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Original Article

Preventive effects of shiitake mushroom extract on candida stomatitis

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Purpose: The purpose of this study was to investigate antifungal activity of shiitake mushroom yeast and hyphal type of *Candida albicans*. **Materials and Methods:** The extract from shiitake mushroom was collected by drying the supernatant after soaking shiitake mushrooms in water or ethanol. The antifungal activity of the extracts against yeast type of *C. albicans* was investigated by the susceptibility assay using microplate. *C. albicans* biofilm was formed on 12-well plate using Ham's F-12 medium in CO_2 incubator and treated with the ethanol extract. Furthermore, *C. albicans* biofilm was formed on denture base resin disk and treated with or without the ethanol extract in the presence of denture cleanser. Live *C. albicans* in biofilm was counted by cultured colony forming unit value after inoculated on agar plate. **Results:** Ethanol extract from shiitake mushroom showed stronger antifungal activity against yeast type of *C. albicans* compared to its water extract. The ethanol extract significantly reduced count of *C. albicans* in hyphal biofilm ($P < 0.05$). Also, the ethanol extract showed synergistically antifungal effect with denture cleanser on candidal biofilm on denture base resin disk ($P < 0.05$). **Conclusion:** The ethanol extract of shiitake mushroom may be a candidate for preventing candidal stomatitis as well as denture-related stomatitis. **(J Dent Rehabil Appl Sci 2021;37(3):123-9)**

Key words: *C. albicans*; candidal stomatitis; extract of shiitake mushroom

Introduction

Shiitake mushrooms are one of the most popular mushrooms worldwide. Among shitake mushroom, Lentinula edodes is the most famous and has been most commonly used food and traditional medicine.[1] Shiitake mushroom has shown to present medicinal compounds such as polysaccharides, sterols, terpenoids, and lipoids, by which has anti-inflammatory, antimicrobial, anti-tumor effects.[2,3] Also, the extract from this mushroom showed antimicrobial and anti-biofilm activity against oral bacteria and biofilm.[4,5]

Candida species are detected up to 90% in healthy persons and present in the oral cavity of up to 75% of the population.[6,7] Furthermore, *Candida albicans* was identified about 80% of the isolated Candida species.[8] The characteristic related to the virulence of *C. albicans* is the change of morphology which grow either budding yeast and hyphal form by growth condition.[9] The morphology of this fungus is changed by environmental pH, physiological temperature, serum and CO_2.[6] The yeast forms are commonly found on the mucosal surface, and the hyphal forms are detected in epithelial layer.[10] Furthermore, hyphal form but not yeast form are found in epithelial layer at sites of infection.[11] Therefore, the transition between yeast and hyphal forms is termed dimorphism, that is an important pathogenicity for oral candidiasis. Also, when the virulence of *C. albicans* is analyzed by global analysis using 177 mutant strains tested,

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attenuation of the virulence was significantly correlated with reduced hyphal morphogenesis.[12] Another characteristic of *C. albicans* is possible to form biofilm of hyphal type on denture. The biofilm formed on denture induces denture related stomatitis.

The elderly population is gradually increasing due to the development of medical technology and equipment. This phenomenon can be expected to increase the use of dentures. Therefore, the aim of this study was to investigated antifungal activity of shiitake mushroom against yeast and hyphal type of *C. albicans*.

Materials and Methods

Shiitake mushroom was used Lentinula edodes. Slide shiitake mushroom (300 g) was soaked in 500 ml of distilled water or ethyl alcohol with magnetic bar. The prepared mushroom was incubated at room temperature for 24 h on magnetic stirrer. The extract from the mushroom was collected by filtering with Whatman filter paper (GE healthcare, Chicago, USA), and the solvent was evaporated with vacuum evaporator (IKA, Staufen, Germany). The weight of the dried extract was measured and solved at 1 g/ml with distilled water and ethyl alcohol. The solution was filtrated with polyvinylidene fluoride (PVDF) filter (0.25 μm of pore size) (Millipore, Billerica, USA).

Candida albicans ATCC 10231 was used in this study. The fungus was cultivated in trypticase soy broth (TSB; BD biosciences, San jose, USA) at 37°C in shaking incubator. Furthermore, to form *C. albicans* biofilm with hyphal type, *C. albicans* was cultivated with Ham's F-12 medium (Hyclone, Logan, USA) at 37°C in an incubator under 5% CO_2 condition.

The antifungal activity of shiitake extract was processed by the methods by protocol of Clinical Laboratory Standard.[13] 180 μl of TSB was dispensed into the well of a 96-well plate (SPL Lifescience, Pocheon, Korea), and the solution of the shiitake extracts were added into the 12th row of well containing TSB. The extract was performed 2-fold serial dilutions from 11th column to 2nd column with micropipette. The fungus was counted with a hemacytometer (Marienfeld, Lauda-Konigshöfen, Germany) and adjusted the concentration to 1×10^6 cells/ml in TSB. 20 μl of the suspension was inoculated into the prepared well. The plate was incubated 37°C under aerobic condition for 36 h. The fungal growth was measured with optical density at a 600 nm wavelength by a microplate reader (Biotek, Winooski, USA). Hyphal *C. albicans* was formed on 12-well plate using Ham's F-12. After observe the hyphal type using a phase contrast microscope, the antifungal activity of the shiitake extract was investigated. The ethanol was diluted with phosphate buffered saline (PBS, pH 7.2) at 12.5, 25, and 50 mg/ml of concentration. The extract was treated on hyphal *C. albicans* for 1 min, and hyphal *C. albicans* on the well was disrupted with a scraper (SPL bioscience). The fungal suspension was transferred into 1.5 ml tube and serially diluted from 10 to 10^5 fold. 50 μl of the diluted suspension was inoculated on trypticase soy agar (TSA) plate. The plate was incubated 37°C for 36 h, and the fungal colonies were counted. To evaluate effect of the extract on denture-related stomatitis, A specimen was fabricated using a 3D printer (Form 2; formlabs Co., Somerville, USA) and acrylic resin (digital Denture resins; formlabs Co.). The specimens were printed with a diameter of 12 mm with a thickness of 2 mm parallel to the bottom of the LASER. Next, the surface of the specimens was washed with isopropyl alcohol and cured using ultra violet and heating. Enzyme based denture cleanser (Dongahwa, Seoul, Korea) solved with 100 ml of sterile tap water, and the extract (25 mg/ml) was solution was added into the solution. Candidal biofilm formed disk were placed into the solution. After incubating for 5 min, the disks were washed with sterile tap water and placed into each well of 12-well polystyrene plate (SPL Lifescience) containing 1 ml of TSB. Candidal biofilm on the disk was physically disrupted with a scraper (SPL bioscience). The fungal suspension was transferred into 1.5 ml tube and serially diluted from 10 to 10^5-fold. 50 μl of the diluted suspension was inoculated on trypticase soy agar plate. The plate was incubated 37°C for 36 h, and the fungal colonies were counted.

IBM SPSS Statistics Ver. 23 (IBM, Armonk, USA) was used for statistical analysis. In order to analyze the statistical difference in the data, data distribution

was determined using the Kolmogorov-Smirnov test. The values among groups were analyzed by a non-parametric Kruskal-Wallis test and Mann-Whitney U-test, and statistical significance was defined by a *P* value of less than 0.05.

Results

First, Ethanol and water extract from shiitake mushroom was investigated against yeast type of *C. albicans*. The ethanol extract significantly inhibited the growth of *C. albicans* above 6.25 mg/ml of concentration ($P < 0.05$), and the water extract inhibited the candidal growth above 25 mg/ml of concentration ($P < 0.05$, Fig. 1). The ethanol extract showed more antifungal activity compared to the water extract.

As the concentration of the ethanol extract increased, it showed strong fungicidal effect on hyphal biofilm (Fig. 2). However, the water extract did not show antifungal activity against hyphal biofilm (data not shown). The ethanol extract significantly showed antifungal activity against candidal biofilm above 25 mg/ml of concentration ($P < 0.05$).

Generally, most elderly people use denture cleansers to remove fungus and debris on denture. Therefore, the antifungal effect of a mixture of denture cleanser and the shiitake extract was investigated. Denture cleanser has strong antifungal activity against candidal biofilm on denture. However, the cleanser did not completely remove *C. albicans* (Fig. 3). The denture cleanser solution containing the shiitake extract showed more antifungal activity against candidal biofilm on denture compared to single condition of the cleanser.

Discussion

C. albicans causes oral candidiasis, which is especially common in immune compromised patients or the elderly.[14,15] Also, denture stomatitis occurs in people who wear dentures and is associated with *C. albicans*.[16] The elderly population is gradually increasing due to the development of medical technology and equipment. This phenomenon can be expected to increase the use of dentures. Therefore, the importance of denture hygiene is emphasized, and the need for a

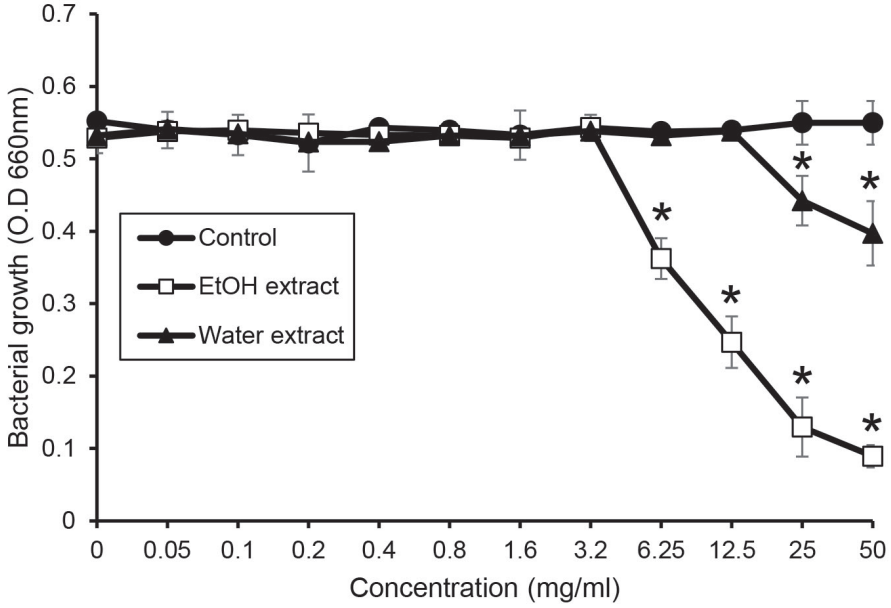

Fig. 1. The antifungal activity of extract from shiitake mushroom on yeast type of *C. albicans*. *C. albicans* was cultivated with or without water or ethanol extract from shiitake in the various concentration. The growth of *C. albicans* was measured by a spectrophometer at 660 nm of wavelength. *symbol indicates significant difference compared to control group ($P < 0.05$).

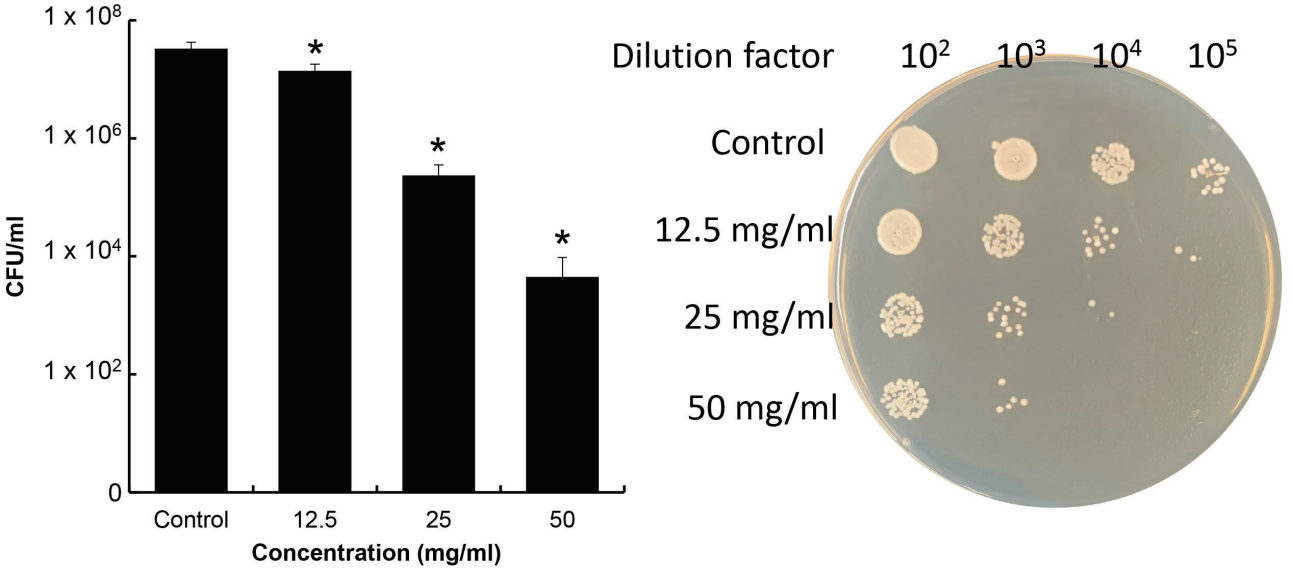

Fig. 2. Effect of the ethanol extract of candidal biofilm. *C. albicans* was formed biofilm on 12-well plate and treated with the ethanol extract for 1 min. After disrupting biofilm mechanically, *C. albicans* in the biofilm was resuspended with TSB and inoculated TSA plate. The plate was incubated, and the colonies on the agar plates were counted. *symbol indicates significant difference compared to control group ($P < 0.05$).

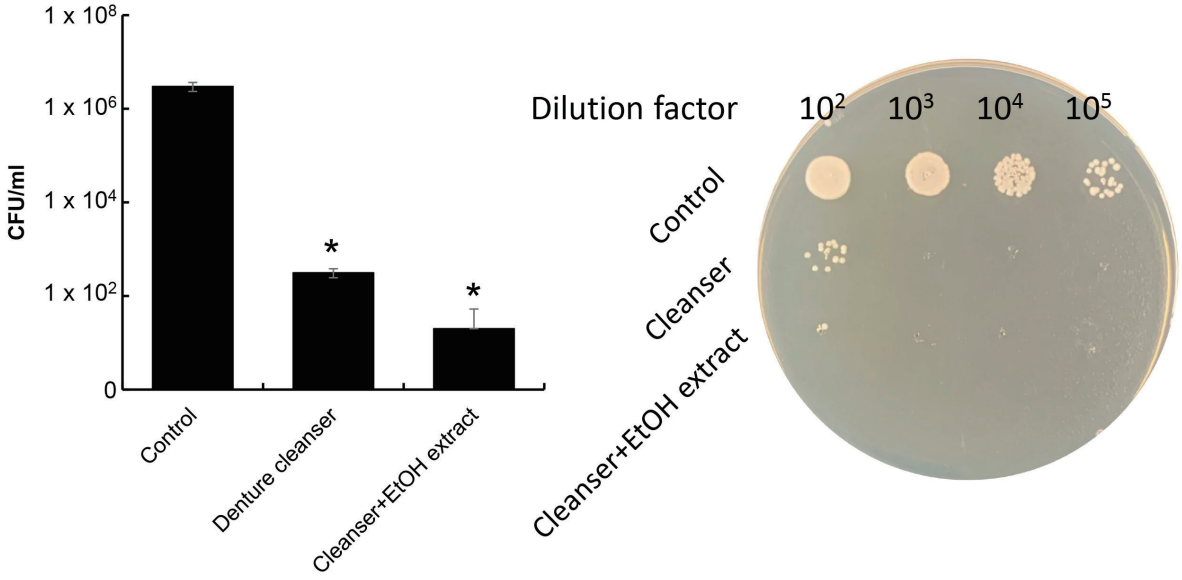

Fig. 3. Synergistic effect of the ethanol extract on reaction of denture cleanser for candidal biofilm on denture base resin. *C. albicans* was formed biofilm on denture base resin disk. The prepared disk was soaked in denture cleanser solution in the presence or the absence of the ethanol extract. After disrupting biofilm mechanically, *C. albicans* in the biofilm was resuspended with TSB and inoculated TSA plate. The plate was incubated, and the colonies on the agar plates were counted. *symbol indicates significant difference compared to control group ($P < 0.05$).

more effective denture cleanser is emerging. This study was investigated antifungal activity of extract from shiitake mushroom to prevent oral candidiasis.

Shiitake mushroom is used general food and is recognized medical value due to its nutritional components. This mushroom has bio-active polysaccharide (lentinan, heteroglucan, xylomannan, and β -glucan), free sugar (arabinose, arabitol, glycerol, mannitol, mannose, and trehalose), vitamins (B2, B12, C, D and E), organic acid (cinnamic acid, phenolic acid, and benzoic acid), and fiber.[17,18] These components of shiitake mushroom show antitumor, antiinflammation, antioxidant and antimicrobial activity.[1-3,17] When extract from shiitake mushroom using water and ethanol was investigated antifungal activity against *C. albicans*, the ethanol extract showed stronger antifungal activity compared to the water extract. These results indicates that hydrophobic components of shiitake mushroom may be suitable to prevent oral candidiasis. Also, comparing other studies. Mushroom extracts with antifungal activity were reported as organic acid such as benzoic acid, cinnamic acid, and phenolic acid, which is consistent with the results of this study.[18] Basis of these results, the ethanol extract was investigated antifungal activity against hyphal type of *C. albicans*. Since the hyphal type forms biofilm, *C. albicans* was formed biofilm on 12-well plate to investigated antifungal activity against hyphal type. The ethanol extract showed antifungal activity against hyphal *C. albicans*. In this study, hyphal *C. albicans* was evaluated for two reasons. First, the hyphal type forms a biofilm, which forms a protective barrier on the outside of the biofilm with exopolysaccharide.[19] The protective barrier makes it resistant to antifungal agents, and the resistance to antifungal agents is greater than that of the yeast type. Another reason is that *C. albicans* mainly exists as a hyphal type in oral cavity. Therefore, in order to investigate the antifungal effects in clinical area, it is necessary to use the hyphal type of *C. albicans*. Recently, resistance to antifungal agents of human disease-related fungi is increasing, and thus a treatment is being sought using antifungal agents in crop.[20,21] Shiitake mushroom is also a crop and may be a candidate to be an agricultural fungicide.

Next, to investigate the effect on denture-related stomatitis, a candidal biofilm was formed on denture resin and its removal ability was tested. Since the antifungal effects of the ethanol extract on candidal biofilm was investigated, the effect when mixed with a denture cleanser was investigated next. The ethanol extract showed a synergistic effect with the denture cleanser on candidal biofilm. This result indicates that the denture cleanser containing enzymes and other constituents had no effect on the ethanol extract. Denture cleanser has been reported to induce oral mucosal injury, chemical burn, and gastric perforation.[22-24] Therefore, it may be safer denture users by reducing or pulling out the strong toxic substances of denture cleanser and using the extract of shiitake mushroom. In addition, a further study is necessary to investigate antifungal activity against candidal biofilm on denture base resin by using the extract of shiitake mushroom and changing the composition of denture cleanser.

Conclusion

The present study showed that the ethanol extract from shiitake mushroom has strong antifungal activity against yeast and hyphal type of *C. albicans*. Also, the ethanol extract showed a synergistic effect with the denture cleanser on candidal biofilm on denture. The extract from shiitake mushroom may be a candidate to prevent oral candidiasis.

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칸디다성 구내염에 대한 표고버섯 추출물의 예방효과

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목적: 본 연구의 목적은 효모형 또는 균사형 *Candida albicans*에 대한 표고버섯 추출물의항진균효과를 살펴보기 위함이다.
연구 재료 및 방법: 표고버섯 추출물은 표고버섯을 물 또는 에탄올에 담근 후, 상층액을 건조시켜서 얻었다. 효모형 *C. albicans*에 대한 추출물의 항진균활성은 마이크로플레이트를 이용한 감수성 시험을 이용하여 조사되었다. *C. albicans* 생물막을 CO_2 배양기에서 Ham's F-12 배지를 이용하여 12-well 플레이트에 형성시키고 에탄올 추출물로 처리하였다. 또한 *C. albicans* 생물막을 의치상용 레진 디스크에 형성시키고 의치세정제를 에탄올 추출물이 포함 또는 포함되지 않은 조건에서 처리하였다. 두 조건의 항바이오필름 효과 시험에서의 바이오필름내 살아있는 *C. albicans*를 조사하기 위해서 한천고체배지에 접종한 후 집락 형성 단위(CFU) 값을 측정하였다.
결과: 효모형 *C. albicans*에 대해서 표고버섯으로부터 물 추출물보다 에탄올 추출물이 강한 항진균력을 보였다. 에탄올 추출물은 균사형 *C. albicans* 바이오필름에 대해서도 유의적인 항진균력을 보였다($P < 0.05$). 또한 에탄올 추출물은 의치세정제와 의치에 형성된 *C. albicans* 바이오필름에 대한 항진균력에 대해서 동반상승효과를 보였다($P < 0.05$).
결론: 표고버섯 에탄올 추출물은 구강 칸디다증 예방뿐만 아니라 의치관련 구내염에 대해서 예방할 수 있는 후보물질로 사료된다.

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주요어: *C. albicans*; 구강 칸디다증; 표고버섯 추추물

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