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Antifungal activity in plants from Chinese traditional and folk medicine

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ABSTRACT

Ethnopharmacological relevance: From over 100 Chinese clinical trial publications, we retrieved 22 commercial preparations and 17 clinical prescriptions used as Traditional Chinese Medicine (TCM) for treating mycotic vaginitis, typically caused by *Candida albicans*. The 8 most frequently used plants as well as another 7 TCM and 18 folk medicinal plants used in the South of China for antifungal therapy were investigated for *in vitro* antifungal activity.

Materials and methods: For each plant we tested 4 extracts prepared with different solvents (water, ethanol, acetone, and n-hexane) for inhibition of *Candida albicans* and *Saccharomyces cerevisiae* growth in liquid culture.

Results: Some plants have quite strong antifungal activity, such as Tujinpi (*Pseudolarix kaempferi* Gord.), of which each extract could significantly inhibit the growth of both tested fungi. In addition, the acetone extract of Kushen (*Sophora flavescens* Ait.), the ethanol, acetone, and hexane extracts of Guanghuoxiang (*Pogostemon cablin* (Blanco) Benth.) and Gaoliangjiang (*Alpinia officinarum* Hance), the hexane extract of Dingxiang (*Eugenia caryophyllata* Thunb.), and the ethanol and acetone extracts of Kulianpi (*Melia toosendan* Sieb. et Zucc.) and Laliao (*Polygonum hydropiper* L.), all inhibited *Candida albicans* growth by more than 50%. In some cases growth inhibition was even comparable to that by the clinically used antifungal miconazole, which we used as our positive control.

Conclusions: The majority of plants, whose clinical use for antifungal treatment is well supported within TCM or Chinese folk medicine, show *in vitro* antifungal activity against *Candida albicans*. Since *Candida* species represent the most common fungal pathogen of humans, these results provide more scientific evidence supporting the clinical application of these plants, and can serve as a starting point for new drug discovery from TCM and Chinese folk medicine.

1. Introduction

Traditional Chinese Medicine (TCM), with its clinical practice for thousands of years in China and the wider Eastern Asian area, continues to play a significant role in the Chinese health care system even today. Extracting and isolating bio-active compounds from TCM have also proved an attractive strategy for drug discovery. In China many plants are still used as folk medicine to treat various diseases. Especially in Southern China, where the weather is warm and humid, fungal infections are common. Numerous plants are used there by the inhabitants as folk medicines to treat fungal infections. After use for many decades or even centuries, the efficacy of some TCM preparations, prescriptions and folk medicine has been firmly established.

Recently, Tripathi et al. (2008) screened 26 essential oils against *Botrytis cinerea* (a necrotrophic fungus that affects many plant species) and 10 oils were found to have absolute fungitoxic activity (*i.e.* 100% growth inhibition). Zhou and Li (2008) investigated the *in vitro* antifungal activity of 23 kinds of TCM against *Microsporum gypseum* and *Trichophyton rubrum*, 2 fungi from the genera that constitute the main cause of superficial dermatophytoses, and some TCM showed strong antifungal activity against one or several species of these fungi. Gong et al. (2002) screened for antifungal activity against *Candida albicans* (Robin) Berkh.,

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Saccharomyces cerevisiae Hansen GL-7, and *Prototheca wickerhamii* in the 95% ethanol extracts of 164 plants used as TCM in China, and the extracts from 22 plants exhibited potent antifungal activity. From *Alpinia officinarum* Hance, Gui et al. (2005) reported that the volatile oil from its rhizome showed strong *in vitro* antifungal effects against 16 strains of dermatophytes. Some active components such as flavones, alkaloids (Rao et al., 2009), volatile oils (Zhang et al., 2009; Gong et al., 2009; Yang et al., 1996), phenols (Guha et al., 2005), and resins (Wang et al., 1995), as well as metabolites from endophytic fungi (Wang et al., 2010; Li et al., 2005; Huang et al., 2001) have been isolated and identified, which have strong potential for development as clinical drugs.

Although there are many reports of antifungal activity in TCM preparations, few systematic surveys exist, and those mostly neglect the rich tradition of folk medicine in China. Moreover, many surveys do not take full advantage of evidence of clinical efficacy when selecting their plants. Based on published clinical trial data as well as advice from TCM practitioners, we selected 33 TCM and Chinese folk medicine plants, whose efficacy for the treatment of mycoses was strongly supported, for a systematic study of *in vitro* antifungal activity.

The objective of the present paper is therefore to evaluate the *in vitro* antifungal activities of these 33 plants in a systematic and consistent manner. The results can provide more scientific evidence supporting the clinical application of these plants and can serve as a starting point for new drug discovery from TCM and Chinese folk medicine. Since *Candida albicans* remains the most frequent cause of fungal infections in an expanding population of immunocompromised patients, and candidiasis is now the third most common infection in US hospitals (Pierce et al., 2010), this species was employed in the present study. In addition, the model organism *Saccharomyces cerevisiae* was used as well to confirm or compare the activity so that later mechanistic studies would be facilitated.

2. Methods and materials

2.1. Chemicals and reagents

Absolute ethanol (SZBA0290) and acetone (S76776-259) of analytical grade were purchased from Sigma-Aldrich Co. (Bornem, Belgium). N-hexane (09953580) of analytical grade was purchased from Acros Co. (Acros, Belgium). Sterile deionised water was produced by a water purification system (Milli-Q Reagent Water System, MA, USA). Yeast extract and peptone were purchased from Lab M Ltd. (Lancashire, UK). Dextrose and glucose were purchased from Fluka—Sigma-Aldrich (MO, US). Yeast nitrogen base without amino acids and complete synthetic medium were purchased from BD Diagnostics (Sparks, MD, US).

2.2. Botanical materials

The information on these materials such as the Chinese (Pinyin) and official (Latin) taxonomic name (according to the International Plant Names Index), medicinal part, origin (Chinese province where the plant was collected), and batch number is shown in Table 1. All the botanical materials were identified according to the Chinese Pharmacopeia (2005 version) or the local Flora, such as Flora of Guangdong (volume 1–9, Guangdong Science and Technology Press, 1987–2009) and Flora Yunnanica (volume 1–16, Science Press, 1977–2006).

2.3. Preparation of the extract samples for antifungal test

The dried raw botanical material was ground to a fine powder. Five gram of powder was transferred into each of four 100 mL flasks, and 50 mL of sterile water, absolute ethanol, acetone, and hexane were added, respectively. The flasks were left standing for 24 h at ambient temperature. The flasks were then placed in a bath sonicator for 4 times 15 min each. After each sonication, there was an interval of 15 min to let the suspension cool to ambient temperature. Each sample was transferred into a 100 mL polyvinyl chloride bottle and centrifugated at 530 *g* (2000 rpm) for 20 min. The supernatant was transferred to a fresh bottle. One millilitre was transferred into a 2.5 mL polyvinyl chloride tube and the solvent was evaporated in a Savant Speedvac Concentrator (SVC 200H, Stratech Scientific, London, UK). For the sample extracted with water, the residue was re-dissolved in 0.5 mL of water using a vortex shaker. For the samples extracted with organic solvents (ethanol, acetone, and hexane), the residue was dissolved in 0.5 mL of DMSO. The samples were stored at 4 °C till the antifungal test.

2.4. Antifungal test

2.4.1. Fungal strains

Candida albicans (SC5314) (American Type Culture Collection, Manassas, Virginia, USA) (Fonzi and Irwin, 1993) and *Saccharomyces cerevisiae* (BY4741) (Invitrogen, Merelbeke, Belgium) were used for the antifungal test. The colonies were inoculated on agar plates and stored in a cold room (4 °C).

2.4.2. Preparation of pre-culture

A single colony of *Candida albicans* and *Saccharomyces cerevisiae* was inoculated from an agar plate in 5 mL of YPD medium (1% yeast extract, 2% peptone, and 2% dextrose) in a reaction tube under aseptic conditions. The reaction tubes were incubated overnight while shaking at 200 rpm at 30 °C.

2.4.3. Preparation of minimal medium (MM)

MM was prepared according to the following formulation (for 1 L medium): 100 mL of yeast nitrogen base without amino acids (6.7 g/100 mL), 100 mL of complete synthetic medium (0.8 g/100 mL), 50 mL of glucose (40 g/100 mL), and 750 mL of demineralised H_2O. All the ingredients were prepared separately and mixed after sterilisation by autoclave, except for the glucose stock which was filter (0.45 μm) sterilised.

2.4.4. Antifungal test

Twenty microlitre of the test sample was transferred into the wells of a 96-well plate, as well as the positive control (miconazole, 2 mg/mL in DMSO) and blank (solvent) controls (DMSO and water). All the samples were diluted with 20 μL H_2O. Five microlitre of each diluted sample was transferred into another 96-well plate. Forty and 100 μL of a *Candida albicans* or *Saccharomyces cerevisiae* pre-culture, respectively, were diluted in 10 mL of MM. Ninety five microlitre of this suspension was added to the wells with the test sample, yielding a final volume of 100 μL. The plate was incubated for 24 h, at 37 °C for *Candida albicans* and 30 °C for *Saccharomyces cerevisiae*. The OD was then measured at a wavelength of 595 nm. The test was carried out in duplicate and the average OD value was calculated. The relative inhibition (%) of the test sample was calculated by dividing the OD value of the test sample by the average OD of the solvent control, and multiplying by 100.