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An *in vitro* study on the anti-adherence effect of *Brucea javanica* and *Piper betle* extracts towards oral *Candida*

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ABSTRACT

Objective: The adherence of *Candida* to mucosal surfaces is the initial step for successful invasive process of the oral cavity. The study aimed to investigate the effect of two plant extracts on the non-specific and specific bindings of oral candida.

Methods: In the former, adsorption to hexadecane was used to measure the hydrophobic interaction of the candida cells. In the later, glass beads coated with saliva represented the experimental pellicles in specific adhesion of oral candida to hard tissue surface.

Results: *Candida krusei*, *Candida dubliniensis* and *Candida tropicalis* showed the highest adsorption to hexadecane at 30.23%, 26.19% and 19.70%, respectively, while the others within the range of 7–10%. All candidal species were significantly affected by the extracts ($P < 0.05$) with *Brucea javanica* exhibited more than 60% reduction of CSH than *Piper betle*. *Candida parapsilosis* showed the highest affinity in specific-bindings to pellicle with $18.72 \pm 0.71 \times 10^5$ CFU/ml. Exposing to *P. betle*-treated pellicle has drastically reduced the adherence of *C. tropicalis*, *Candida albicans* and *C. krusei* by 86.01%, 61.41% and 56.34%, respectively. *B. javanica* exhibited similar effect on *C. tropicalis* (89.86%), *Candida lusitaniae* (88.95%), *C. albicans* (79.74%), *Candida glabrata* (76.85%) and *C. krusei* (67.61%).

Conclusion: The extracts demonstrated anti-adherence activities by modifying the CSH and the characteristics of the experimental pellicle.

1. Introduction

Human oral cavity constitutes a multitude of substrata to which microorganisms such as candida may adhere. The ability of candida to adhere to dentures is crucial in the development of an infection because the presence of this resistant microorganism may perturb the stability of the oral microflora.[1–3] To establish successful colonisation, both the non-specific and specific binding mechanisms are involved. In the former, adhesion depends very much on the non-polar component of the cell wall structure while in the later, more specific receptors are involved.

The surface hydrophobicity of a cell wall (CSH) has great influence on its ability to adhere and forms biofilm on inert surfaces.[4] CSH is characterised by the presence of hydrophobic proteins that are embedded in the cell wall matrix of the candida. This cell wall which is found beneath an outer fibrillar layer provides the hydrophobic interactions between the candida and host surfaces.[5] Several studies have reported that

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the hydrophobicity of yeast cells correlate positively with *Candida*-endothelial cell, *Candida–Candida* and *Candida*-immobilised binding proteins.[6,7]

In the oral cavity, the adherence of candida to tissue surfaces is also mediated by specific adhesion mechanism that occurs between the acquired pellicle and the candidal cells. Salivary components of the acquired pellicle promote the adhesion of pioneer candida by providing many specific receptors. Like most microorganisms, *Candida* possess a dynamic cell wall structure with components that are specifically designed to bind to a variety of ligands on the host cell surface via protein–protein interactions, protein–carbohydrate interactions and candida mannoprotein ligands.[8] These interactions endowed with hyphae, enables candida to attach and invade tissues of the host.

Malaysia is blessed with natural products with potent and unique medicinal properties that have been reported to exhibit various biological activities. Their usage as traditional remedies is popular as there have been minimal reports on side-effect towards the host.[9] *Piper betle* and *Brucea javanica* are two local plants that have been reported to possess several biological activities and used as a common ingredient in local medicines. *P. betle* exhibits antibacterial, anti-adhesion[10,11] and antifungal[12] activities, while *B. javanica* possess cytotoxic effect on cancer cell lines.[13] Data on the antifungal effect of these plants on oral *Candida* is still scarce.

The study was designed with the objectives of evaluating the effect of *B. javanica* and *P. betle* aqueous extracts on the non-specific and specific adherence mechanisms of seven species of oral candida. The study focused mainly at the responses of candidal cells at the active growth stage as the physiological growth and metabolic activity of the cells are at their optimum during the log phase. Data obtained may contribute to a better understanding of the antifungal properties of these plants which can be promoted as an alternative antifungal agent.

2. Materials and methods

2.1. Plant collection and extract preparation

Fresh *P. betle* leaves (100 g) and *B. javanica* seeds (100 g) were collected from a rural area in Sekinchan Selangor, Malaysia. The specimens were identified by a botanist from the Institute of Biological Science, Faculty of Science University of Malaya. The voucher specimens were deposited at the Herbarium of Rimba Ilmu, University of Malaya. Crude aqueous extract of the specimens was prepared according to Himratul-Aznita et al.[12] The specimens were washed and oven-dried at 60–65 °C for two days. The dried specimens were homogenised in distilled water at a ratio of specimens to water of 1:10. The homogenate was heated at high temperature and concentrated to one-third of the original volume. The concentrate was filtered through a filter paper (Whatman No. 1) before it was further concentrated to a final volume of 100 ml. The decoction was then freeze-dried overnight (EYELA FDU-1200, Tokyo) and the powder was kept in sterile Falcon tubes and stored at 4 °C. A stock solution of the extract was prepared in sterile distilled water at a concentration of 200 mg/ml. Following centrifugation (Jouan A14, France) for 10 min at 8000 × *g*, the stock was then diluted to concentrations required for the experiment. The extract was sterilised by filtration using 0.2 μm nylon syringe filter (Millipore, USA).

2.2. Candidal strains

Seven strains of oral *Candida* that includes *Candida albicans* ATCC 14053, *Candida dubliniensis* ATCC MYA-2975, *Candida glabrata* ATCC 90030, *Candida krusei* ATCC 14243, *Candida lusitaniae* ATCC 64125, *Candida parapsilosis* ATCC 22019 and *Candida tropicalis* ATCC 13803 were purchased from the American Type Culture Collection (ATCC), USA for use in the study. Yeast Peptone Dextrose (YPD) broth (BD Difco™) was used to revive the cultures.

2.3. Determination of cell surface hydrophobicity (CSH)

2.3.1. Preparation of candidal suspension

A loopful of candidal colonies was inoculated in fresh YPD broth and incubated at 37 °C for 8 h where the log phase is attained. Following this, the cells were harvested by centrifugation at 8000 × *g*. Cell pellet was washed twice with PBS and resuspended in the same buffer. The cell density was adjusted at an absorbance of 0.450 at 550 nm which is equivalent to 1×10^7 cells/ml. Following examination using light microscope, at this log phase all cells were observed to be oval and most were in the budding stage presenting characteristic of yeast.

2.3.2. CSH of Candida strains

Determination of CSH was carried out following the protocol of Klotz et al.[14] To measure the non-specific adhesion, adsorption to hexadecane was used to measure the hydrophobic interaction of the candida cells. A volume of 2 ml of cell suspension (10^7 cells/ml) of each strain was respectively dispensed into sterile glass tubes. To each tube, 2 ml of sterile saline was added to give a final volume of 4 ml. Sterile saline was used as a blank control. 200 μl of hexadecane which represented the hydrophobic surface was then added and the tubes were vigorously agitated for 1 min. The tubes were then left to stand at room temperature for 15–20 min to allow for separation of hexadecane from the aqueous phase. The lower aqueous phase of the mixture was gently aliquoted out into cuvette and the absorbance (A_u) was read at OD 550 nm. Hydrophobicity was expressed as a percentage of adsorption of the candidal cells to hexadecane. Each experiment was carried out in three independent experiments performed in triplicate to ensure reproducibility. The relative CSH was determined by the following equation:

$$\%\ \text{change in}\ A_{550} = [(A_t - A_u)/A_t] \times 100$$

where A_t is absorbance of the total cell suspension in the absence of hexadecane and A_u is absorbance of the total cell in the presence of hexadecane.

2.3.3. CSH of Candida strains treated with B. javanica and P. betle extracts

Two millilitres of candidal suspension was aseptically dispensed into sterile tubes and appropriate volume of the