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SHORT COMMUNICATION

The Effects of Xanthorrhizol on the Morphology of *Candida* Cells Examined by Scanning Electron Microscopy

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The effects of xanthorrhizol, a natural anticandidal agent isolated from the rhizome of temulawak or java turmeric (*Curcuma xanthorrhiza* Roxb.) on the morphology of four human pathogenic *Candida* species, i.e., *C. albicans*, *C. glabrata, C. guilliermondii*, and *C. parapsilosis* was examined by scanning electron microscopy (SEM). The SEM analysis showed that, unlike control cells representing normal oval to spherical with smooth surface, treatment of *Candida* strains with xanthorrhizol at 1 x MICs (minimum inhibitory concentration) significantly affected the external morphology, exhibiting deformation, and protrusions on the cell surface. The potent anticandidal activity of xanthorrhizol may support the use of medicinal plants for the treatment of candidal infections.

Key words: anticandidal, *Candida* sp., scanning electron microscopy, xanthorrhizol

The incidence of invasive fungal infections, particularly those caused by *Candida* sp., has increased over the past few decades (Hsueh *et al.* 2005). Recently, strains of *Candida* sp., such as *C. albicans*, *C. glabrata*, *C. guilliermondii*, and *C. parapsilosis* are showing increased resistance to traditional antifungal agents (Hawser and Dauglas 1995; Nguyen *et al.* 1996; Barchiesi *et al.* 1999; Dauglas 2003). This demonstrates the great importance of identifying novel antifungal agents (Ficker *et al*. 2003). Recent years have seen a growing interest in the use of natural antifungal agents isolated from the medicinal plants.

Xanthorrhizol, a novel bioactive compound isolated from the rhizome of an indigenous Indonesian medicinal plant, temulawak or java turmeric (*Curcuma xanthorrhiza* Roxb.) has been previously reported to possess an antibacterial activity against several oral pathogens (Hwang *et al*. 2000a, 2000b) and has the ability to prevent and remove *Streptococcus mutans* biofilm formation (Rukayadi and Hwang 2006a, 2006b, 2006c). Xanthorrhizol also has anticandidal activity (Rukayadi *et al*. 2006), anti-*Malassezia* activity (Rukayadi and Hwang 2007a) and antimycotic activity against opportunistic filamentous fungi (Rukayadi and Hwang 2007b). This short communication reports the effect of xanthorrhizol on the morphology of *Candida* cells examined using the scanning electron microscopy (SEM).

The *Candida* strains (*C. albicans* ATCC 10231, *C. glabrata* ATCC 50044, *C. guillerimondii* ATCC 9058, and *C. parapsilosis* ATCC 22019), used in this study, were obtained from the American Type Culture Collection (Rockville, MD, USA). The strains were cultured on Sabouraud dextrose broth (SDB) or Sabouraud dextrose agar (SDA) (Difco, Becton Dickinson and Company, USA) for 48 h at 35 °C. The standardized inoculum (a McFarland standard) for each isolate used was 5×10^6 cfu ml^{-1} (NCCLS 2002).

Xanthorrhizol (Fig 1) (Hwang *et al*. 2000a) was isolated from the ethyl acetate fraction of the methanol extract from *Curcuma xanthorrhiza* according to the method of Hwang *et al*. (2000a). Briefly, the rhizomes of *C. xanthorrhiza* (100 g) were ground and extracted with 75% MeOH (v/v; 400 ml), and further fractioned consecutively with ethyl acetate (4.8 g), n-butanol (1.7 g), and water (1.1 g). Xanthorrhizol was isolated from the ethyl acetate fraction using silica-gel-column chromatography (Merk; 70-230 mesh; 5 x 43 cm; n-hexane/ethyl acetate, 10:1) yielding 0.2 g. Xanthorrhizol was dissolved in 10% (vol/vol) dimethylsulfoxide (DMSO) to obtain 1 mg ml^{-1} stock solutions. DMSO at 10% (vol/vol) was found not to kill *Candida*.

Samples for scanning electron microscopy were provided as follows: 10 ml of cultures of *C. albicans*, *C. glabrata*, *C. guilliermondii*, and *C. parapsilosis* were exposed to 1 x MICs of xanthorrhizol (15, 10, 8, and 25 µg ml^{-1}, respectively) (Rukayadi *et al*. 2006). After 1 hour of incubation at 35 °C, 1 ml of each culture, and a negative control of a corresponding culture were aliquoted and centrifuged at 3,900 x g for 10 min. Cell pellets were resuspended in 10 ml of sterile water and fixed overnight in 4% (vol/vol) glutaraldehyde. The samples were washed twice with 2-ml portions of sterile water and centrifuged at 3,900 x g for 10

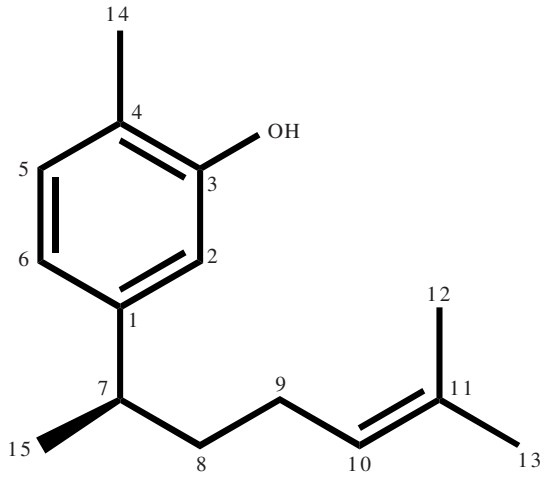

Fig 1 Structure of xanthorrhizol (Hwang *et al*. 2000a).

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min. The final pellets were then resuspended in sterile water. A drop of each suspension was transferred onto glass cover slips and fixed onto aluminium SEM stubs (Agar Science Ltd, Standstead, UK). The drop was spread thinly on the slip and dried in the air for 2 hour at room temperature. Graded concentrations of ethanol (70, 80, 90, and 95%, vol/vol), were applied, 2-5 min each, to ensure complete dehydration of specimens. The specimens were coated with gold in a low-pressure argon atmosphere employing a model E5000 Polaron Sputter Coating Unit (Polaron Equipment Ltd, New Haven, West Sussex, UK). A JEOL JSM-840 scanning electron microscope (Jeol Technics Ltd, Tokyo, Japan) was used to evaluate samples, operating at accelerating voltages of 20-25 kV (Helal *et al*. 2006).

SEM analysis showed that, unlike control cells (antifungal-agent free) showing normal oval to spherical shapes with smooth surfaces, treatment of *Candida* species with MIC of xanthorrhizol affected the external morphology of these yeasts (Fig 2). Control cells displayed well-formed cells with smooth unadulterated surface (Fig 2a, c, e, g). In contrast, cells incubated in the presence of xanthorrhizol demonstrated a greater tendency to clump compared with the control cultures (e.g., *C. albicans* - Fig 2b). Xanthorrhizol-treated *C. glabrata* cells showed minor abnormalities without a smooth or a slightly awkward surface (Fig 2d). Xanthorrhizol-treated *Candida* cells exhibited deformation and protrusions on the cell surface, which was more clearly demonstrated with *C. guilliermondii* and *C. parapsilosis* (Fig 2f, h).

Electron micrographs revealed the existence of a recognizable affected external morphology of *Candida* cells caused by xanthorrhizol. Visible deformation, protrusion, or clumping was noted for each species at concentration MICs for 1 h treatment. In general, *Candida* exposed to xanthorrhizol at concentrations 1 x MICs exhibited substantial ultrastructural abnormalities such as shape deformation, protrusion, rugged cells surface, and clumping. Although, we were not able to identify the underlying molecular changes caused by the compounds by scanning electron microscope after 1 h treatment, we were able to show that the observable cell wall changes were generally obtained following exposure of the isolates to concentrations of xanthorrhizol equal to 1 x MICs. Analysis of electron micrograph at the appropriate exposure time and higher concentrations (2 x MICs or 4 x MICs) may result in more detailed analyses of the activities and effects of antifungal agents (Klepser *et al*. 1998). Further studies have been conducted examining the effect of xanthorrhizol on the morphology *Candida* cells at 2 x MICs and 4 x MICs for 2 and 4 h of incubation.

In summary, the potent anticandidal action of xanthorrhizol against strains of four human pathogenic *Candida* species was demonstrated by scanning electron microscopy analysis. The results showed the usefulness of xanthorrhizol, a promising new antifungal agent for the topical treatment of candidiasis.

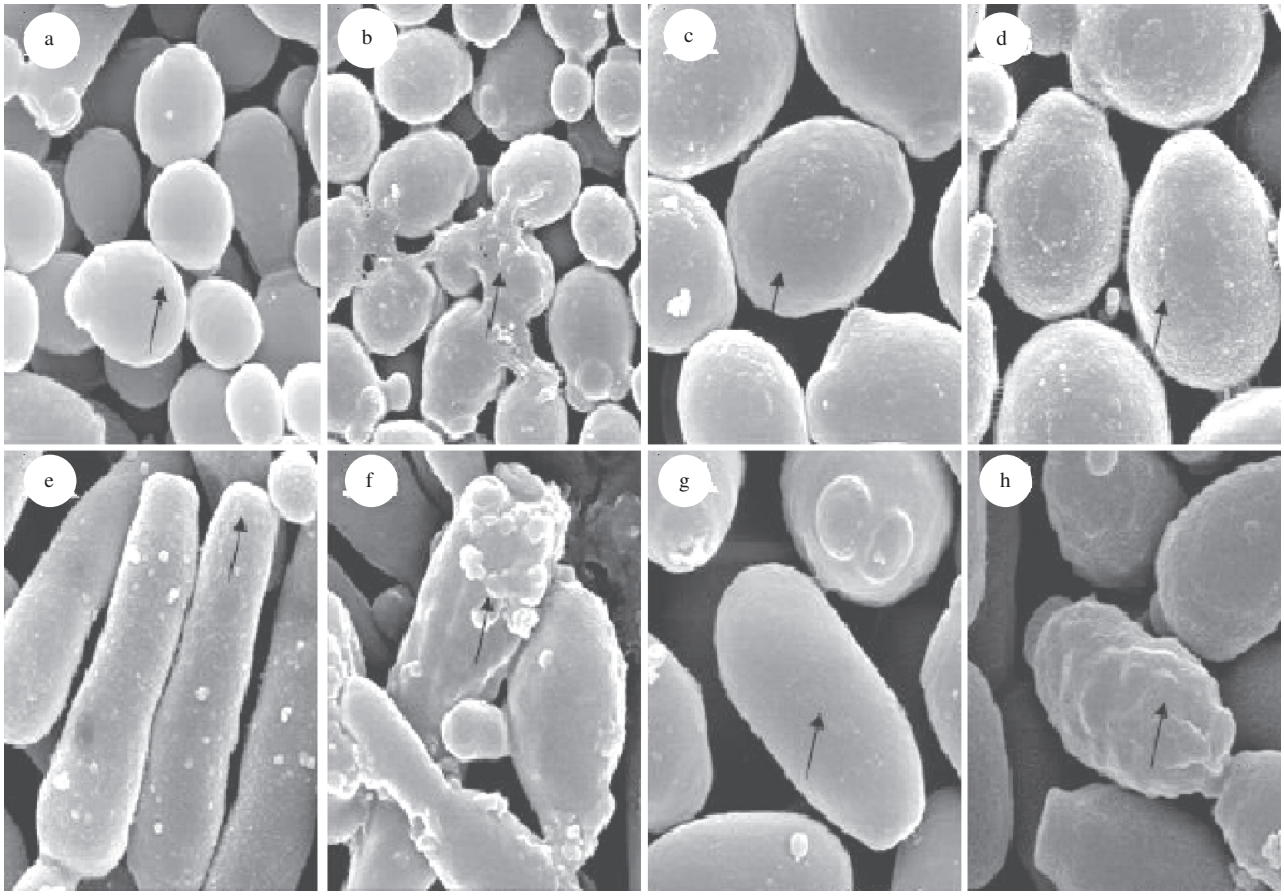

Fig 2 SEM of *Candida albicans* (a and b), *C. glabrata* (c and d), *C. guilliermondii* (e and f), and *C. parapsilosis* (g and h) after treating by xanthorrhizol at 1 x MIC for 1 h of incubation.

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