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Short Communication

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Antibacterial Effects of *Plectranthus amboinicus* (Lour.) Spreng (Lamiaceae) in Methicillin Resistant *Staphylococcus aureus* (MRSA)

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***SUMMARY*. The present study describes some *in vitro* experiments with hydroalcoholic extract of leaves from *Plectranthus amboinicus* (Lour.) Spreng (Lamiaceae) in several Methicillin resistant *Staphylococcus aureus* (MRSA) strains in the attempt to determine whether the popular use corroborates with pharmacological properties. The antimicrobial activity was determined by the agar diffusion assay method. The evaluation of the minimum inhibitory concentration was determined using the agar dilution method. The time-kill curve was used to determine bactericidal and bacteriostatic effects. In accordance with the results, the hydroalcoholic extracts of leaves of *P. amboinicus* have shown a promising activity in MRSA strains. The minimum inhibitory concentration ranged from 18.7 to 9.3 mg/mL and the time-kill curves suggests a bactericidal and bacteriostatic effects, ranging with the concentration of the extract. These results corroborate with the use of *P. amboinicus* in folk medicine for the treatment of infections caused by *S. aureus*.**

INTRODUCTION

The use of medicinal plants for the treatment of several diseases is a primary health care in Latin America. In Brazil, especially in Northeast Region, the use of the plants in form of crude extracts, essential oils and infusions is a practice widely used for the treatment of several diseases, including infections. However, many of these medicinal species are used without evidence of pharmacological effects [1]. Furthermore, these medicinal plants are used in association with antimicrobial drugs employed in clinical practice. These associations can increase or inhibit the pharmacological effects of drugs, interfering substantially in the therapy [1-3]. Therefore, it is necessary to investigate the pharmacological effects of these plants commonly used in the folk medicine in Brazil.

Among the mentioned commonly species in ethnomedicinal reports, the medicinal species of genus *Plectranthus* are always cited as antimicrobial agents used to treat several infections [4-6]. The genus *Plectranthus* contains about 300 species, being found in Tropical America, Africa, Asia and Australia [7]. *Plectranthus amboinicus* (Lour.) Spreng (Lamiaceae) is native from Asia and distributed in America. It is commonly known in Brazil as "orégano" or "hortelã da folha grossa", and is often used in the folk medicine to treat inflammations and respiratory infections [8]. In Brazil, several ethnomedicinal studies have shown that *P. amboinius* is used for treatment of diseases, such as inflammations and fungal and bacterial infections [9,10].

Previous pharmacological studies have reported that *P. amboinicus* extracts possesses antiepileptic, antioxidant [11], leishmanial [12] and antimicrobial properties [2,8,13]. The leaves of this specie secrete essential oils, flavonoids and terpens 8, which have inhibitory effects in several microorganisms [14].

These previous studies encouraged us to investigate the antimicrobial properties of *P. amboinicus* in order to determine whether the

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medicinal uses are supported by pharmacological effects. We report here the results of an *in vitro* experimental study with extract of leaves of *P. amboinicus* in methicillin-resistant *Staphylococcus aureus* strains.

MATERIALS AND METHODS

Plant material

The leaves of *P. amboinicus* were collected in the Laboratory of Pharmaceutical Technology of the Federal University of Paraíba (Brazil) and identified by Dra. Rita de Cássia A. Pereira. A voucher specimen was deposited in the IPA herbarium (Empresa Pernambucana de Pesquisa Agropecuária), under the IPA 65501 number.

Plant extracts and Phytochemical screening

Crude hydroalcoholic extracts (HE) were prepared by maceration of 2,74 kg remnants of the leaves, with ethanol 70% (v/v), for 72 h. The obtained extract was filtered and concentrated in a rotary evaporator at 45 °C under reduced pressure, yielding 32.5 g.

Plant material was screened to detect alkaloids, monoterpenoids, diterpenoids, sesquiterpenoids, triterpenoids, iridoids, saponins, flavonoids, condensed proanthocyanidins and leucoanthocyanidins using methods described in Table 1.

The Chromatography was performed by TLC on Si gel (MERCK – Germany, 105553) developed by different solvent systems: EtOAc-HCOOH- AcOH-H_2O (100:11:11:26 v/v), Benzene-AcOEt (97:3 v/v) and EtOAc- HCOOH-AcOH-H2O (100:0.5: 0.5:0.5 v/v).

Microorganisms

The microorganisms used in this study were *Staphylococcus aureus* MRSA (ATCC 6538) *Staphylococcus aureus* (ATCC 25923) and 9 MRSA strains isolated from patients at the University Hospital Lauro Wanderley, João Pessoa, State of Paraiba, Brazil. All strains were obtained from the culture collection of the Department of Molecular Biology, Federal University of Paraíba, Brazil.

Antibacterial activity

The antibacterial activity was determined by the Agar Diffusion Assay Method [15] with some modifications. Petri plates containing 20 mL of medium (Agar Müeller Hinton, DIFCO) were seeded with selected microbial strains. Afterwards, were made cavities of 6 mm of diameter and in each cavity were inoculated 50 µL

Metabolits	TLC solvent	Reagent	Presence/Absence
Alkaloids [23]	EtOAc-HCOOH- AcOH-H_2O (100:11:11:26 v/v)	Dragendorff	-
Cinnamics derivatives (chlorogenic acid) [24,25]	EtOAc-HCOOH- AcOH-H_2O (100:11:11:26 v/v)	2-Aminoethyldiphenyl borinate	+
Flavonoids (quercetin, caffeic acid, luteolin) [24]	EtOAc-HCOOH- AcOH-H_2O (100:11:11:26 v/v)	2-Aminoethyldiphenyl borinate	+
Condensed proanthocyanidins and leucoanthocyanidins [23]	EtOAc-HCOOH- AcOH-H_2O (100:11:11:26 v/v)	Vanillin chloridric	+
Monoterpenoids (carvacrol), sesquiterpenoids and diterpenoids [23]	Benzeno-AcOEt (97:3 v/v)	Vanillin sulphuric acid	+
Terpenoids and Steroids (β-Sitosterol, β-Amirin) [26]	EtOAc- HCOOH- AcOH-H_2O (100:0,5:0,5:0,5 v/v)	Lieberman / Burchard	+
Iridoids [27]	EtOAc-HCOOH- AcOH-H_2O (100:11:11:26 v/v)	Vanillin sulphuric acid	-
Saponins [23]	EtOAc-HCOOH- AcOH-H_2O (100:11:11:26 v/v)	Anisaldehyde	-

Table 1. Pytochemical screening of hydroalcoholic extract of leaves from *Plectranthus amboinicus* (Lour.) Spreng.

aliquots of dilution extracts. 70% alcohol was used as negative control and each test was carried out in duplicate. The assessment of antimicrobial activity was based on measurement of inhibition zones formed around the cavities. The diameters of these zones were recorded. The media was incubated for 24 h at 37 °C.

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) was determined by Agar Dilution Method [16]. On Petri plates were placed 18 mL of medium agar accrued from 2 mL of dilutions of the extract. The strains of *S. aureus* MRSA were maintained on Mueller-Hinton broth (Brain Heart Infusion, DIFCO), incubated at 37 °C for 18-20 h, diluted at 10^{-2} colony forming-units (CFU/mL) in saline solution and inoculated. The concentrations used ranged from 1200 to 2.3 mg/mL of plant extract. Alcohol 70% was used as negative control and Norfloxacin® 10 µg as positive control. The experiments were performed in duplicate.

Tim-kill curve

Rates of killing were determined by measuring the reduction in viable bacteria (log10 CFU/mL), exposed to concentrations of 1.200 mg/mL 18.7 mg/mL (two times the MIC) and 37.5 mg/mL (four times the MIC) of the extracts during 0, 2, 4, 6, 8, 10 and 24 hours. Experiments were performed in duplicate. Petri plates containing less than 2 log 10^{-1} CFU/mL were considered to be under the limit of quantification [14]. The bactericidal effects were considered when values of rates of killing were higher than 2 log 10^{-1} CFU/mL. There were bacteriostatic effects when values of rates of killing were lower than 2 log 10^{-1} CFU/mL. In the attempt to minimize the drug carryover, dilution of at least 32-fold (solely in the kill curve of sample ATCC 6538) were performed.

RESULTS

The results of phytochemical screening of leaves of hydroalcoholic extract from *P. amboinicus* (shown in Table 1), indicated the presence of flavonoids (caffeic acid, quercetin and luteolin), monoterpenoids (carvacrol), sesquiterpenoids, diterpenoids, triterpenoids, steroids, and condensed proanthocyanidins, leucoanthocyanidins and cinnamics derivatives (chlorogenic acid).

The antibacterial activity of the extract in MRSA strains have shown a promising activity against all tested MRSA samples, with holes diameters ranged from 23 to 10 mm, in the concentrations of 150 at 9.3 mg/mL (data not shown).

The MICs ranged from 18.7 to 9.3 mg/mL (Table 2). The strain 19 Lab showed sensible in all concentrations tested. These results indicate the efficiency of the HE of *P. amboinicus* as a promising antibacterial agent, once all tested samples are resistant to Penicillin, Eritromicin, Chloramphenicol, Gentamicin and some metallic ions such as Cd and Hg.

Three strains of *S. aureus* (MRSA) were treated with HE of *P. amboinicus,* in different concentrations. In the strain 171, treated with concentration of 1.200 mg/mL, bactericidal effect with reduction of 6 $\log_{10}$ in the first hour of exposition was verified (Fig. 1). The ATCC 6538 strain was treated with values that correspond to two times of MIC, 18.7 mg/mL, and four times of MIC, 37.5 mg/mL. These concentrations have shown bacteriostatic effects, as can be noted in Figure 2.

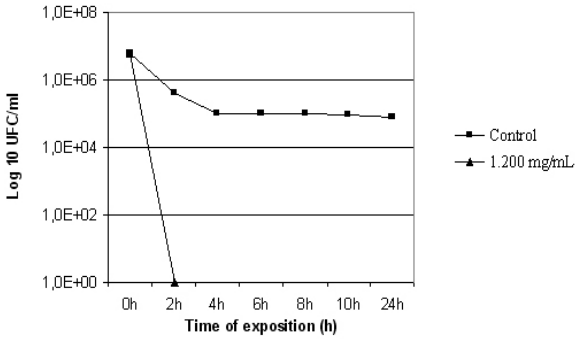

Figure 1. Time-kill curve analyses of hydroalcoholic extract of *P. amboinicus* against *S. aureus* 171C MRSA with 1.200 mg/mL extract concentration.

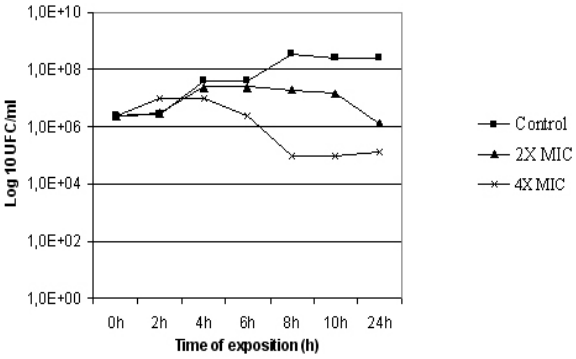

Figure 2. Time-kill curve analyses of hydroalcoholic extract of *P. amboinicus* against *S. aureus* ATCC 6538 MRSA at two and four times the MIC for 24 h.

Bactéria strains	Concentration of HE of leaves from *Plectranhus amboinicus,* in mg/mL											
	Ethanol 70%	Norfloxacin 10 µg	2.3	4.6	9.3	18.7	37.5	75	150	300	600	1.200
149L	+	-	+	+	+	-	-	-	-	-	-	-
171CC	+	-	+	+	+	-	-	-	-	-	-	-
10CC	+	-	+	+	-	-	-	-	-	-	-	-
02H	+	-	+	+	-	-	-	-	-	-	-	-
06C	+	-	+	+	+	-	-	-	-	-	-	-
259I	+	-	+	+	+	-	-	-	-	-	-	-
19L	+	-	-	-	-	-	-	-	-	-	-	-
192C	+	-	+	+	-	-	-	-	-	-	-	-
ATCC 25923	+	-	+	+	-	-	-	-	-	-	-	-
ATCC 6538	+	-	+	+	-	-	-	-	-	-	-	-

Table 2. Minimum inhibitory concentration of hydroalcoholic extract of leaves from *P. amboinicus* against microorganisms *S. aureus* MRSA. (+) Growth; (-) Inhibition.

DISCUSSION AND CONCLUSION

Although the genus *Plactranthus* comprises many plants of medicinal interest [7] its chemistry is poorly known [18]. Such observed antibacterial activity occurs due to the presence of several phytocompounds with known antimicrobial properties. Several studies have reported that carvacrol and essentials oils, present in *P. amboinicus,* modifiy the constitution and increase the fluidity of the cell membrane, typifying a dose-dependent effect, as fully demonstrated in this study [19-21]. Other phytocompounds found in the HE of *P. amboinicus* also possess antibacterial activity. Some studies describe damage to the membrane and lyses cells caused by cinnamic derivatives and terpens [14,21].

The bactericidal action of the extract seems promising, once the speed of cell kill, in the first hours of exposition of the HE of *P. amboinicus,* is similar to fluorquinolone, perfloxacin, ofloxacin and norfloxacin (wide spectrum antimicrobials very used in Human and Veterinary medicine). These results are consistent with others *in vitro* experimental findings. Oliveira *et al.* [5] and Nogueira *et al.* [13] have reportedthe antimicrobial effects of leaves from *P. amboinicus* in the form of essential oil and crude extracts, respectively. Moreover, some studies demonstrated absence of toxicity *in vitro* and *in vivo* [8,22], which makes the use of this species very safe in the treatment of multi-resistant infections.

The results indicated a promising antimicrobial action of HE of leaves of *P. amboinicus* in methicillin-reistante strains. The time-kill curve have shown that the extract possesses bactericidal or bacteriostatic action in the first hours of treatment, varying according to the concentration. Such results are very important once MRSA strains possess a great resistance spectrum.

The preliminary results of this study have corroborated with the use of *P. amboinicus* in folk medicine for the treatment of infections caused by *S. aureus* pathogen. However, future studies should investigate whether the use of this medicinal specie interferes with drugs in the antimicrobial therapy.

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