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SOCIAL SCIENCES

Foreword

This is the first time that ESTEEM Academic Journal UiTM Pulau Pinang has come up with 2 publications in a year! Previously, ESTEEM was published once biennially.

For these publications to materialise, I would like to thank Associate Professor Mohd Zaki Abdullah, the Director of UiTM Pulau Pinang for his unflinching support and who always told me, "Go ahead, don't worry about the money!".

Both the Associate Professor Mohd Zaki Abdullah and Dr. Mohamad Abdullah Hemdi, the Deputy Director of Academic Affairs really provided me with a great deal of assistance in ensuring that there are sufficient articles for publishing. Both of them have emphasized the need for lecturers to embark on journal writing. Incidentally this is one of the prerequisites for promotion among the academic staff members of UiTM Pulau Pinang.

I do not think I can run the show alone without the help from the editorial board, reviewers and the cooperation from University Publication Centre (UPENA) of UiTM Malaysia. My special thanks to Mr. Mohd Aminudin Murad for his efficiency in editing articles and to Dr. Khairil Iskandar Othman for speeding up the final stage of printing process.

Since writing is an important criterion in rating a university, I feel it is a great responsibility for me to produce a good journal. Fellow colleagues, let's work closely to put UiTM Pulau Pinang in the final list of Anugerah Kualiti Naib Canselor (AKNC) and Anugerah Kualiti Perdana Menteri (AKPM) by submitting more quality articles to ESTEEM!

Lastly, let me end by thanking all of you for giving your unwavering support to UPENA.

The Chief Editor
November, 2008

ESTEEM, Vol. 4, No. 2, 2008, 31–38

The Structural Modifications of *Candida albicans* Cells After Treatment with *Cinnamomum zeylanicum* Blume Crude Extract

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Siti Nurdijati Baharuddin

ABSTRACT

The fungal species Candida albicans is an opportunistic pathogens which causes serious infections in humans, particularly in immunocompromised patients. Cinnamomum zeylanicum (cinnamon) or locally known as "kayu manis" has been reported to possess potent antifungal activity. Thus, this study evaluated the effects of C. zeylanicum chloroform extract on cell morphology of C. albicans by observing the extract-treated yeast cells through scanning electron microscopy (SEM). Candida albicans cells morphology was observed to be lysed after 48 hours at various time intervals of exposure to the extract. The results indicated that the severity effect of cinnamon extract on the yeast cells was dependent on the time of exposure, and that the C. zeylanicum can inhibit filamentous growth of C. albicans cells. Electronic microscopy observations revealed that the sodium chloride (NaCl) crystals were probably primarily produced due to the extract-yeast reactions, which then contributing to the cells death. It is concluded that the cytoplasmic membrane is involved in the toxic action of the cinnamon crude extract.

Keywords: *Candida albicans, Cinnamomum zeylanicum, SEM, cells morphology, antifungal activity*

Introduction

Cinnamomum zeylanicum Blume belong to Lauraceae family, has showed many biological properties as analgesic, antiseptic, antispasmodic, aphrodisiac, astringent, carminative, insecticide, and parasiticide. Its branch peel without the epidermis and subereous layer is marketed as the commercial cinnamon, which has been used a long in perfumery, culinary and native medicine systems (López Diaz, González, Moreno,

& Otero, 2002). *Cinnamomum zeylanicum* was the most sought after spice in Europe from the 16th to the 18th century. The commercial products of cinnamon are derived from its bark and leaves (Indu Bala & Ng, 2000). In Malaysia, the bark, both in the whole and in the ground form, is used in domestic culinary especially for its flavoring properties. Other than its culinary purposes, oil obtained from the cinnamon bark is also popularly used in flavoring, perfumery, and in dental and pharmaceutical products. Cinnamon is high in antioxidant activity (Shan, Cai, Sun, & Corke, 2005) and also believed to possess some antifungal properties (Indu Bala & Ng, 2000). The essential oil of cinnamon also has antimicrobial properties (López, Sánchez, Batlle, & Nerín, 2005), which aid in the preservation of certain foods.

However, there is lacking of *in vitro* investigations conducted on the effects of the *C. zeylanicum* crude extract against yeast cells. Therefore, in this communication we would like to highlight the effects of the stem bark extract of *C. zeylanicum* on *C. albicans*, a pathogenic yeast cells, which can caused various serious diseases in man and animals. *Candida albicans* is an opportunistic pathogen that can cause local and systemic infections in predisposed persons, commonly affecting immunologically compromised patients and those undergoing prolonged antibiotic treatment (Pfaller & Yu, 2001).

Materials and Methods

Preparation of Cinnamon Extract

The stem barks of *C. zeylanicum* were purchased from the wet market in Penang Island. The clean, dried, and finely chopped materials of 100 g were then extracted with 250 ml of chloroform ($CHCl_3$) using the Soxhlet Apparatus. The crude extract was dried using a rotary evaporator.

Test Organisms

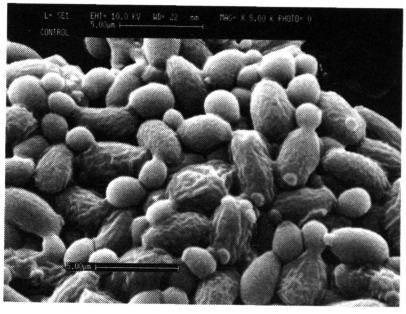
The primary isolate of *C. albicans* used throughout this study was obtained from the Medical Microbiology and Parasitology Department, School of Medical Sciences, Universiti Sains Malaysia, Kelantan and was grown and maintained on Sabouraud Dextrose Agar (SDA) slant at 37°C for 48 hours.

SEM Studies

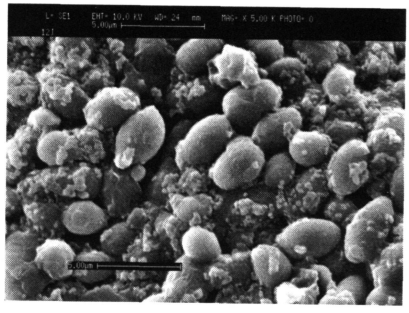
One milliliter of 4 × 10^6 cells/ml yeast cell suspension was added into Sabouraud Dextrose broth containing extract to give a final concentration of 12.5 mg/ml. The mixture was inoculated on a SDA plate. Two milliliters of the extract at the concentration of 100 mg/ml was then dropped onto the inoculated agar and incubated at 37°C for 48 hours. Chloroform treated culture was used as a control. A small block of yeast containing agar was withdrawn from the inoculate culture at various time intervals (12, 24, 36, and 48 hours) and was fixed for scanning (Borgers, Van De Ven, & Van Cutsen, 1989) electron microscopy works.

Results and Discussion

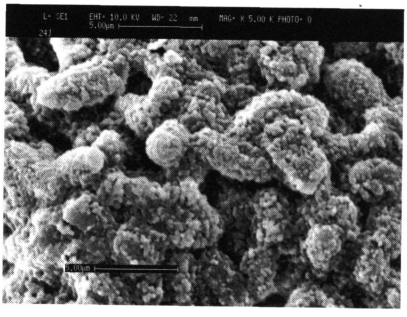
Figure 1 shows the SEM photomicrographs of the control and extract treated cells of *C. albicans* at various time of exposure to the crude extract of *C. zeylanicum*. In Figure 1(a), the control cells, which represent for normal yeast cells showed typical oval-shaped and smooth cell surface in appearance with some at a budding stage, showing spherical of slightly elongated daughter cells that emerged from the parent cell. On the outer surface of the cells was a thin layer of mucous secreted by the cells itself as a protection. After 12 hours of exposure (Figure 1[b]), the cells appeared to be covered with unknown crystallized materials, causing the cells to stick together to a stage of gross alteration and distortion. The mucous layer of the cells became rigid and fragile. The condition of the 24 hours treated cells became worst when the cells were heavily covered with crystallized materials. After 36 hours of exposure (Figure 1[d]), it appears that the cells stuck together and the existence of the small crystallized materials seems to be reduced. At 48 hours of exposure (Figure 1[e]), the cells membrane demonstrate invaginations and the yeast cells appeared significantly collapsed and lysed without any sign of crystallized materials.



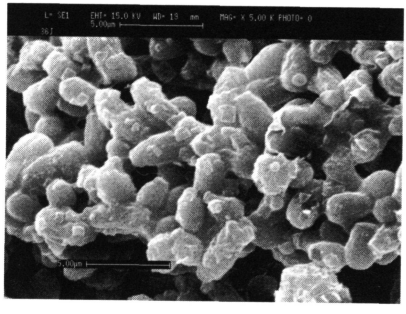
(a) 5.00 µm



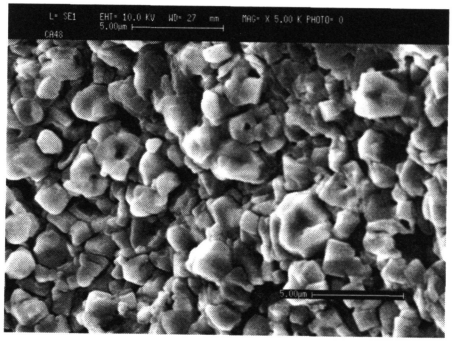
(b) 5.00 µm



(c) 5.00 µm



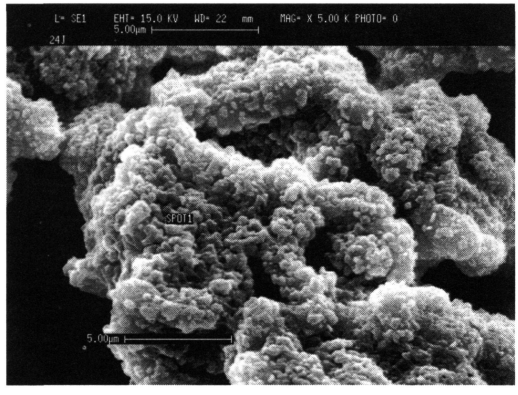
(d) 5.00 µm



(e) 5.00 µm

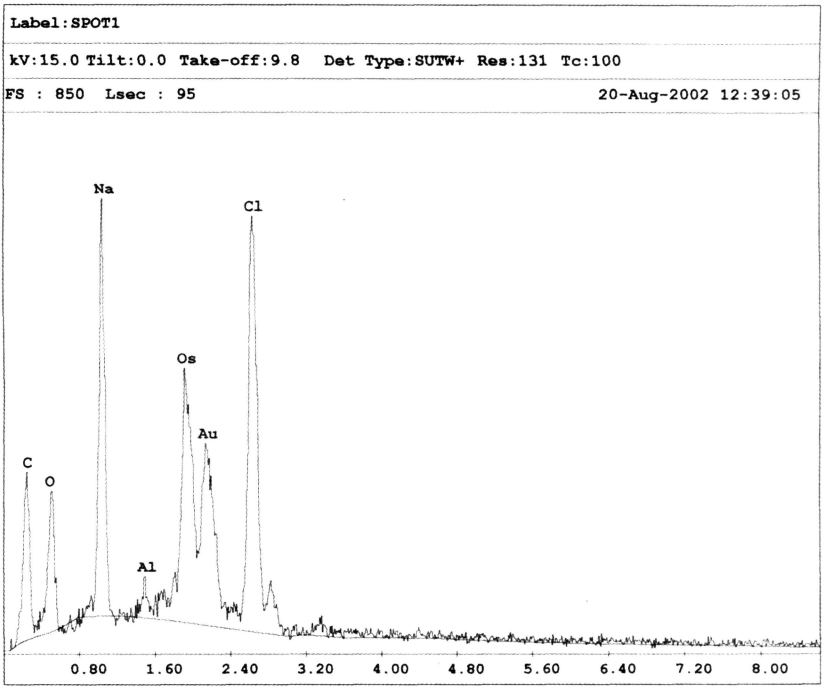
Figure 1: SEM Photomicrograph of *C. albicans* Showed the Structural and Morphological Changes on Yeast Cells After the Extract Treatment. (a) Control (b) 12 Hours Exposure (c) 24 Hours exposure (c) 36 Hours Exposure (c) 48 Hours Exposure

From the SEM results, we suggested that the cells had undergone some distinct morphological and cytological alterations. Normal *C. albicans* undergoes reversible morphological transition between budding pseudohyphal and hyphal growth forms (Brown & Gow, 1999). All forms are present in clinical disease tissue specimens. Yeast cells may be disseminated more effectively, whereas hyphae are thought to promote invasion of epithelial and endothelial tissue and help evade macrophage engulfment (Sherwood, Gow, Gooday, Gregory, & Marshall, 1992). By comparing to the control cells, it showed that the cell's growth of budding pseudohyphal and hyphal was inhibited during the treatment of the cinnamon crude extract. This might happen because of the alterations in the cell membrane permeability, which leads release of the cell constituents and cell lyses. It was also speculated that the cell constituents was leaking due to the present of the crystallized materials. By performing Energy Dispersive X-ray (EDX) analysis on the specimen sample of 24 hours extract-treated yeast cells (Figure 2), it was confirmed that the crystallized materials was sodium chloride (NaCl) crystals (Figure 3). It was suggested that the initial extract-yeast reaction had produce NaCl crystals on the outer surface of the yeasts cells, resulting the alteration of the cell membrane permeability which allowing extracellular substances to cross easily into the cells. This lead to the swollen and lyses process of the yeast cells, which contribute to releasing of the cell constituents.



5.00 µm

Figure 2: SEM Photomicrograph Showing the
Yeast Cells Covered by Unknown Crystallized Materials



Element	Wt %	At %
C K	40.56	63.22
O K	14.67	17.17
NaK	13.39	10.90
AlK	0.44	0.30
OsM	18.48	1.82
ClK	12.46	6.58
Total	100.00	100.00

(b)

Figure 3: EDX Chromatogram Confirmed the Existence of NaCl Crystals

Conclusions

This investigation has opened up the possibility of the use of this plant in drug development for human use. The effect of this plant on more pathogenic organisms and toxicological investigations and further purification however, needs to be carried out in the future.

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