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Antibacterial activity of Lamiaceae plant extracts in clinical isolates of multidrug-resistant bacteria

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ABSTRACT

The antibacterial activity of plant extracts of the Lamiaceae family was evaluated against clinical isolates of multi-resistant Gram-negative bacteria by broth microdilution technique. Promising results were obtained considering that all extracts were active for at least two bacterial species with MIC ranging from 0.5 to 2.0 mg/mL.

Key words: antibacterial, clinical isolates, Lamiaceae extracts, multidrug-resistant.

INTRODUCTION

Antibiotic resistance is spreading faster than the introduction of new compounds into clinical practice, causing a public health crisis (Ling et al. 2015). Nosocomial bacterial infections have increased significantly, resulting in prolonged hospitalization, morbidity and treatment costs and hospital stay (Vincent et al. 2009). The increase of bacterial resistance to the main antibiotics used in therapy emphasize the need to develop new and more effective antibacterial drugs (Cataneo 2010, Kumarasamy et al. 2010).

The plant extracts are presented as a promising source for the search of new substances, because they have a higher molecular diversity when compared to products synthetic chemically (Novais et al. 2003, Veiga Junior 2008, Surendra et al. 2016a). To be invaded by bacteria, fungi, parasites, viruses or other agents, plants synthesize molecules defense with antimicrobial activity (Haida et al. 2007). Plants contribute to a variety of chemical compounds with antimicrobial properties and scientific research to determine the therapeutic potential of these substances is relevant for possible antibiotic properties against resistant pathogens (Duarte et al. 2005, Surendra et al. 2016b, Dinesh et al. 2017). Thus, this study aims to evaluate the antimicrobial activity of ethanol extracts in prevalent multidrug-resistant Gram negative bacteria in nosocomial infections.

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MATERIALS AND METHODS

The Lamiaceae species were collected in Carmópolis de Minas, Minas Gerais, Brazil, in April 2011. The plants materials were identified by Dr. Alexandre Salino and the voucher specimens were deposited at the Instituto de Ciências Biológicas Herbarium, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil (*Mentha* sp. BHCB 147244, *Ocimum basilicum* BHCB 147240*, Plectranthus barbatus* BHCB 147241*,* and *Rosmarinus officinalis* BHCB 147245).

The fresh plant material was extracted by cold maceration in ethanol P.A (Vetec, Brazil) for a period of 10 days at room temperature. After it was filtrate and concentrated in a rotary evaporator (IKA RV10, Germany) at 40 °C under reduced pressure and lyophilized (Líotop K105, Biobrás, Brasil) to yield ethanol extract (*Mentha* sp. 10.37 g, *Ocimum basilicum* 2.78 g, *Plectranthus barbatus* 3.17 g, and *Rosmarinus officinalis* 6.30 g) (Araújo et al. 2014).

Twelve clinical isolates of multidrug-resistant Gram-negative bacteria were selected for this study, three of each species: *Acinetobacter baumannii, Klebsiella pneumoniae, Escherichia coli* and *Pseudomonas aeruginosa*. Isolates from clinical samples including urine, tracheal aspirate and broncoalveolar lavage, were provided by Laboratório de Microbiologia do Hospital São João de Deus, Divinópolis, MG, Brazil. Underwent identification and antimicrobial susceptibility testing using the VITEK-2 (Bio-Merieux, Marcy l'Etoile, France) automated system.

The minimum inhibitory concentration **(**MIC) was determined by the microdilution broth method performed in accordance with the guidelines of the Clinical and Laboratory Standards Institute, with modifications (CLSI 2012). The extracts were diluted in dimethylsulfoxide (DMSO) (Sigma-Aldrich, USA) 20% in at concentrations 2, 1, 0.5 and 0.25mg/mL. Mueller Hinton broth (MH) medium (Himedia, India) without samples or solvents was used as a control for sterility. The antibiotics streptomycin 1.0 mg/mL plus penicillin 0.6 mg/mL (Sigma-Aldrich, USA) and DMSO (Sigma-Aldrich, USA) used in the dilution of the compounds were included as positive and negative controls, respectively. The MIC was assessed based on the lowest concentration of sample required to complete inhibit microbial growth detected as the lack of visible turbidity plus spectrophotometer (625 nm). The experiments were performed in triplicate and repeated three times in independent experiments.

RESULTS

The he profile resistance of clinical isolates performed by VITEK-2 (Bio-Merieux, Marcy l'Etoile, France) automated system were showed in Table I. The antimicrobial activity of ethanol extracts in prevalent multidrug-resistant Gram negative bacteria in nosocomial infections was evaluated. The values of MIC are shown in Table II.

The *P. barbatus* extract showed activity against strains of all species, with best results, with MIC ranging from 0.5 to 2.0 mg/mL. Mean while, *O. basilicum* extract was active against the A2 (*A. baumannii*) and E2 strains (*E. coli*), with both showing a MIC of 2.0mg/mL. The *R. officinalis* extract inhibited the growth of two *A. baumannii* strains, A1 and A2, with MIC of 1.0 and 2.0 mg/mL, respectively, and two *K. pneumoniae* strains, K2 and K3, with MIC of 2.0 and 0.5 mg/mL, respectively. *Mentha* sp. extract inhibited all *P. aeruginosa* strains and the *A. baumannii* A2 strain, showing MICs of 2.0 mg/mL. Negative control with DMSO 20% showed no activity.

DISCUSSION

In this work, have been used clinical samples that show resistance to major classes of antibiotics

TABLE I

Resistance profile of clinical isolates *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa* against three antibiotic classes used in medical clinic.

	A. baumannii			*K. pneumoniae*			*E. coli*			*P. aeruginosa*		
	A1*	**A2***	**A3***	**K1***	**K2***	**K3***	**E1***	**E2***	**E3***	**P1***	**P2***	**P3***
β-lactams												
Ampicilin	R	R	R	R	R	R	R	R	R	R	R	R
Cefepime	R	I	R	I	R	R	R	R	S	I	S	S
Cefotaxime	R	R	R	R	R	R	R	R	S	R	R	R
Ceftazidime	R	R	R	R	R	R	R	R	S	R	I	R
Cephalothin	R	R	R	R	R	R	R	R	S	R	R	R
Meropenem	R	S	R	S	S	R	S	S	S	S	R	S
Piperaciclin+Tazobactam	R	R	R	S	R	R	S	R	S	R	S	R
Aminoglycosides												
Amikacin	I	S	I	S	S	S	S	S	S	S	S	S
Gentamicin	R	R	S	R	R	R	S	R	R	S	S	S
Fluoroquinolones												
Ciprofloxacin	R	R	R	R	R	R	R	R	R	R	S	R

S – Susceptible, I – Intermediate, R – Resistant.
*Strains of *A. baumannii* (A), *K. pneumoniae* (K), *E. coli* (E) and *P. aeruginosa* (P).

TABLE II

Minimal inhibitory concentration (MIC) from crude extracts of Lamiaceae family against *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa* multidrug-resistant bacteria strains.

		Plectranthus barbatus	*Ocimun basilicum*	*Rosmarinus officinalis*	*Mentha* sp.
		MIC	**MIC**	**MIC**	**MIC**
	A1*	>2.0	>2.0	1.0	>2.0
A. baumannii	**A2***	2.0	2.0	2.0	2.0
	A3*	>2.0	>2.0	>2.0	>2.0
	K1*	1.0	>2.0	>2.0	>2.0
K. pneumoniae	**K2***	1.0	>2.0	2.0	>2.0
	K3*	>2.0	>2.0	0.5	>2.0
	E1*	>2.0	>2.0	>2.0	>2.0
E. coli	**E2***	1.0	2.0	>2.0	>2.0
	E3*	>2.0	>2.0	>2.0	>2.0
	P1*	>2.0	>2.0	>2.0	2.0
P. aeruginosa	**P2***	0.5	>2.0	>2.0	2.0
	P3*	1.0	>2.0	>2.0	2.0

MIC – Minimal Inhibitory Concentration (mg/mL).
*Strains of *A. baumannii* (A), *K. pneumoniae* (K), *E. coli* (E) and *P. aeruginosa* (P).

used in medicine, and the results show promising activity of the studied extracts. The activities of Lamiaceae species were superior than that of synthetic compounds Isoquinolin-1-yl-2-(cycloalk2-enylidene) hydrazines derivatives evaluated by Manivel et al. (2009), which were inactive against *E. coli*, *Proteus mirabilis*, *Salmonella typhi* and *S. aureus*. In the present study, the best result of inhibition of antibacterial activity was obtained by extract of *P. barbatus*, which showed activity against all tested samples of species, presenting MIC of 0.5 mg/mL against a strain of *P. aeruginosa.* The extract of *R. officinalis* was active against two samples *of A. baumannii* and two samples of *K. pneumoniae*, with MICs ranging from 0.5 to 2.0 mg/mL, the results are also considered promising, however, the antimicrobial activity of the natural compounds of the Lamiaceae family could be potentiated through the use of silver nanoparticles, as described by Madhumitha et al. (2015).

Many studies have demonstrated antibacterial activity of plants commonly used by traditional medicine (Rakholiya and Chanda 2012, Tekwu et al. 2012). Ríos and Recio (2005) suggested that the presence of activity is very interesting in the case of concentrations below 0.1 mg/mL for extracts. On the other hand, Fabry et al. (1998) defined as active the crude extract with MIC < 8 mg/mL. In a study carried out by Holetz et al. (2002) was considered that if the extracts displayed an MIC less than 0.1 mg/mL, the antimicrobial activity was good; from 0.1 to 0.5 mg/mL the antimicrobial activity was moderate; from 0.5 to 1.0 mg/mL the antimicrobial activity was weak; over 1.0 mg/mL the extract was considered inactive. In this study, however, MIC lower than 1.0 mg/mL were considered as promising.

Several studies have demonstrated antibacterial activity of plant extracts. Gemechu et al. (2013) tested the methanol extract of *Ocimum basilicum* against *Mycobacterium tuberculosis* and *M. bovis*. The MIC ranged from 0.025 to 0.1 mg/mL, and from 0.025 to 0.050 mg/mL, respectively. Extract of *P. betle* was evaluated against multidrug-resistant bacteria, *P. aeruginosa*, *A. baumanni*, *E. coli* and *K. pneumoniae*, and exhibited MIC ranging from 0.312 to 0.625 mg/mL (Valle et al. 2015).

In a study conducted Kowero et al. (2016) *P. barbatus* displayed a relatively wide MIC range of 3.12 to 12.5 mg/mL. The *P. barbatus* extracts demonstrated MIC values of 3.12 mg/mL against *S. typhi* and *K. oxytoca*. Additionally, *P. barbatus* exhibited MIC value of 3.12 mg/mL against *P. aeruginosa*. Likewise, essential oils of *P. barbatus* showed antibacterial activity against *E. coli*, *Proteus vulgaris*, *Bacillus cereus*, *P. aeruginosa*, *Staphylococcus aureus*, and *S. aureus* (multidrug-resistant) (Galvão et al. 2013). These findings corroborate with the present findings, where promising antibacterial activity was found for *P. barbatus* extract against *P. aeruginosa* with MIC (0.5 to 1.0 mg/mL) lower than that reported by Kowero et al. (2016).

Additionally, Araújo et al. (2014) observed that ethanol extracts exhibited a synergetic effect with streptomycin. Data showed synergistic effects of *P. barbatus* extract and streptomycin against *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 43895 serotype O157:H7, as well as, synergistic effect of *R. officinalis* extract and streptomycin against *E. coli* ATCC 43895 serotype O157:H7.

In order to identify the compounds present in ethanol extracts of Lamiaceae family, Araújo et al. (2014) investigated these extracts by gas chromatography/mass spectrometry (GC/MS). Phytol was identified in all ethanol extracts, camphor and verbenone compound are present in *P. barbatus* and *R. officinalis*, cadinene was identified in *Mentha* sp. and *O. basilicum* (Araújo et al. 2014). Santoyo et al. (2005) observed that camphor and verbenone showed activity against *S. aureus* and *Escherichia coli*. Vukovic et al. (2007) demonstrated activity of cadinene against

several microorganisms including *P. aeruginosa* and *K. pneumoniae* (Vukovic et al. 2007). These findings explain the activity of extracts against Gram negatives bacteria.

In view of the clinical importance of the bacteria studied in this work, the results obtained with the four extracts of Lamiaceae encourage new studies to isolate compounds and studying them to better elucidate its antimicrobial effect. This report probably enables the discovery of new antimicrobial compounds of those used in medical clinical.

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