SCREENING FOR ANTIYEAST ACTIVITIES FROM SELECTED MEDICINAL PLANTS

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DARAH, I., JAIN, K., SURAYA, S., LIM, S. H., HAZARINA, N. & SITI NOOR ADNALIZAWATI, A. 2006. Screening for antiyeast activities from selected medicinal plants. Plants synthesize a vast array of secondary metabolites that are gaining importance for their biotechnological applications. Methanolic extracts of 22 medicinal plants in Malaysia were tested for their antifungal activities against four species of pathogenic yeasts, namely, *Candida albicans, Rhodotorula rubra, Cryptococcus neoformans* and *Torulopsis glabrata*. The extracts studied displayed remarkable and significant antifungal activities against *C. albicans, R. rubra* and *T. glabrata* but not against *C. neoformans*.

Keywords: Antifungal activity, methanolic extracts, yeasts, pathogenic yeasts, Malaysia

DARAH, I., JAIN, K., SURAYA, S., LIM, S. H., HAZARINA, N. & SITI NOOR ADNALIZAWATI, A. 2006. Menyaring aktiviti antiyis daripada tumbuhan ubatan terpilih. Tumbuhan mensintesis banyak metabolit sekunder yang penting untuk kegunaan bioteknologi. Aktiviti antikulat ekstrak metanol daripada 22 tumbuhan ubatan Malaysia telah diuji keberkesanannya terhadap empat spesies yis patogen iaitu *Candida albicans, Rhodotorula rubra, Cryptococcus neoformans* dan *Torulopsis glabrata*. Kesemua ekstrak 22 tumbuhan yang diuji mempamerkan aktiviti antikulat yang menakjubkan dan signifikan terhadap *C. albicans, R. rubra* dan *T. glabrata* tetapi tidak terhadap *C. neoformans*.

INTRODUCTION

The incidence of systemic candidiasis infections caused by pathogenic yeasts especially *Candida* spp. has increased considerably in the past few decades. This is mainly due to the increased number of immunocompromised-patients as a result of new and more aggressive therapies in the treatment of cancer and tumour. Other factors are the increase of immunosuppressive drugs and number of organ transplant recipients. Studies of AIDS all over the world show that about 58–81% of all patients contract a fungal infection especially candidiasis (Motsei *et. al.* 2003) at some time during the primordial stage or after developing AIDS. On top of that about 10–20% of the patients died as a direct consequence of fungal infections (Drouhent & Dupont 1989). These facts coupled with the resistance to antifungals and with the toxicity during prolonged treatment using several antifungal drugs have been reasons for an extended search for newer drugs to treat candidiasis.

Although there are several synthetic- and natural product-based drugs available for treating candidiasis, they are not consistently effective against pathogenic yeast infections. Furthermore, the development of resistance in fungi against most of the drugs has been reported (Sanglard 2002).

Medicinal plants constitute an important source of bioactive compounds and the use of medicinal plants in the treatment of infections is an age-old practice. Plants produce many types of secondary metabolites; many of them with antifungal activities. Examples of these compounds include flavonoids, phenolics and phenolic glycosides, unsaturated lactones, sulphur compounds, saponins, cyanogenic glycosides and glucosinolates (Cowan 1999). In many cases, these substances serve as plant defense mechanisms against predation by microorganisms, insects and herbivores.

This paper reports the *in vitro* antifungal

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screening of crude extracts of 22 medicinal plants against four species of human pathogenic yeasts. The plant species selected are commonly used as traditional medicines in Malaysia for the treatment of candidiasis.

MATERIALS AND METHODS

Plant materials

All plant materials were collected from their natural habitat around Penang Island, Malaysia. Voucher specimens have been deposited at the Herbarium of the School of Biological Sciences, Universiti Sains Malaysia, Penang. All plant materials were washed thoroughly under running tap water and oven dried at 50 °C for two to three days. The selection of plant parts was based on the local traditional medicinal practices where leaves were mainly used. However, for *Thyphonium flagelliforme* the whole plant was used.

Extraction of plant materials

Dried, powdered plant materials were successively extracted using a Soxhlet apparatus with 95% methanol as solvent, at 55 °C for three hours. The extract was then evaporated to dryness in a rotary evaporator under reduced pressure at a temperature of 40 °C until an oily dark paste was formed. A stock solution containing 100 mg ml^{-1} (w/v) extract was prepared in 95% methanol, sterilized using membrane filter (pore size 0.47 µm, Millipore) and then diluted in sterile distilled water.

Yeast cultures

Clinical isolates of pathogenic yeasts (*Candida albicans, Cryptococcus neoformans, Rhodotorula rubra* and *Torulopsis glabrata*) used in this study were obtained from the University Hospital, School of Medical Sciences, Universiti Sains Malaysia Health Campus, Kelantan, Malaysia. Cultures were grown and maintained on Sabouraud glucose agar slopes at 37 °C for 24–48 hours.

Antifungal activity test

Antifungal activity tests were performed using agar diffusion method. One milliliter of yeast cell suspension (1×10^6 cells ml^{-1}) from the 24-hour-old cultures was incorporated into 15 ml molten Sabouraud glucose agar maintained at 45 °C. The agar was then poured into sterile Petri plates and allowed to solidify at room temperature. Extract impregnated disc (20 µl of extract per disc) 100 mg ml^{-1} at a concentration of was placed on the agar surface and incubated at 37 °C for 24–48 hours. Sterile distilled water and methanol were used as controls. Antifungal activity was indicated by a clear zone of growth inhibition formed around the disc. For comparative purposes, ketoconazole (5 µg ml^{-1}) was used as a positive control. The experiments were carried out in six replicates.

Effects of extract on *Candida albicans* cells

Little information is available on the structure of cells after being treated with the extracts. Thus, we performed a series of additional investigations. The Petri plate which showed a clear zone of inhibition was selected to study the effects of extract on *C. albicans* cells. A 1×1 cm agar block was cut and withdrawn from separate sections of the *C. albicans* culture plate. The first section was from the clear inhibition zone, the second from the border between the edge of the clear and the growth zones, and the third, from the growth zone only (Figure 1). The agar blocks were then fixed and observed under a scanning electron microscope (SEM). Cell identification was according to Borgers *et al.* (1989).

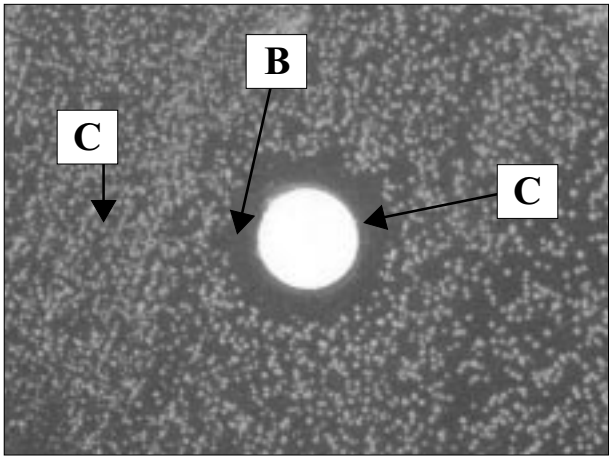

Figure 1 Sampling sections on the agar diffusion for investigation under scanning electron microscope—A: clear inhibition zone; B: border between the edge of the clear and growth zones; and C: growth zone

RESULTS

Positive results of antiyeast activities of the plants used in this study were indicated by the presence of clear zones around extract-impregnated discs. All of the methanolic extracts used showed significant antifungal activity against *C. albicans, R. rubra* and *T. glabrata* (Table 1). However, there was no significant antifungal activity showed by any of the extracts against *C. neoformans*.

From the SEM micrographs, we could see a few collapsed cells of *C. albicans* on the clear inhibition zone area (Figure 2a). Micrographs of the inner part of the border area, which was at the edge of the clear zone, showed that the *C. albicans* cells were slightly altered and elongated and smaller in size compared with the control (Figures 2b and c). However, cells from the growth area showed typical, oval shape cells of *C. albicans* (Figure 2c). Distinct cells were observed with the presence of buddings which emerged from the surface of mother cells. These buds were seen as spherical or slightly elongated cells.

DISCUSSION

The predominant factor that has stimulated the search for safer and more effective antifungal agents has been the increasing evidence of systemic mycoses in immunocompromised patients and the unfortunate incidence of certain strains of *C. albicans* becoming resistant to certain antifungal. Hence, there is a need for developing wider variety of antifungal agents for the treatment of fungal diseases.

Search and screening programmes for naturally occurring compounds with antifungal activity revealed that many plant species possess antifungal properties. This explains the use of the plants in traditional medicine for treatment of various diseases which symptoms may involve fungal, especially yeast infections. The results of this study also underline the importance of ethnobotanical approach for selection of plants in the discovery of new bioactive or lead compounds. However, further phytochemical research is needed to identify the active principles responsible for antifungal effects of some of the medicinal plants studied.

Based on the electron micrographs shown, it is clear that the extracts are able to kill yeast cells by causing lysis on the growing cells. Cells from the clear inhibition zone had undergone autolysis due to potency of the extract. In principle, cell lysis may be caused either by interference with cell membrane function or by disturbance of the delicate balance between synthesis and

Table 1 Growth inhibition of methanolic extracts against pathogenic yeasts

Botanical name	Family	Used parts	CA	CN	RR	TG	Keto
Carica papaya Linn.	Caricaceae	Leaves	+	_	+	+	+
Cassia alata Linn.	Leguminosae	Leaves	+	_	+	+	+
Cinnamomun zeylanicum Blume.	Lauraceae	Leaves	+	_	+	+	+
Costus specious Koenig.	Zingiberaceae	Leaves	+	_	+	+	+
Curculigo latifolia Dryand.	Hypoxidaceae	Root	+	_	+	+	+
Drymaglossum piloselloides Linn.	Polupodiaceae	Leaves	+	_	+	+	+
Elettariopsis triloba Gagneb.	Zingiberaceae	Leaves	+	_	+	+	+
Chromolaena odorata Linn.	Compositae	Leaves	+	_	+	+	+
(*Eupatorium odoratum*)	Araceae	Leaves	+	_	+	+	+
Homalomena rubra Hassk.	Melastomaceae	Leaves	+	_	+	+	+
Melastoma malabathricum Linn.	Rutaceae	Leaves	+	_	+	+	+
Micromelum pubescens Blume	Rutaceae	Leaves	+	_	+	+	+
Murraya koenigii Linn.	Acanthaceae	Leaves	+	_	+	+	+
Orthosiphon grandiflores Bold.	Zingiberaceae	Leaves	+	_	+	+	+
Phaemoria imperialis	Piperaceae	Leaves	+	_	+	+	+
Piper caninum Blume	Piperaceae	Leaves	+	_	+	+	+
Piper nigrum Linn.	Piperaceae	Leaves	+	_	+	+	+
Piper sarmentosum Roxb.	Piperaceae	Leaves	+	_	+	+	+
Piper spPyrossia lanceolata	Cyatheaceae	Leaves	+	_	+	+	+
Rhinacanthus nasutus Linn.	Acanthaceae	Leaves	+	_	+	+	+
Thyphonium flagelliforme Lodd.	Menispermaceae	Whole plant	+	_	+	+	+
Tinospora crispa Linn.	Menispremaceae	Stem	+	_	+	+	+

CA: *Candida albicans*; CN: *Cryptococcus neoformans*; RR: *Rhodotorula rubra*; TG: *Torulopsis glabrata*; Keto: Ketoconazol; -: no inhibition zone formed; +: inhibition zone formed

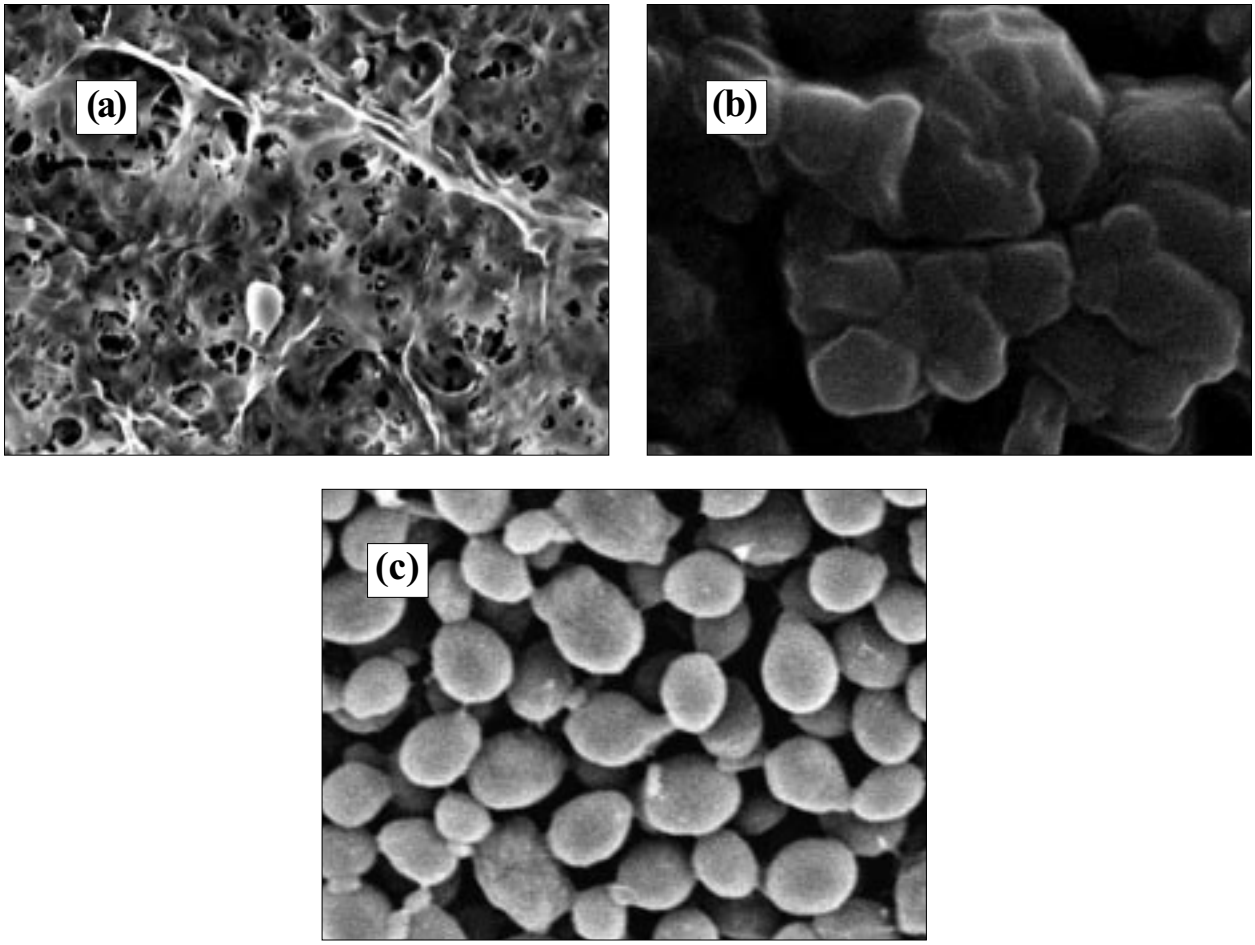

Figure 2 Micrographs of the sampling sections: (a) clear growth inhibition zone; (b) border between the inhibited and the non-inhibited areas; and (c) good growth area without any effect of the extract, and serves as a control. (Magnification ×8500)

degradation of compounds involved in the syntheses of cell membrane and cell wall (Long *et al.* 1998). In this study, the latter could be a result of exposure to the extracts. The majority of natural antifungal and antiyeast agents act on sterols and other compounds located in the cell membrane (Sanglard 2002). In fact most microorganisms which contain sterols are susceptible to these agents. However, other plants may involve other mechanisms such as interaction with lipophilic compounds and proteins (Mason & Wassermann 1987, Cowan 1999). This study showed that the extracts were not effective against *C. neoformans*. This could be due to the presence of capsule surrounding its cell that blocks the penetration of extract. Polysaccharide capsules in pathogen can protect cells from adverse conditions (Long *et al.* 1998).

Plants have an almost limitless ability to synthesize aromatic compounds, most of which are phenolics and polyphenols, terpenes and terpenoids, alkaloids, lectins and polypeptides, and other compounds such as polyamines, isothiocyamates, thiosulfinates and glucosides with various mechanism of actions including disrupting the cell membranes. The results of the present work indicated that plant extract investigated possessed antiyeast properties. Thus the extracts are suitable to be used to treat yeast infections especially those caused by *C. albicans, T. glabrata* and *R. rubra*. The extracts could be an important source of biologically active lead compounds useful for developing new antiyeast drugs.

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