Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading

Inhibition of candida adhesion to denture acrylic by *Boesenbergia pandurata*

Thaweboon Sroisiri*, Thaweboon Boonyanit

Department of Microbiology, Faculty of Dentistry, Mahidol University, Bangkok 10400, Thailand

ARTICLE INFO

Article history:
Received 27 January 2010
Received in revised form 27 February 2010
Accepted 27 March 2010
Available online 27 April 2010

Keywords:
Candida
Adhesion
Denture
Boesenbergia pandurata

ABSTRACT

Objective: To investigate effect of *Boesenbergia pandurata (B. pandurata)* rhizome extract on adhesion of *Candida albicans (C. albicans)* to acrylic surface. **Methods:** Transparent acrylic strips were prepared and divided into three groups with pretreatment by extract solution of *B. pandurata* rhizome at concentration of 25, 50 and 100 mg/mL, respectively. After washing, the strips were then inoculated with two strains of *C. albicans* (ATCC13803 and the clinical isolate) (10^7cells/mL). Normal saline solution and 0.2% chlorhexidine gluconate were used as negative and positive controls, respectively. Stained the strips with modified Gram stain without counterstain. Adherent yeast cells were direct counted under microscope (Olympus-CX31, Japan) in 20 randomly selected fields on each strip. The statistical significance was calculated by Kruskal-Wallis and Mann-Whitney non-parametric tests at a significance level of $P < 0.05$. **Results:** Pretreatment with *B. pandurata* extract significantly reduced the adhesion of both strains of *C. albicans* to acrylic surfaces in a dose dependent manner. **Conclusions:** This observation indicates that *B. pandurata* extract has an inhibitory effect on the ability of *C. albicans* to adhere to denture acrylic and could be employed as an antifungal agent for preventing denture stomatitis.

1. Introduction

*Boesenbergia pandurata (B. pandurata)*belongs to the Zingiberaceae family, which is a medicinal and culinary herb commonly used in Southeast Asia. The fresh rhizome has a characteristic aroma and slightly pungent taste. It has been used for the treatment of several diseases, such as apthous ulcer, dry mouth, stomach discomfort, leucorrhea and dysentery. In addition, antifungal activity against food spoilage fungi has been demonstrated[1]. Recent studies showed that the ethanolic extract of *B. pandurata* has anti-inflammatory effects and anti-biofilm formation of multi-species oral bacteria[2,3].

Candida albicans (C. albicans) is the predominant fungus found in the oral cavity. Candida-associated denture stomatitis, chronic inflammatory changes of the denture-bearing mucosa, is a common recurring disease observed in 10% to 67% of denture wearers among several populations[4,5]. Adhesion of candida to oral surfaces is considered an essential step for the successful colonization of the mouth and therefore an important determinant in the development of infection. Current treatment regimens for denture stomatitis include the use of topical antifungal agents applied to affected mucosa and prostheses.

Therefore, this study was designed to investigate the inhibitory effect of *B. pandurata* rhizome extract on the adhesion of *C. albicans* to denture acrylic surface.

2. Materials and methods

2.1. Preparation of plant extract

Rhizomes of *B. pandurata* were air-dried, ground, extracted with 95% ethanol at room temperature for 48 h and then freeze dried. Approximately 7 g of extract was obtained from 300 g of dried ground rhizomes. The extract was dissolved in distilled water at 400 mg/mL and further diluted two-fold with normal saline solution (NSS) to the desired concentration.

2.2. Preparation of acrylic strips

*Corresponding author: Sroisiri Thaweboon, Department of Microbiology, Faculty of Dentistry, Mahidol University, 6 Yothi Road, Rajthewee, Bangkok 10400, Thailand.
Tel: +66 2 2036410
E-mail: dtstw@mahidol.ac.th

The acrylic strips for the adhesion assay were prepared as described by Samaranayake and MacFarlane (1980)[6]. Briefly, self-polymerized acrylic powder and monmer liquid were mixed in accordance with manufacturer instructions (Takilon, Rodent, s.r.l., Milano, Italy). The mixture was placed between two glass slides firmly secured at both ends with two binder clips. The acrylic was then polymerized in a hydroflask at 50 ℃ for 5 mins. Subsequently, the firmed transparent acrylic sheet was cut into strips (5×5 mm square and 0.3 mm thick). The strips were immersed in distilled water for one week to leach excess monomer, disinfected by dipping in 70% alcohol for 1 min, and washed with sterile distilled water. The strips were then ultrasonicated (VWR B9500E-DTH, VWR International,USA) for 20 mins, washed again in sterile distilled water, dried and checked for sterility.

2.3. Preparation of *C. albicans* suspension

Two strains of *C. albicans* were used. ATCC 13803 and the clinical isolate, which was recently isolated from the oral lesion of a patient with denture stomatitis in the Faculty of Dentistry, Mahidol University. Identification of this isolate was established by germ tube formation and use of the API-20C system (bioMerieux, UK). Candida were cultured in Sabouraud dextrose broth (Difco Laboratories, Detroit, USA) at 37 ℃ for 18 h, harvested by centrifugation, and washed three times in phosphate buffered saline (PBS)(0.01 M, pH 7.2). Yeast cells were counted with a hemocytometer and resuspended in PBS at 10^7cells/mL.

2.4. Adhesion assay

The adhesion assay was based on the method of Samaranayake and MacFarlane (1980) [6] with modifications. The prepared acrylic strips were pretreated with extract solutions (at concentrations 25, 50 and 100 mg/mL) for 30 mins at room temperature. After washing with PBS, the strips were placed vertically in the wells of a sterile serology plate (Nunclon, Denmark). Approximately 400 μL of the yeast-cell suspension was added to each well, which completely soaked the acrylic strips. The strips were then placed in a shaker incubator for 1 h at 37 ℃ with gentle agitation at 120 rev/min. Normal saline solution and 0.2% chlorhexidine gluconate were used as negative and positive controls, respectively. After incubation, the acrylic strips were removed from the wells and washed three times by dipping in PBS to dislodge the loosely attached yeast cells. Subsequently, the strips were stained using a modified Gram stain without counterstain. After air-drying at room temperature, they were mounted on glass slides with glycerol and the adherent yeasts were quantified under microscope (Olympus CX-31, Japan). Twenty-fields at ×400 magnification were randomly counted for each strip[7].

The mean number of yeasts per 20 fields was finally expressed as counts per square millimeter. Though blastospores were the predominant form of yeast in both candida strains, occasional hyphae were found. In order to standardize the measurement of adherent cells, filamentous forms were not counted and budding daughter cells were counted as individual yeast. All experiments were repeated on three separate occasions with duplicate determinations on each occasion.

2.5. Statistical analysis

Since the data obtained from adhesion assay were not normally distributed, Kruskal-Wallis and Mann-Whitney non-parametric tests were used. In all comparisons, statistical significance was declared if the *P*-value was less than 0.05.

Table1

Effect of *B. pandurata* extract on the adhesion of *C. albicans* to acrylic surfaces.

C. albicans	Chlorhexidine	NSS	Concentrations (mg/mL)		
			25	50	100
ATCC 13803	0.84±0.05*	10.05±0.50	5.54±0.36*	3.42±0.23*	2.52±0.32*
Clinical isolate	0.94±0.07*	12.27±0.57△*	6.01±0.19△*	3.97±0.21△*	3.04±0.14△

Data are expressed as mean number of yeasts/mm^2 + SEM;
*:$P < 0.001$ compared with NSS, a negative control;
△:$P < 0.001$ compared with ATCC 13803 strain in the same column.

Table 2

Mean percentage inhibition of *C. albicans* adhesion to acrylic surfaces after pretreatment with *B. pandurata*.

C. albicans	Chlorhexidine	Concentrations (mg/mL)		
		25	50	100
ATCC 13803	91.63	43.84	65.97	74.90
Clinical isolate	92.34	50.99 △	67.62	75.21

△: $P < 0.001$ compared with ATCC 13803 strain in the same column.

3. Results

Pretreatment of denture acrylic surfaces with *B. pandurata* rhizome extract at concentrations of 25, 50 and 100 mg/mL significantly reduced the adhesion of two strains of *C. albicans* (ATCC 13803 and clinical isolate) compared with those treated with NSS, which was used as negative control. The mean values for adhesion of candida were presented in Table 1. It was found that the inhibitory effect of the extract was dose-dependent. Additionally, a higher level

of adhesion was clearly observed in the clinical isolate strain compared with the ATCC 13803 strain. Table 2 showed the percentage inhibition of adhesion achieved in different concentrations of the extract. Greatest inhibition was observed at 100 mg/mL of the extract when the number of adherent yeasts was reduced by approximately 75%. Chlorhexidine gluconate, a positive control, produced more than 90% inhibitory effect. Despite the fact that the clinical isolate strain elicited higher adhesion, no significant differences in percentage inhibition were observed between the two strains except at the concentration 25 mg/mL.

4. Discussion

C. albicans is generally accepted to be an opportunistic fungus which is the most frequent cause of denture stomatitis in denture wearing patients. It is the most adherent Candida species due to its ability to adhere to a variety of materials including oral prostheses. Additionally, *C. albicans* possesses various virulence factors, such as the secretion of proteolytic enzymes and the ability to inhibit the immune defense of the host, giving it a growth advantage over other yeasts[8].

There is evidence that adhesion to a surface is regarded as a critical prerequisite for the permanent colonization or infection of a site exposed to a constant flow of fluid[9]. The process of adhesion to denture acrylic surface may be especially important in denture stomatitis where *C. albicans* can adhere to the fitting surface of the upper denture to form a reservoir for chronic dissemination of yeast cells and infection of the palate[10]. Several methods have been used to investigate the adhesion of *C. albicans* to acrylic surfaces. Two main methods commonly used are the visual and radio-labelling techniques. In the present study, the microscopic visual method was used to quantify the adhesion of candida in vitro. Although more time-consuming, this method could allow for the estimation of the differential adhesion of yeast and filamentous forms.

In the visual method, it is worth noting that a number of inherent variables cannot be easily controlled. Firstly, it is difficult to count the adherent yeast cells, which may aggregate on acrylic surfaces during drying of the acrylic material following staining process, as a result of surface tension forces[11]. Secondly, there is also evidence that the composition of the medium used for culturing yeasts prior to use in the assay in vitro can have a marked effect on the adhesion and hydrophobicity of the yeasts[12].

By using the visual method, it is demonstrated that 30 min pretreatment of acrylic surfaces with *B. pandurata* extract significantly reduced the ability of both strains of *C. albicans* to adhere to these surfaces compared with those treated with NSS, the negative control, and the effect was dose-dependent. The mean percentage of inhibition of two strains after pretreatment with 100 mg/mL, 50 mg/mL and 25 mg/mL of the extract were approximately 75%, 66% and 47%, respectively.

Boesenbergia pandurata, also known as fingerroot, Chinese ginger or krachai, has been used in folk medicine in Southeast Asia for the treatment of swelling, wounds and diarrhea. The rhizome of this plant has a strong typical odor and is frequently added in Thai foods as curry pastes. It contains 1 to 3% of essential oil. Several aroma constituents have been identified, 1-8 cineol, d-borneol, camphor and methyl cinnamate. For the non-volatile components, flavones and flavonoids, chalcones (cardamonin, cardomin) and dihydrochalcones (panduratin A) have been identified[13-15]. Panduratin A has been reported to have many biological effects, such as anti-inflammatory[16], anti-cancer[17] and anti-oxidant[18] properties. In dentistry, though the mechanisms of action still remain unclear, the potential for inhibiting periodontal pathogen[19] and caries-associated bacteria[20], as well as eliminating multi-species oral biofilm[3] have been demonstrated.

The reduction of *C. albicans* adhesion on acrylic surfaces by *B. pandurata* extract may be explained in several ways. Firstly, an important factor affecting candidal adhesion is the surface free energy of acrylic as this may be interfered with the extract components adsorbed[21]. Another factor influencing the adhesion is the cell surface hydrophobicity of the microorganism[12]. It may be speculated that *B. pandurata* extract establishes changes in cell surface properties of the yeasts, such as interfering with the production of extracellular matrix or a fibrillar floccular layer, leading to modulation of the cell surface hydrophobicity. Furthermore, the attachment of *C. albicans* to acrylic surfaces may be related to the surface adhesins. Adhesins are the fungal surface moieties that mediate binding of *C. albicans* to inert polymers, other cells or proteins. Some strains of *C. albicans* are more adherent than others and possess adhesins with different specificities[22]. Most adhesins identified to date are mannoproteins, and both the protein and carbohydrate portions have been implicated in adhesion. Therefore, it is possible that *B. pandurata* extract decreases *C. albicans* adhesion to acrylic surfaces by blocking adhesins present on cell wall surfaces of the yeasts.

In the previous study, it is recognized that adhesion of *C. albicans* to acrylic is strain dependent[23]. albicans isolated from active lesion of the patients were more adherent to acrylic surfaces than other strains. However, these strains showed more sensitive to antifungal agent than others. The results presented in this study confirm this strain dependent effect since *C. albicans* isolated from the patient exhibited higher adhesion compared with that of ATCC 13803 strain, giving the mean number of adherent yeast of 12.27 cells/mm^2 and 10.05 cells/mm^2, respectively. However, the percentage inhibition of the clinical isolate strain and ATCC strain by chlorhexidine and nearly all concentrations of the extract were not statistically different despite the clinical isolate strain showing a higher percentage.

The results indicate that soaking acrylic dentures in *B. pandurata* rhizome extract for 30 mins would have potential to reduce candidal adhesion and may be a useful adjunct to treat candida-associated denture stomatitis and help

to prevent recurrence of the infection. Further studies are necessary for elucidating the mechanism of action of *B. pandurata* extract at the molecular level as well as the actual effect in clinical tests.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1]Wanchitanawong P, Chaungwanit P, Poovarodom N, Nitisinprasert S. *In vitro* antifungal activity of Thai herb and spice extracts against food spoilage fungi. *Kasetsart J* 2005; **39**: 400-5.

[2]Tewtrakul S, Subhadhirasakul S, Karalai C, Ponglimanont C, Cheenpracha S. Anti-inflammatory effect of compounds from *Kaempferia parviflora* and *Boesenbergia pandurata*. *Food Chemistry* 2009; **115**: 534-8.

[3]Yanti, Rukayadi Y, Lee KH, Hwang JK. Activity of panduratin isolated from *Kaempferia pandurata* Roxb. against multi-species oral biofilms *in vitro*. *J Oral Sci* 2009; **51**: 87-95.

[4]Daniluk T, Fiedoruk K, Sciepuk M, Zaremba ML, Rozkiewicz D, Cylwik-Rokicka D, et al. Aerobic bacteria in the oral cavity of patients with removable dentures. *Adv Med Sci* 2006; **51**: 86-90.

[5]Zaremba ML, Daniluk T, Rozkiewicz D, Cylwik-Rokicka D, Kierklo A, Tokajuk G, et al. Incidence rate of Candida species in the oral cavity of middle-aged and elderly subjects. *Adv Med Sci* 2006; **51**: 233-6.

[6]Samaranayake LP, MacFarlane TW. An in vitro study of the adherence of Candida albicans to acrylic surfaces. *Arch Oral Biol* 1980; **25**: 603-9.

[7]Weibel ER. Sampling of tissue. In: *Stereological methods: practical method for biological morphometry*. London: Academic Press; 1979, p. 63-100.

[8]Romani L, Bistoni F, Puccetti P. Adaptation of *Candida albicans* to the host environment: the role of morphogenesis in virulence and survival in mammalian hosts. *Curr Opin Microbiol* 2003; **6**: 338-43.

[9]Ramage G, Mowat E, Jones B, Williams C, Lopez-Ribot J. Our current understanding of fungal biofilms. *Crit Rev Microbiol* 2009; **35**: 340-55.

[10]Kanli A, Demirel F, Sezgin Y. Oral candidosis, denture cleanliness and hygiene habits in an elderly population. *Aging Clin Exp Res* 2005; **17**: 502-7.

[11]Coimbra CD, Rufino RD, Luna JM, Sarubbo LA. Studies of the cell surface properties of Candida species and relation to the production of biosurfactants for environmental applications. *Curr Microbiol* 2009; **53**: 245-51.

[12]Szabelska M, Gospodarek E, Ciok-Pater E. Influence of incubation conditions on cell surface hydrophobicity of Candida species fungi. *Med Dosw Mikrobiol* 2006; **58**: 253-60.

[13]Jaipetch T, Kanghae S, Pancharoen O. Constituents of *Boesenbergia pandurata* (syn. *Kaempferia pandurata)*: isolation, crystal structure and synthesis of (+) boesenbergia A. *Aust J Chem* 1982; **35**: 351-6.

[14]Pancharoen O, Picker K, Reutrkul V. Constituents of Zingiberaceae. Diasteromers of [7-hydroxy-5-methoxy-2-methyl-2-(4´-methylpent-3´-enyl)-2H-chromen-8-yl] 3"-methyl-2"-3"-methylbut-2"-enyl)-6"-phenylcyclo-hex-3"-enyl] methanone (panduratin B), a constituent of the red rhizomes of a variety of *Boesenbergia pandurata*. *Aust J Chem* 1987; **40**: 455-9.

[15]Pandji CW, Grimm C, Wray V. Insecticidal constituents from four species of the Zingiberaceae. *Phytochemistry* 1993; **34**: 415-9.

[16]Yun JM, Kwon H, Hwang JK. In vitro anti-inflammatory activity of panduratin isolated from *Kaempferia pandurata* in RAW264.7 cells. *Planta Med* 2003; **69**: 1102-8.

[17]Yun JM, Kwon H, Mukhtar H, Hwang JK. Induction of apoptosis by Panduratin isolated from *Kaempferia pandurata* in human colon cancer HT-29 cells. *Planta Med* 2005; **71**: 501-7.

[18]Sohn JH, Han KL, Lee SH, Hwang JK. Protective effects of panduratin against oxidative damage of tert-butylhydroperoxide in human HepG2 cells. *Biol Pharm Bull* 2005; **28**: 1083-6.

[19]Park KM, Choo JH, Sohn JH, Lee SH, Hwang JK. Antibacterial activity of panduratin isolated from *Kaempferia pandurata* against *Porphyromonas gingivalis*. *Food Sci Biotechnol* 2005; **14**: 286-9.

[20]Hwang JK, Chung JY, Baek NI, Park JH. Isopanduratin from *Kaempferia pandurata* as an active antibacterial against cariogenic *Streptococcus mutans*. *Int J Antimicrob Agents* 2004; **23**: 377-81.

[21]Pereira-Cenci T, Cury AA, Cenci MS, Rodrigues-Garcia RC. In vitro Candida colonization on acrylic resins and denture liners: influence of surface free energy, roughness, saliva, and adhering bacteria. *Int J Prosthodont* 2007; **20**:388-10.

[22]Angiolella L, Stringaro AR, Bernardis FD, Posteraro B, Bonito M, Toccacieli L, et al. Increase of virulence and its phenotypic traits in drug-resistant strains of *Candida albicans*. *Antimicrob Agents Chemother* 2008; **52**: 927-36.

[23]Hasan F, Xess I, Wang X, Jain N, Fries BC. Biofilm formation in clinical Candida isolates and its association with virulence. *Microbes Infect* 2009; **11**: 753-61.