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Antibacterial activity of the ethanolic extract of Brazilian red propolis against multidrug-resistant extended-spectrum β-lactamase and carbapenemase-producing bacteria

Atividade antibacteriana do extrato etanólico da própolis vermelha brasileira contra bactérias produtoras de β-lactamase e carbapenemase de espectro estendido multidroga-resistentes

A. de L. Xavier[1]; N. C. de O. Silva[1]; R. R. Soares[2]; S. Abreu[3]; E. A. de Araújo[1]; J. B. Oliveira Júnior[4*]; A. B. S. Siqueira[1]

[1]Universidade Federal de Pernambuco, Departamento de Ciências Farmacêuticas, Laboratório de Microbiologia Clínica, 50740-520, Recife-Pernambuco, Brasil
[2]Hospital das Clínicas, Universidade Federal de Pernambuco, Laboratório de Bacteriologia Clínica, 50670-901 Recife-Pernambuco, Brasil
[3]Néctar Farmacêutica Ltda., 30130-151, Belo Horizonte, Minas Gerais, Brasil
[4]Universidade Federal de Pernambuco, Pós-graduação em Medicina Tropical, 50670-901, Recife-Pernambuco, Brasil

**junniorbiologia@gmail.com*
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Propolis is a resinous product derived from honeybees and has been widely used by folk medicine throughout the years for several purposes. A wide biological potential has been attributed to the use of Brazilian red propolis, especially its antimicrobial activity which represents the mean of protection of honeybees against microbial pathogens. This study aimed to assess the *in vitro* antibacterial activity of the ethanolic extract of Brazilian red propolis produced by *Apis mellifera* against multidrug-resistant bacteria, acquired from hospital infections. Five *Escherichia coli* isolates and seven *Klebsiella pneumoniae* ones were used in this study, all of them presented either ESBL and/or KPC phenotypes. *In vitro* antimicrobial assay was performed by microdilution method. The minimum inhibitory and bactericidal concentrations (MIC and MBC) of the ethanolic extract of red propolis were determined for each bacterial strain and exhibited bacteriostatic and bactericidal activities against multi-drug resistant strains of *E. coli* and *K. pneumoniae*, presenting MIC of 2.05 and 0.13 mg/mL and MBC of 15.63 and 3.91 mg/mL, respectively. These results confirmed the antibacterial activity of the Brazilian red propolis against multidrug-resistant strains, highlighting its use as a potential therapeutic target for adjuvant treatment of multidrug-resistant bacterial infections.
Keywords: antibacterial, propolis, folk medicine.

A própolis é um produto resinoso derivado das abelhas e tem sido amplamente utilizada pela medicina popular ao longo dos anos para diversas finalidades. Um amplo potencial biológico tem sido atribuído ao uso da própolis vermelha brasileira, principalmente sua atividade antimicrobiana, que representa o meio de proteção das abelhas contra patógenos microbianos. Este estudo teve como objetivo avaliar a atividade antibacteriana *in vitro* do extrato etanólico da própolis vermelha brasileira produzida por *Apis mellifera* contra bactérias multidroga-resistentes adquiridas em infecções hospitalares. Cinco isolados de *Escherichia coli* e sete de *Klebsiella pneumoniae* foram utilizados neste estudo, os quais apresentaram os fenótipos ESBL e/ou KPC. O ensaio antimicrobiano *in vitro* foi realizado pelo método de microdiluição. As concentrações mínimas inibitórias e bactericidas (CIM e CBM) do extrato etanólico da própolis vermelha foram determinadas para cada cepa bacteriana e exibiu atividade bacteriostática e bactericida contra cepas multidroga-resistentes de *E. coli* e *K. pneumoniae*, apresentando a CIM de 2,05 e 0,13 mg/mL e CBM de 15,63 e 3,91 mg/mL, respectivamente. Estes resultados confirmaram a atividade antibacteriana da própolis vermelha brasileira contra cepas multidroga-resistentes, destacando seu uso como um potencial alvo terapêutico para o tratamento adjuvante de infecções bacterianas multidroga-resistentes.
Palavras-chaves: antibacteriano, própolis, medicina popular.

1. INTRODUÇÃO

The occurrence of resistance to antibacterial drugs in bacteria isolated from hospitalized patients has considerably increased, as well as from other health care units, which worsens even more the public health scenario [1, 2]. In this context, enterobacteria have been noticed as the responsible agents for more than 50% of cases of infection related to health care. Usually being associated to urinary tract infections, pneumonia, and sepsis [3-5] besides the fact of the therapeutic challenges arisen due to an increase in the occurrences of hospital multi-drug resistant bacterial strains [6, 7].

Some strains of enterobacteria can synthesize enzymes capable of hydrolyzing different classes of antibiotics, and according to the work of Bush, Jacoby, and Medeiros [8] bacteria that can hydrolyze high spectrum beta-lactams as ceftazidime, cefotaxime, or aztreonam, are classified in the 2b/be group, which corresponds to the extended spectrum beta-lactamases bacteria (ESBL).

The main producers of ESBLs are *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter* spp., and *Proteus* spp. [9-11]. Which have already been disseminated worldwide in countries such as Turkey [12], Spain, Portugal, Italy, United Kingdon, Poland, Bulgaria [13], Argentina, Chile, Brazil [14], the United States and Canada [15], among others. This scenario highlights the emergency in containing the spread of these pathogens into the community and hospital settings.

Besides the ESBL phenotype, another important resistance profile is represented by the carbapenemase-producing Enterobacterales whose susceptibility to antibacterial drugs is extremely limited, due to their ability to hydrolyze most beta-lactams drugs, such as carbapenems, cephalosporins, penicillin and aztreonam, not to mention their capacity of commonly being resistant to aminoglycosides, fluoroquinolones, ciprofloxacin [16]. The main representative of carbapenemase enzyme and most prevalent is the *Klebsiella pneumoniae*-carbapenemase (KPC) enzyme, first described in a *K. pneumoniae* isolate [17] but can also be found in different bacterial species [18].

It is even more worrying the co-occurrence of both resistance phenotypes in the same bacterial strain, a condition relatively common in the hospital setting and that demands a greater interest when it comes to treatment options and patient care [19, 20].

In Brazil, it has been raised concern about the incidence of resistant enterobacteria, of which *E. coli* and *K. pneumoniae* consist as the most prevalent bacteria species associated to hospital infections [20]. Due to their ESBL and KPC phenotypes, the therapeutic approach applied to these infections is very limited thus leading to patient's death [21-25].

According to the actual perspective of bacterial resistance, it is needed the development of new therapeutic alternatives that may fulfill the lack of pharmaceutical treatment options for these resistant infections. In this regard, great effort has been attributed to find novel therapeutic options in natural herbal products and their chemical constituents [26-28].

Propolis is a complex resinous mixture produced by *Apis mellifera* bees and that has been extensively implemented in popular medicine throughout the years. The botanical source used by bees to create the resin such as the period of collection altogether determine its chemical constituents and directly influence its biological properties, thus classifying this resin into various types such as green, brown, yellow, and red propolis [29-32]. Great biological potential has been verified with the use of Brazilian propolis. Such as antibacterial activity [33], antifungal [34], antiviral [35], antiparasitic [36, 37], anti-inflammatory [38], immunomodulatory [39, 40], antitumoral [41], antioxidant [42, 43], cytotoxic [44], among others.

With reference to the Brazilian red propolis one of its most exploited biological properties is its antibacterial potential due to its high content of isoflavones, which consist of molecules belonging to the class of flavonoids, whose antimicrobial activity is attributed [45-48]. Such feature highlights this type of propolis to the development of new therapeutic strategies against bacterial infections if compared to other variants of propolis [49].

The antibacterial activity of Brazilian red propolis has been tested against bacterial agents of clinical relevance such as *Staphylococcus aureus* [48, 50], *Streptococcus mutans* [51], *Enterococcus faecalis* [52], *E. coli* and *Pseudomonas aeruginosa* [53]. Already suggesting its promising use as a therapeutic target to treat infections concerning both, Gram-positive and Gram-

negative bacteria. However, literature lacks studies that have approached the antibacterial activity of Brazilian red propolis against multidrug-resistant bacteria isolated from hospital environment.

Therefore, the aim of this study was to evaluate the susceptibility of *E. coli* and *K. pneumoniae* isolates, with ESBL and KPC phenotypes, to the ethanolic extract of Brazilian red propolis from patients admitted to a teaching public hospital from Recife, Pernambuco/Brazil.

2. MATERIALS AND METHODS

Bacteria strains

Twelve recent isolated bacterial strains were used for the susceptibility assay. They were obtained from urine samples, rectal swab, tissue fragment, surgical wound secretion, and tracheal secretion. Which five of them were *E. coli* (three ESBL type, one KPC type, and one strain with both resistance phenotypes) and seven *K. pneumoniae* (five ESBL type, one KPC type, and one strain with both resistance phenotypes), collected between February and March of 2015. The abovementioned strains were kindly donated by the laboratory of bacteriology of one hospital from Recife, Pernambuco/Brazil (Table 1).

Table 1: Clinical samples and resistance phenotypes of enterobacteria isolated from cases of hospital infection in Recife-PE/Brazil, from February to March of 2015.

Bacterial Strain	Clinical Sample	Resistance Phenotype
Escherichia coli 043 and 048	Urine	ESBL
E. coli 055	Urine	ESBL+KPC
E. coli 072	Tissue Fragment	KPC
E. coli 073	Surgical Wound Secretion	ESBL
Klebsiella pneumoniae 032	Urine	ESBL
K. pneumoniae 033	Rectal Swab	ESBL
K. pneumoniae 070	Tracheal Secretion	KPC
K. pneumoniae 071	Rectal Swab	ESBL+KPC
K. pneumoniae 082, 083 and 084	Rectal Swab	ESBL

ESBL: extended spectrum beta-lactamase; KPC: *Klebsiella pneumoniae* carbapenemase.

Propolis sample

The powder extract of red propolis utilized (25% of pure red propolis; Lot Code: PADE0113-SR) was kindly provided by PharmaNectar®. This propolis sample was produced by *Apis mellifera* honey bees and has *Dalbergia ecastophyllum* (checked at: http://www.theplantlist.org/tpl1.1/record/ild-1824 on February, 2019) as its botanical source. The chemical composition of the propolis used in this work (Table 2), and the chemical constituents of its botanical source (Table 3) have already been identified and quantified, through HPLC methodology, by PharmaNectar® according to its COA - certificate of analyses and characterized by Daugsch et al. (2008) [46].

Table 2: Chemical constituents of the ethanolic extract of Brazilian red propolis (Cod: PADE0113-SR).

No.	Compounds	Retention Time (min)	Area	Results (g L^{-1})	Results (%)
1	Rutin	11.193	2123954	0.4	0,04
2	Liquiritigenin	17.157	1165116	0.4	0,04
3	Daidzein	20.225	7201263	0.4	0,04
9	Formononetin	31.819	22507429	2.6	0,26
12	Biochanin A	40.409	5216525	0.6	0,06
					Total = 0,44 %

Source: PharmaNectar®

Table 3: Chemical constituents of Dalbergia ecastophyllum, botanical source of the ethanolic extract of Brazilian red propolis (Cod: PADE0113-SR)

No.	Compounds	Retention Time (min)	Area	Results (mg/mL)
1	Rutin	13.423	7344432	1.3
2	Liquiritigenin	16.991	19804160	7.1
3	Daidzein	22.347	83385299	4.3
4	Pinobanksin	23.199	8886438	6.0
5	Quercetin	24.593	9451717	1.9
6	Luteolin	28.395	17510819	2.1
7	Dalbergin	32.154	5049072	0.9
8	Isoliquiritigenin	34.619	34084540	12.1
9	Formononetin	36.967	167980291	19.5
10	Pinocembrin	42.296	8620282	7.1
11	Pinobanksin-3-acetate	42.950	4279493	2.6
12	Biochanin A	46.446	13339728	1.5

Source: PharmaNectar®

Antibiogram screening test

The antibiogram screening test was performed by disk diffusion methodology according to standard protocol M100-S25 of the clinical and laboratory standards institute [54]. The antibacterial drugs used are described as it follows: ampicillin (10 µg), amoxacillin + clavulanic acid (20/10 µg), piperacillin + tazobactam (100/10 µg), cefepime (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), cefuroxime (30 µg), meropenem (10 µg), nitrofurantoin (300 µg), ciprofloxacin (5 µg) and gentamicin (10 µg). The bacterial inoculum was standardized at the 0.5 McFarland scale, corresponding to 10^8 CFU/mL. Subsequently the inoculum was smeared onto agar Mueller-Hinton plates in petri dishes and the antibiotic disks were placed on the inoculated plates. The measurement of diameters of the inhibition halo formed around the antibiotic disks was evaluated to determine the antimicrobial sensibility into three categories: sensible, intermediate, or resistant.

The identification and confirmation of the ESBL and KPC resistance profiling were performed by the laboratory of bacteriology of one hospital, using automated approach trough the BD PhoenixTM automated microbiology system.

Microdilution assay

The entire microdilution assay was performed according to the M07-A9 protocol of the clinical and laboratory standards institute [55]. Brain heart infusion (BHI) broth was the culture medium used to maintain viable bacterial growth.

About 2.5 g of red propolis powder ethanolic extract were diluted in 10mL of 70% ethanol to obtain an initial concentration of 250 mg/mL. Then, the ethanolic extract of red propolis was diluted in dimethylsulfoxide (DMSO) 20% to obtain a range of 20 concentrations from 0.24 mg/mL to 125 mg/mL and from 0.004 mg/mL to 2.05 mg/mL.

Appropriately covered microtitration plates (TPP, Switzerland) containing 96 round-bottomed wells were used in the study. A volume of 100 µL of BHI culture medium was placed in each well of the plate. Additionally, 100 µL of the propolis extract were added to the wells, varying the concentration from the first to the tenth well, according to the serial dilution previously prepared. In the eleventh well there were placed 100 µL of broth BHI and the standardized inoculum, whereas in the twelfth well it was added only broth BHI, which corresponded to the bacterial growth culture and negative sterile controls, respectively.

The bacterial inoculum was adjusted to 0.5 McFarland scale and diluted in sterile distilled water (1:20) to obtain a final concentration of 10^4 to 10^6 CFU/mL. Each horizontal column of the microtitration plate corresponded to an individual bacterial strain. Aliquots of 10 µL of the inoculum were added from the first to the eleventh wells. The plates were incubated at 37°C for 24h to verify the susceptibility to the ethanolic extract of Brazilian red propolis. Considering total growth of the positive inoculum control, it was evaluated the bacterial growth reduction in wells containing propolis extract. The inhibition of bacterial growth was verified through visual observation, and it was also examined by measurement of the optical density of each well suspension with a microplate spectrophotometer, second to the skanlt software 3.1. for multiskan FC (Thermo Scientific) at 620 nm.

Minimum inhibitory and bactericidal concentration

The minimum inhibitory concentration (MIC) was determined by the lowest concentration of propolis capable of total inhibition of macroscopic bacterial growth in the microtitulation plates. To posterior evaluation of the bactericidal effect, aliquots of 50 µL of each MIC suspension were smeared onto the surface of agar Mueller-Hinton petri plates, which were incubated at 37°C for 24h. Total absence of bacterial growth in petri plates represented by the lowest tested concentration, determined the minimum bactericidal concentration (MBC). There were determined the geometric mean of minimum inhibitory and bactericidal concentrations (GMMIC and GMMBC, respectively) regarding the species and its respective resistance phenotype.

3. RESULTS

Results concerning the antibiogram screening test (AST) are described in table 4. All the *E. coli* isolates were resistant to cefotaxime, and the *E. coli* 043/ESBL and *E. coli* 048/ESBL were resistant to ceftazidime. Two isolates, *E. coli* 055/ESBL+KPC and *E. coli* 072/KPC, were both resistant to meropenem. All the *K. pneumoniae* isolates were resistant to cefotaxime and to ceftazidime, whilst the *K. pneumoniae* 070/KPC, *K. pneumoniae* 071/ESBL+KPC and K. PNEUMONIAE 082/ESBL isolates were resistant to meropenem. All the *E. coli* and *K. pneumoniae* isolates were resistant to ampicillin and to cefotaxime. The sensibility to ceftriaxone was confirmed only to *E. coli* 048/ESBL. Resistance to at least 50% of the antibiotics used in this approach was found to *E. coli* 043/ESBL. *E. coli* 073/ESBL, however *E. coli* 055/ESBL+KPC was resistant to 83,33% of the antibiotic drugs. The *K. pneumoniae* 070/KPC and *K. pneumoniae* 071/ESBL+KPC isolates were resistant to all drugs tested in this study. *K. pneumoniae* 083/ESBL was resistant to 50% of the drugs and the other isolates showed resistance to at least ten, out of twelve antibacterial drugs used in the ATS.

Table 4: In vitro Antibiogram Screening Test of Escherichia coli-ESBL/KPC and Klebsiella pneumoniae-ESBL/KPC obtained from hospital infections in Recife-PE/Brazil.

Bacterial Strain	Antibacterial Drugs											
	AMP	AMC	PPT	CPM	CTX	CAZ	CRO	CRX	MER	NIT	CIP	GEN
E. coli 043	R	I	I	S	R	R	R	R	S	S	R	S
E. coli 048	R	S	S	I	R	R	S	I	S	S	R	S
E. coli 055	R	R	S	R	R	S	R	R	R	I	R	R
E. coli 072	R	S	S	R	R	I	R	R	R	S	R	S
E. coli 073	R	S	S	R	R	S	R	R	S	S	R	S
K. pneumoniae 032	R	R	R	R	R	R	R	R	I	R	R	S
K. pneumoniae 033	R	S	S	R	R	R	R	R	S	S	S	S
K. pneumoniae 070	R	R	R	R	R	R	R	R	R	R	R	R
K. pneumoniae 071	R	R	R	R	R	R	R	R	R	R	R	R
K. pneumoniae 082	R	R	R	R	R	R	R	R	R	S	S	R
K. pneumoniae 083	R	R	S	I	R	R	R	R	S	S	S	S
K. pneumoniae 084	R	R	I	R	R	R	R	R	S	R	R	R

AMP: ampicillin; AMC: amoxicillin/clavulanic acid; PPT: piperacillin/tazobactam; CPM: cefepime; CTX: cefotaxime; CAZ: ceftazidime; CRO: ceftriaxone; CRX: cefuroxime; MER: meropenem; NIT: nitrofurantoin; CIP: ciprofloxacin; GEN: gentamicin; R: resistant; I: intermediate; S: susceptible.

The ethanolic extract of Brazilian red propolis used in this study showed antibacterial activities against all the isolates with ESBL and/or KPC resistance phenotype. The bacteriostatic activity was determined in the concentration of 2.05 mg/mL for *E. coli*-ESBL+KPC and between 0.24-2.05 mg/mL for *K. pneumoniae*-ESBL+KPC, whose GMMIC for *E. coli* was 2.05 mg/mL and for *K. pneumoniae* it was 0.5 mg/mL. The bactericidal activity was observed ranging from 15.63 to 31.25 mg/mL and from 3.91 to 31.25 mg/mL, respectively, for the same bacterial phenotypes abovementioned. The MBC for all the strains ranged from 3.91 to 31.25 mg/mL, being the GMMBC value of 17.95 mg/mL for *E. coli* and *K. pneumoniae* (Table 5).

Table 5: Bacteriostatic and bactericidal activities of the Ethanolic extract of Brazilian red propolis against clinical isolates of Escherichia coli and Klebsiella pneumoniae positive for KPC and ESBL phenotype production of hospitalized patients from Recife-PE/Brazil.

Bacterial Strain	MIC (mg/mL)	MBC (mg/mL)
E. coli 043	2.05	31.25
E. coli 048	2.05	15.63
E. coli 055	2.05	15.63
E. coli 072	2.05	15.63
E. coli 073	2.0	15.63
K. pneumoniae 032	0.24	3.91
K. pneumoniae 033	2.05	15.63
K. pneumoniae 070	1.02	15.63
K. pneumoniae 071	2.05	31.25
K. pneumoniae 082	0.26	31.25
K. pneumoniae 083	0.26	31.25
K. pneumoniae 084	0.13	15.63

MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentration.

4. DISCUSSION

The determination of *E. coli* and *K. pneumoniae* producers of ESBL is widely documented with worrying occurrence ratios [56]. In countries such as Argentina, Brazil, Chile and Mexico, the percentage of detection of ESBL-producing *E. coli* varied from 12 to 49% and of ESBL-producing *K. pneumoniae* from 49 to 61% [57].

The dissemination of kpc phenotype bacteria in Brazil has been found in various regions of the country [18], as well as by our results in Recife. According to the work of Biberg et al. (2015) [58], in Mato Grosso do Sul, 44 cases of KPC-producing *K. pneumoniae* were documented from infections in hospitalized patients, of whom 43,2% died. According to Lepeule et al. (2014) [59], a great amount of ESBL or KPC phenotypes infectious diseases are treated with carbapenems, without previous antibiogram screening, which can cause the selection of resistant bacterial strains, contributing to the spread of multidrug-resistant bacteria within the hospital setting.

Although the biological activities of propolis have been of worldwide knowledge, this is the first scientific documentation that proves the effective bacteriostatic and bactericidal activities of the ethanolic extract of Brazilian red propolis against ESBL/KPC-producing *E. coli* and ESBL/KPC-producing *K. pneumoniae* isolated from hospitalized patients.

The antibacterial activity of propolis has been one of its most explored biological activities in scientific field due to the possibility of providing an important therapeutic alternative to non-effective antibiotics as well as its therapeutic efficacy against resistant bacterial infections to current treatment courses [60, 61].

The antibacterial effect of Brazilian green and red propolis/PharmaNectar® has already been documented in literature against staphylococcus aureus, whose authors emphasized that the antibacterial activity of red propolis was better than the green one [33]. Regarding the antibacterial activity of the propolis used in this work, we could determine that this effect was obtained in lower concentrations than those related by other authors. The mic of brown propolis from Mato Grosso, Brazil for *E. coli* varied from 125 silvato 1000mg/ml (Bastos, Galbiati, Loureiro & Scoaris, 2011). Also, there was no growth inhibition of e. Coli, k. Pneumoniae and pseudomonas aeruginosa in a concentration of 10mg/ml of the ethanolic extract of red propolis from Alagoas, Brazil [62].

Nonetheless, the need of elevated concentrations of propolis extract to inhibit the growth of Gram-negative bacteria, when compared to the concentrations used to inhibit Gram-positive ones [46, 60, 63] was also noticed by Santos et al. (2015) [64]. These authors demonstrated that the hexane extract of red propolis at concentrations of 1 to 10% were not enough to inhibit the growth of Gram-negative bacteria but inhibited gram-positive ones.

Several authors related that the antibacterial activity of red propolis is very efficient against Gram-positive bacteria and less effective against Gram-negative ones [60, 65-67]. This fact can be partially explained by the presence of the external lipid membrane present in Gram-negative bacteria, which may possibly difficult the permeability to a variety of molecules [68]. Such explanation can somewhat justify the need of higher concentrations of the red propolis extracts to obtain the antibacterial effect against these pathogens. It is important to notice that the resistance phenotypes of the bacterial isolates used in this study as well as their high pathogenicity status for being isolated directly from hospital infections may have influenced somehow the achievement of the antibacterial effect in lower concentrations. In addition, the use of the extract of red propolis and not its chemical constituents per se certainly contributed to the MIC and MBC concentrations obtained in this work.

Although the active compounds of the antibacterial effect of propolis have not been totally elucidated yet and despite the complexity of its composition, its activity may be attributed to the presence of flavonoid compounds, as neovesitol, vesitol, rutin, liquiritigenin, biochanin A and formononetin [69, 70]. A study developed by Liu et al. (2011) [71] assessed the synergism between biochanin A and ciprofloxacin, and found it was able to inhibit the growth of methicillin-resistant *Staphylococcus aureus* (MRSA) reaching lower mic concentrations than the values for biochanin A or the drug alone. In addition, Bueno-Silva et al. (2013) [72] have proven that neovesitol and vesitol showed potent bacteriostatic activity ranging from < 6.25 mg/ml to 25-50 mg/ml and from 25-50 mg/mL to 50-100 mg/ml, respectively, as well as MBCs of 25-50 mg/ml

to 50-100 g/L for neovesitol and a range of 100-200 mg/ml to > 1600 mg/mL for vesitol. Also, das Neves et al. (2016) [73] reported that the antibacterial activity of formononetin isolated from the acetate fraction of Brazilian red propolis against *S. aureus*, *S. epidermidis* and *P. aeruginosa* achieved mic values of 200 mg/ml, whilst the bactericidal activity had only been confirmed in the hexane fraction of the same propolis sample (MBC of 1024 mg/mL) of which pinocembrin, luteolin and formononetin were the most prevailing compounds.

Moreover, efforts to find new therapeutic alternatives to treat a great variety of multidrug-resistant infections have extensively been explored in herbal medicine due to the diversity of molecules and chemicals found in these natural sources, thus demonstrating their biologically active compounds [27, 74, 75]. Al-Mariri and Safi (2014) [76] demonstrated the antibacterial activity of syrian oils and extracts against Gram-negative bacteria, such as *Proteus* spp., *K. pneumoniae*, *Yersinia enterocolitica* and *E. coli*. However, the MIC_{50} for *E. coli* was at least two-folds higher than that found for *K. pneumoniae* isolates. These results agree with our findings, once the antibacterial activity of red propolis ethanolic extract we used was more effective against *K. pneumoniae* isolates than against *E. coli* ones.

5. CONCLUSION

Our study corroborates with previous works in literature abovementioned, suggesting Brazilian red propolis as a promising natural source for development of new synergistic therapeutic adjuvants to treat infections of resistant etiologies. Thus, our results revealed that the Brazilian red propolis ethanolic extract presents significant bacteriostatic and bactericidal potential against ESBL/KPC-producing *E. coli* and ESBL/KPC-producing *K. pneumoniae* from different sources of infections in hospitalized patients, which highlights its possible use as a novel therapeutic adjuvant for treatment of infections caused by these Gram-negative multidrug resistant bacterial species. In this case, further research is needed to identify the constituent of this propolis with antibacterial capacity such as its mechanism of action against multidrug-resistant clinically relevant pathogens.

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