Revista Brasileira de Farmacognosia
Brazilian Journal of Pharmacognosy
17(2): 186-190, Abr./Jun. 2007
Received 12/20/06. Accepted 04/24/07

Artigo

Interference of *Plectranthus amboinicus* (Lour.) Spreng essential oil on the anti-*Candida* activity of some clinically used antifungals

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RESUMO: "Interferência do óleo essencial de *Plectranthus amboinicus* (Lour.) Spreng sobre a atividade anti-*Candida* de alguns antifúngicos utilizados clinicamente". As plantas com propriedades medicinais têm sido utilizadas por um longo tempo no cuidado tradicional de saúde, de modo que algumas vezes seu uso ocorre concomitantemente ao uso de drogas industrializadas. O objetivo deste estudo foi avaliar a interferência do óleo essencial de *Plectranthus amboinicus* sobre atividade anti-*Candida* de alguns antifúngicos utilizados clinicamente através do método de difusão em meio sólido. Os antifúngicos ensaiados foram anfotericina B (100 µg/mL), cetoconazol (50 µg/mL) e itraconazol (50 µg/mL). *C. albicans*, *C. tropicalis*, *C. guilliermondii*, *C. krusei* e *C. stellatoidea* foram utilizadas como microrganismos testes. O óleo essencial de *P. amboinicus* mostrou um valor de CIM de 40 µL/mL para a maioria das cepas de leveduras ensaiadas. O óleo essencial quando ensaiado em sua CIM mostrou alguma interferência sobre a efetividade anti-*Candida* dos antifúngicos testados, sendo notada a formação de zonas de inibição do crescimento fúngico com diferentes diâmetros quando os antifúngicos foram testados isolados e em combinação com o óleo essencial. O óleo essencial de *P. amboinicus* mostrou destacável interferência sobre a atividade antifúngica de itraconazol, sendo observado um efeito sinérgico sobre *C. albicans*, *C. tropicalis*, *C. krusei* e *C. stellatoidea*. Ademais, o óleo essencial interferiu na atividade antifúngica de cetoconazol sobre *C. albicans*, *C. guilliermondii* e *C. stellatoidea* causando, respectivamente, efeito antagônico e sinérgico. De outra forma, foi observada uma pequena interferência do óleo essencial sobre o efeito antifúngico de anfotericina B. Estes dados mostram que o uso combinado de plantas medicinais e/ou seus produtos derivados com drogas industrializadas, particularmente, antimicrobianos podem interferir sobre os efeitos terapêuticos esperados.

Unitermos: *Plectranthus amboinicus*, óleo essencial, antifúngicos.

ABSTRACT: Plants with medicinal properties have been applied for a long in the traditional health care, so that sometimes their use takes place concomitantly to the use of industrialized drugs. The aim of this study was to evaluate the interference of *Plectranthus amboinicus* essential oil on the anti-*Candida* activity of some clinically used antifungals by the solid medium diffusion procedure. Assayed antifungals were amphotericin B (100 µg/mL), ketoconazole (50 µg/mL) and itraconazole (50 µg/mL). *C. albicans*, *C. tropicalis*, *C. guilliermondii*, *C. krusei* and *C. stellatoidea* were used as test microorganisms. *P. amboinicus* essential oil showed MIC value for most assayed yeast strains. The essential oil when assayed in its MIC value showed some interference on the anti-*Candida* effectiveness of the assayed antifungals. It was noted yeast growth inhibition zones with different diameters when the antifungals were tested alone and combined with the essential oil. *P. amboinicus* essential oil showed prominent interference on the anti-yeast activity of itraconazole providing a synergic effect on *C. albicans*, *C. tropicalis*, *C. krusei* and *C. stellatoidea*. Also, the essential oil interfered on the anti-yeast activity of ketoconazole when interacting with *C. albicans*, *C. guilliermondii* and *C. stellatoidea* providing, respectively, an antagonic and synergic effect. On the other hand, it was found a small interference on the anti-yeast effect of amphotericin B. These data showed that combined use of medicinal plants and/or derivatives with industrialized drugs, particularly antimicrobials, could interfere on their expected therapeutic effects.

Keywords: *Plectranthus amboinicus*, essential oil, antifungals.

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ISSN 0102-695X

INTRODUCTION

Candida species are reported as the yeasts most frequently involved in mycotic infections etiology (Wilhelmus, 2005). Candidiasis is the most common mycotic infection being *C. albicans* its most important causing agent, although *C. guilliermondii*, *C. krusei*, *C. parapsilosis*, *C. stellatoidea* and *C. tropicallis* have been also reported as potential etiological agents (Schwartz, 2004; Duarte et al., 2005; Lima et al., 2006a,b).

Plants with medicinal properties have been focus of scientific studies all over the world in order to discover new and effective antimicrobial compounds (Radhrakrishanan-Sridhar et al., 2003; Burt, 2004; Lima et al., 2006c; Packer & Luz, 2007). World Health Organization (WHO) estimates that about 65 to 80 % of the world population trust on traditional health care practices which are mainly based on the use of medicinal plants and derivatives. Still, the WHO has recommended the planning of studies for evaluating the safety and effectiveness of phytotherapeutic products in order to promote their correct use by population (Calixto, 2000; Aguiar, 2004).

The concomitant use of medicinal plants and derivatives with industrialized drugs has been observed in some ethnopharmacological studies (Dorigoni, 2001; Veiga Júnior et al., 2005). This practice has been found as consequence of self-medication or even of health professional advising and includes the use of decocts, infusions, teas, extracts, macerates, plasters, essential oils and powders from medicinal plants (Sucar, 2003; Oliveira et al., 2006). Drugs association means the possibility of interactions which could result in enhanced therapeutic answer, antagonic effect to the used drug or decreased side effects (Brinker, 1998; Seidil, 2000).

Plectranthus amboinicus (Lour.) Spreng, Lamiaceae, is a perennial, aromatic, juicy and erect plant with length varying from 5 cm to 1 m. The plant is found in all Tropical America since the Antilles up to Brazil South (Castillo; Gonzáles, 1999). *P. amboinicus* is widely used in the Brazilian folk medicine to treat skin diseases and its leaves are topically used for the treatment of furuncles and superficial mycosis. Still, it is popularly used for treating flue, constipation, cephalea, cough, hoarseness, fever and digestive diseases (Lorenzi; Matos, 2002; Sellar, 2002; Tôrres et al., 2005; Morais et al., 2005).

Regarding the lack of scientific studies about the interference of medicinal plants and derivatives on the effectiveness of industrialized drugs, particularly antimicrobials, this study aimed at evaluating the interference of *P. amboinicus* essential oil on the anti-*Candida* activity of some clinically used antifungals.

MATERIAL AND METHODS

Plant material

Plectranthus amboinicus (Lour.) Spreng leaves were collected from the plant collection of the Laboratório de Tecnologia Farmacêutica, Universidade Federal da Paraíba (João Pessoa, Brazil) in May, 2004. Whole plant was sent to Herbário IPA - Dárdamo de Andrade Lima, Empresa Pernambucana de Pesquisa Agropecuária (Recife, Brazil) for its botanic identification. A voucher specimen was deposited at the same herbarium under a number of 65001 IPA.

P. amboinicus essential oil was obtained by hydrodistillation procedure using the Clevenger apparatus. Essential oil solutions at concentrations ranging from 320 to 2.5 µL/mL prepared according to Souza et al. (2005) were used in the antimicrobial assays.

Microbial strains

Candida albicans ATCC-90028, *C. tropicalis* LM-69, *C. guilliermondii* LM-28, *C. krusei* LM-07, *C. stellatoidea* LM-46 and *C. parapsilosis* CM-01 strains were used as test microorganisms. These strains were obtained from the microorganism collection of the Laboratory of Mycology, Health Science Center, Federal University of Paraíba (João Pessoa, Brazil). Stock cultures were kept on Sabouraud agar slants at ± 7 °C. Yeasts inocula, were prepared by inoculating a loopfull of overnight cultures (cultivated on Sabouraud agar slants at 28 °C) in sterile saline solution (NaCl 0.85% w/v) followed by shaking using vortex for 5 minutes. Yeast suspensions had their turbidity compared and adjusted to the McFarland 0.5 tube turbidity providing an inoculum of approximately 10^6 colony forming unity per mL.

Antifungals

Amphotericin B (100 µg/mL), ketoconazole (50 µg/mL) and itraconazole (50 µg/mL) were chosen for inclusion in the antimicrobial assays based on their availability in the Centers of Basic Assistance in Health of the Brazilian Health Public System. The sensitivity of the *Candida* strains to the tested antifungals were assessed by the solid medium procedure using paper discs (Sensibiodisc - Cecon, Saõ Paulo, Brazil) (Bauer et al., 1966).

Determination of the essential oil Minimum Inhibitory Concentration - MIC

Solid medium diffusion procedure using wells in dishes was used to determine the *P. amboinicus* essential oil MIC. For this, 1 mL of the yeast inoculum (approximately 10^6 cfu/mL) was uniformly spread on sterile Sabouraud agar Petri dishes. After inoculum absorption by Sabouraud agar, wells were made using sterile glass tubes (diameter 6 mm) which were filled with 50 µL of the essential oil solutions with different

concentrations (Hadaceck; Greger, 2002; Souza et al., 2007). The system was incubated at 28 °C for 48 hs. At the end of the incubation period, the MIC was the lowest essential oil concentration showing growth inhibition zones with diameters equal to or greater than 10 mm. Controls included in this assay were essential oil replaced by sterile water.

Interference of the essential oil on the antifungal anti-*Candida* activity

Interference of *P. amboinicus* essential oil on the antifungal anti-*Candida* activity was assessed by the solid medium diffusion procedure using paper discs. Prior the anti-yeast assays, the antifungals paper discs were soaked with 20 µL of the essential oil solution adjusted to have a concentration similar to the MIC value previously found. After that, 1 mL of the yeast suspension (approximately 10^6 cfu/mL) was uniformly spread on sterile agar Sabouraud Petri dishes. After inoculum absorption by Sabouraud agar, the paper discs (Whartman n. 1, diameter 6 mm) were placed on the inoculated agar. The system was incubated at 28 °C for 48 hours. At the end of the incubation period, the interference of the essential oil on the antifungal anti-*Candida* activity was evaluated. Synergic effect was regarded when observed yeast growth inhibition zone resulting of the combined use of antifungal and essential oil, with diameter equal to or higher than 2 mm in comparison to the one found when the antifungal was assayed alone. Antagonic effect was regarded when observed yeast growth inhibition zone resulting of combined use of antifungal and essential oil, with diameter smaller than the one found when the antifungal was assayed alone. No interference was regarded when observed no change in the yeast growth inhibition zone diameter when the antifungal was assayed alone and combined with the essential oil (Cleeland; Squires, 1991; Oliveira et al., 2006). All assays were carried out in triplicate and the results were expressed as average of the three assays.

RESULTS AND DISCUSSION

Table 1 shows the sensitivity of *Candida* species to some clinically used antifungals. The assayed strains presented sensitivity behavior to the *P. amboinicus* essential oil however it was depending on the yeast strain and assayed antifungal. *C. albicans* was the only strain sensitive for all tested antifungals showing yeast growth inhibition zones with diameter between 10 and 16 mm. On the other hand, *C. krusei a*nd *C. stellatoidea* presented resistance to all tested antifungals.

Table 2 shows the MIC values of *P. amboinicus* essential oil on *Candida* species. *P. amboinicus* essential oil presented MIC value of 40 µL/mL for the most assayed strains providing growth inhibition zones with diameter between 10 and 12 mm. Essential oil highest MIC value (80 µL/mL) was found to *C. tropicalis*.

C. stellatoidea was the only strain that showed resistance to the essential oil at all assayed concentrations. Antimicrobial properties in *P. amboinicus* essential oil on bacteria and/or fungal strains were found for some researchers, although its antimicrobial effectiveness has varied according to the target microorganisms, microbial inoculum, method and essential oil assayed concentrations (Aguiar, 2004; Oliveira et al., 2006).

Thymol and carvacrol, both phenolic compounds, have been reported as the majority compounds of *P. amboinicus* essential oil and are considered to be responsible for its antimicrobial property (Alves, 2000). Carvacrol and thymol present capability of dissolving into the cytoplasm membrane aligning among the fatty acid chains providing an increase in the cytoplasm membrane passive permeability (Lambert et al., 2001; Souza et al., 2007).

Table 3 shows the interference of *P. amboinicus* essential oil on the anti-*Candida* activity of some clinically used antifungals. The essential oil was assayed at concentration of 40 µL/mL because it was the MIC value found to the most *Candida* strains (Table 2). *P. amboinicus* essential oil showed some interference on the anti-*Candida* activity of the assayed antifungals noted by the formation of yeast growth inhibition zones with different diameters when the antifungal was assayed alone (Table 1) and combined with the essential oil (Table 3). Amphotericin B showed the smallest interference of the essential oil.

P. amboinicus essential oil presented a high interference on the anti-*Candida* property of itraconazole providing an enhancing (synergic effect) of its inhibitory effect on *C. albicans*, *C. tropicalis*, *C. krusei* and *C. stellatoidea*. On the other hand, it was observed an antagonic effect of the essential oil on the anti-*C. krusei* activity of itraconazole. Ketoconazole presented an interference of the essential oil in the interactions with *C. albicans*, *C. guilliermondii* and *C. stellatoidea* being found, respectively, an antagonic and synergic effect. Moreover, *C. stellatoidea* presented resistance to *P. amboinicus* essential oil (Table 1) and all tested antifungals (Table 2), however when the antifungals were assayed combined with the essential oil it was noted a change in its behavior (resistance to sensitivity) to ketoconazole and itraconazole.

Researchers emphasizing the interactions of industrialized drugs with medicinal plants are still little explored. Figueiredo et al. (2000) studying the associate use of medicinal plants used in the folk medicine of different Brazilian regions reported a lack of scientific data on the possible pharmacological interactions when different medicinal plants are used concomitantly to industrialized drugs. Some researches have noted some interference of medicinal plants and/or derivatives on the pharmacological effects of antimicrobial, anti-hypertensive, anti-coagulating and hypo-glycemic drugs

Table 1. Sensitivity of *Candida* species to some clinically used antifungals [a].

Yeasts	Antifungicals			
	amphotricin B	ketoconazole	itraconazole	strain viability
C. albicans	10	16	12	+
C. tropicalis	10	0	0	+
C. guilliermondii	0	15	11	+
C. krusei	0	0	0	+
C. stellatoidea	0	0	0	+
C. parapsilosis	0	18	12	+

+: yeast growth in Sabouraud agar without adding essential oil or synthetic antifungal
[a] results expressed in yeast growth inhibition zones diameters (mm)

Table 2. MIC of *P. amboinicus* essential oil on *Candida* species.

Yeasts	MIC (µL/mL)	Yeast inhibition zone diameter (mm)
C. albicans	40	11
C. tropicalis	80	10
C. guilliermondii	40	12
C. krusei	40	10
C. stellatoidea	R	0
C. parapsilosis	40	10

R: resistant for all assayed concentrations

Table 3. Interference of *P. amboinicus* essential oil on the anti-*Candida* activity of some clinically used antifungals [a, b].

Yeasts	Association		
	amphotericin B + essential oil	ketoconazole + essential oil	itraconazole + essential oil
C. albicans	10 *	12 ↓	15 ↑
C. tropicalis	10 *	0 *	12 ↑
C. guilliermondii	0 *	20 ↑	8 ↓
C. krusei	13 ↑	0 *	10 ↑
C. stellatoidea	0 *	15 ↑	14 ↑
C. parapsilosis	0 *	18 *	13 *

[a] results expressed in yeast growth inhibition zones diameters (mm)
[b] *P. amboinicus* essential oil assayed at concentration of 40µL/mL
+ : yeast growth in Sabouraud agar without adding essential oil or synthetic antifungal
* no interference
↑: synergic effect
↓: antagonic effect

(Alonso, 1998; Nascimento et al., 2005; Veiga Júnior et al., 2005).

Regarding our results *P. amboinicus* essential oil presents capability of interfering on the anti-*Candida* effectiveness of some clinically used antifungals, mainly itraconazole and ketoconazole. However, this interference depended on the *Candida* strain and assayed antifungal. Concomitant use of medicinal plants and/or derivatives with industrialized drugs is still a wide research field to be explored in order to discover the occurrence and ways by which plant products can interfere on the treatment of many diseases, particularly, microbial diseases.

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