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SHORT COMMUNICATION/COURTE COMMUNICATION

Pothomorphe umbellata: Antifungal activity against strains of *Trichophyton rubrum*

Pothomorphe umbellata : *activité antifongique sur des souches de* Trichophyton rubrum

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Summary *Trichophyton rubrum* is a dermatophyte, which can cause infections in human skin, hair and nail. *Pothomorphe umbellata* (L.) Miq. (Piperaceae) is a native Brazilian plant, in which phytochemical studies have demonstrated the presence of steroids, 4-nerolidylcatechol, sesquiterpenes and essential oils. The objective of this study was to analyze the in vitro activity of extracts and fractions of *P. umbellata* on resistant strains of *T. rubrum*. The microdilution plate method was utilized to test Tr1, H6 and Δ*TruMDR2* strains of *T. rubrum*; Δ*TruMDR2* strain was obtained from H6 by *TruMDR2* gene rupture, which is involved in multiple drugs resistance. The highest antifungal activity to all strains was observed for dichloromethane and hexane fractions of the 70% ethanolic extract which showed minimal inhibitory concentration (MIC) and minimal fungicide concentration (MFC) of 78.13 μg/mL. This antifungal activity was also obtained by 70% ethanolic extract, which presented MIC and MFC of 78.13 μg/mL to Δ*TruMDR2*, whereas the MIC values for Tr1 and H6 were 78.13 and 156.25 μg/mL, respectively. Our results suggest the potential for future development of new antifungal drugs from *P. umbellata*, especially to strains presenting multiple resistance.

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MOTS CLÉS
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Résumé *Trichophyton rubrum* est un dermatophyte qui peut provoquer des infections de la peau humaine, des cheveux et des ongles. *Pothomorphe umbellata* (L.) Miq. (Piperaceae) est une plante originaire du Brésil où les études phytochimiques ont démontré la présence de stéroïdes, de 4-nerolidylcatechol, de sesquiterpènes et d'huiles essentielles. L'objectif de cette étude est analyser l'activité in vitro d'extraits et de fractions de *P. umbellata* sur les souches de *T. rubrum*. La méthode de plaque de microdiluition a été utilisée pour faire les essais sur les souches Tr1, H6 et Δ*TruMDR2* de *T. rubrum* ; la souche Δ*TruMDR2* a été obtenue à partir de H6 par la délétion du gène *TruMDR2*, qui est impliqué dans la résistance aux multiples médicaments. La plus forte activité antifongique pour toutes les souches a été observée pour les fractions dichlorométhane et hexane de l'extrait éthanolique 70 % qui ont présenté une concentration minimale inhibitrice (CMI) et une concentration minimale fongicide (CMF) de 78,13 μg/mL. Cette activité antifongique a été aussi obtenue pour l'extract éthanolique à 70 %, qui a présenté CMI et CMF de 78,13 μg/mL pour la souche Δ*TruMDR2*, tandis que les valeurs de CMI pour Tr1 et H6 ont été 78,13 et 156,25 μg/mL respectivement. Nos résultats suggèrent le possible développement de nouveaux médicaments antifongiques à partir de *P. umbellata*, en particulier pour les souches présentant une résistance multiple.

Introduction

The dermatophytes are a group of fungi that presents the capacity to invade several keratinized tissue (skin, hair, and nails) where these substrates are used as carbon, nitrogen and sulfur source. In humans and other animals these fungi can produce an infection called dermatophytosis [29].

Among the dermatophytes can be distinguished the genus *Trichophyton* and within this, the species *Trichophyton rubrum*, one of the more adapted to humans [29], being the most common cause of tinea pedis, nail infection, tinea cruris, and tinea corporis world-wide [28]. Drakensjö and Chryssanthou [9] in study conducted in Sweden in the County of Stockholm showed that *T. rubrum* was the predominant pathogen isolated from these cases (83%). In a study by Aquino et al. [2] in Porto Alegre, Brazil, with 5077 samples, 2033 were positive for dermatophytes, in which 62.2% were from *T. rubrum*.

The increase of the incidence of fungi infections has become one of the main problems in the therapeutics. In the medical practice the availability of antifungal agents is relatively small, sometimes inefficient and most of them present certain toxicity. Besides the growth of those infections, the problem of the microbial resistance also showed an accentuated increase [12]. All these reasons have motivated the search of new antifungal or targets for this action.

Plants are a large source of new bioactive compounds with therapeutic potential. Medicinal plants have been used for several purposes including its use as antimicrobial agents and they have been presented inhibition of fungi growth [18]. Only a small percentage of living plants on earth have been phytochemically investigated. Thus, plants are an enormous reservoir of pharmaceutically valuable molecules to be discovered [3,13]. Based on the great biodiversity of the Brazilian flora, there is significant interest in species with pharmacologic potential.

Pothomorphe umbellata (L.) Miq. (Piperaceae), a plant belonging to the Brazilian flora, is a small green shrub erect, perennial, very ramified, with articulate stems, with 1.0 to 2.5 m of height, presenting wide leaves, with pleated bases seeming peltate, with 15 to 23 cm of length. The species produces small and discreet flowers, of cream-greenish color, joined in tall axillaries inflorescences of 4—8 cm of length and multiples seeds. The species belongs to the group of the sciophyte, a plant of obligatory shadow [19]. It can be found growing spontaneously from the South of Brazil to the Amazonas, mainly in the states of São Paulo, Minas Gerais, Espírito Santo and south of Bahia.

Phytochemical studies on aerial parts demonstrated the presence of ß-sitosterol, stigmasterol and campesterol [27]. In essential oil were identified, several compounds as spathulenol, caryophyllene, caryophyllene oxide germacrene D, bicyclogermacrene, β-elemene, β-pinene, α-cadinol, δ-cadinene, α-copaene, limonene, linanool, E-nerolidol [22,25]. Baldoqui et al. [5] isolated in the hexane extract of *P. umbellata* 4-nerolidylcatechol, 4-5-hydroxy-7, 3′, 4′-trimethoxy-flavone and sesamin (lignan). Among demonstrated effects there is the antimicrobial action to *Helicobacter pylori* [14], *Mycobacterium tuberculosis* [15] and anti-inflammatory property [24]. In the popular medicine it is used for swelling in the legs, fever, headaches and rheumatism [19].

The objective of this study was to evaluate the action of extracts and fractions of *P. umbellata* on resistant strains of *T. rubrum* in vitro using the microdilution plate method supported by bioguided analysis.

Material and methods

Plant extract

The aerial parts of *P. umbellata* were collected, 15 December 2005, in the experimental field of the Plant Biotechnology Unity at the ''Universidade de Ribeirão Preto - UNAERP'' in Ribeirão Preto, São Paulo, Brazil. A voucher specimen was deposited under number HPMU-831 in the Herbarium of Medicinal Plants of ''Universidade de Ribeirão Preto - UNAERP''. The aerial parts of the plant were dried in greenhouse at 42 °C and powdered in mill knives. The plant material (PM) was extracted by maceration separately with methanol, chloroform (800 g of PM) and ethanol/water (7:3, v/v) named 70% ethanolic extract (500 g of PM). The extracts were filtered, concentrated and dried. The yields of extraction (w/w) were 15.0, 8.1 and 9.5%, respectively.