use. However, further studies regarding the growth inhibition mechanism and toxicity are found to be of paramount relevance in order to evaluate the potential of these compounds in therapeutic application.

Authors' contributions

AFS contributed in running the laboratory work (biological studies), analysis of the data and drafted the paper. DFB (MSc student), LRVF (PhD student), NAC (MSc student) and GRA contributed in running the laboratory work (synthesis of the chemical compounds). MB contributed to biological studies and analysis of the data. AAC, AN and DCMR (PhD student) contributed to analysis of the data and to critical reading of the manuscript. AA designed the study, supervised the laboratory work, contributed to biological studies, analysis of the data and contributed to critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgment

The authors would like to express their sincerest gratitude to the Post-graduation Program PGRN-UEMS and to the following

Brazilian agencies for offering the needed financial support in making this study a reality: CAPES and FUNDECT-MS.

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