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Research Article

The Comparison between Black Cumin Extract and Betel Leaf Extract As Antifungal Potential to Candida Albicans on Acrylic Resin Denture Base

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Abstract

Porosity in acrylic resin denture base causes a rough surface and can absorb fluid, leading to plaque. Candida albicans can stick to a plaque on the denture base and engender denture stomatitis. Denture base submersion has to be carried out in traditional plant solutions to prevent denture stomatitis. Traditional plants such as black cumin and betel leaf are considered to have anti-fungal properties. This study aims to identify the comparison of antifungal properties between black cumin and betel leaf extract to Candida albicans on the acrylic resin denture base. This study is an experimental laboratory. Treatment group 1 used aquadest as a control negative, treatment group 2 used a black cumin extract with a concentration of 0.25%, while treatment group 3 used betel leaf extract with a concentrate of 50%. In every group, 9 acrylic resin pieces were soaked for 8 hours. The colony calculation was then conducted and analyzed using the Mann-Whitney test to compare the average difference level between the two tested groups. There was no significant difference level among a total colony of Candida albicans in the treatment group with a concentration of 0.25% black cumin and the treatment group with a 50% concentration of betel leaf. The result showed that the black cumin extract is no more effective in hindering the growth of Candida albicans on the acrylic resin denture base than betel leaf extract.

Keywords: antifungal; black cumin; betel leaf; candida albicans; dentures

INTRODUCTION

Dentures are instrument imitations used to replace missing teeth and prevent negative impacts.[1] Denture generally uses a polymer-based material, including polymethyl methacrylate (PMMA) or acrylic resin. Acrylic resin naturally has some weaknesses such as dimension instability, color change, and porosity that cause rough surface and liquid absorption.[2] Acrylic resin absorbs water when contacting saliva and shapes plaque where microorganisms gather, such as *Candida albicans*. It can lead to cleaning difficulty and harm oral and dental hygiene. Plaque causes microorganisms and reacts to the membrane mucosa of the oral cavity, causing denture stomatitis.[3]

Candida albicans is a commensal organism that can lead to opportunistic infection due to supportive predisposition factors. Frequent causes of local *Candida albicans* infection are dentures, especially poor denture cleaning procedures.[6]

Prevention of denture stomatitis, including infection *Candida albicans*, is highly essential. Denture base submersion in a cleaning solution is an effective method to prevent it. However, the cleaning solution circulating in the community, such as chlorhexidine, is less affordable. Thus, it needs affordable alternative materials for base denture cleaning. The alternative

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material can be obtained from traditional plants that play as anti-fungal properties such as black cumin (*Nigella sativa*) and green betel leaves (*Piper betel L.*).[4]

Black cumin seeds are often used as traditional medicine in the Middle East and Asian countries to handle many diseases such as microorganism infection. Black cumin contains anti-fungal properties such as *thymoquinone*, *thymohydroquinone* and *thymol*. Such materials are the main components of black cumin essential oil and have excellent antifungal properties in hindering *Candida albicans*.[5] Furthermore, Saponin compounds have also been reported to have an ability as an antifungal property.[2]

Moreover, green betel leaves are experimentally known to have various pharmacological properties, such as anti-microbial, anti-fungal, and others. [6] the antifungal property in green betel leaf is found in the *hydroxychavicol* compound.[7] Furthermore, the green betel leaves also contain several bioactive compounds as an antifungal property, such as alkaloids and flavonoids, which can hinder the formation of pseudohyphae and disturb nucleic acid biosynthesis.

MATERIALS AND METHODS

This research is an experimental laboratory study conducted in the Microbiology Faculty of Medicine and Health Sciences laboratory, Universitas Muhammadiyah Yogyakarta and Research and Testing Integrated Laboratory (LPPT) Gajah Mada University. This study employed 27 samples for 3 groups; 9 samples for each group. The treatment group utilized the acrylic resin denture base submersion in aquadest for 8 hours. Treatment group 2 utilized the acrylic resin denture base submersion in black cumin extract concentration of 0.25% for 8 hours. Meanwhile, treatment group 3 utilized the acrylic resin denture base submersion in green betel leaf extract concentration of 50% for 8 hours after being contaminated with *Candida albicans* for 24 hours.

The acrylic resin denture base started with making a plate from modeling wax with a diameter of 10 mm and a thickness of 2 mm. The total was 27 plates using wax. The wax was used to create the unpolished plate sample of acrylic resin.

Furthermore, the production of black cumin and green betel leaf extract was carried out using the maceration method. 600 grams of black cumin seeds and green betel leaves were cleaned and dried for 48 hours at a temperature of 45^{o} C. The dried black cumin seed and green betel leaves were ground using a pollinator machine. The two kinds of powder were mixed with methanol and then were stirred for 30 minutes. It was left for 24 hours and filtered. The process was repeated three times. After that, the filtration was done and evaporated. The evaporation produced a thick extract. Next, the thick extract was poured into a porcelain cup and heated until the black cumin and green betel leave extract was obtained. The black cumin extract was then diluted with dimethyl sulfoxide to reach 0.25% concentration, while the green betel leave extract was diluted with dimethyl sulfoxide to reach 50% concentration.

Candida albicans colony was prepared by taking the Candida albicans using ose sterile and then planting it into *Sabouraud ' dextrose agar*. It was then incubated for 24 hours at 37^{o} C. The suspension of *Candida albicans* was customized with a solution standard of McFarland.

Acrylic resin disc with a diameter of 10 mm and a thickness of 2 mm totaling 27 pieces were soaked using aquadest for 48 hours and then sterilized using 70% alcohol. Acrylic resin disc was soaked in sterile saliva for 1 hour and then rinsed with phosphate-buffered saline (PBS) twice, respectively. It was put into a reaction tube containing *Candida albicans* suspension and incubated for 24 hours. It was then soaked in a reaction tube containing aquadest, black cumin extract of 0.25% concentration and green betel leave extract

of 50% concentration previously containing *Candida albicans* suspension for 8 hours. After that, each acrylic resin disc was put into a reaction tube containing 10 ml aquadest, then was shaken with a vortex mixer for 1 minute and diluted up to 10^{-3}. Furthermore, it was put into *Sabouraud's dextrose agar* and incubated for 48 hours at 37^{o} C. The calculation of the total colony of *Candida albicans* was conducted using a calculator.

The data were analyzed using Shapiro Wilk and Levene's test. Test data analysis using SPSS showed that distributed data were not normal and homogeneous; thus, the Mann-Whitney test was conducted.

RESULT

The study on the comparison between black cumin and green betel leave extract as antifungal properties towards the growth of *Candida albicans* on the acrylic resin denture base has been carried out in the Microbiology laboratory Faculty of Medicine and Health Sciences, Universitas Muhammadiyah Yogyakarta with 27 subjects. The total and average data are as follows:

Table 1. Data in the study

Group	n	Amount	Average
Aquadest	9	708	78.6667
Black Cumin extract	9	595	61.1111
Green Betel Leave Extract	9	399	44.3333

Furthermore, a normality test was conducted to identify the data distribution. The total number of the data was 27 samples. Therefore, the Shapiro-Wilk test (small number of the sample) was conducted to identify whether the data distribution was normal. The Shapiro-Wilk column showed the data distribution of *Candida albicans* colony in aquadest and black cumin extract concentration of 0.25% revealed the significance value of 0.498 and 0.114 ($p>0.05$), respectively. It indicated that the data were normally distributed. Meanwhile, the treatment group of green betel leave extract of 50% concentration showed the significance value of 0.002 ($p <0.05$), indicating the data were not normally distributed. Thus, the Mann-Whitney test's non-parametric hypothesis was conducted to identify the comparison between black cumin and green betel leave extract.

Furthermore, the homogeneity test was conducted using Levene's test to identify whether the analyzed data were homogeneous or heterogeneous. The results showed that the total colony of *Candida albicans* on all three treatment groups had a significance value of 0.787 ($p> 0.05$), indicating that the data were homogeneous. Moreover, the non-parametric hypothesis using the Man Whitney test was conducted.

The result of the Mann-Whitney test showed a significance value of 0.659 ($p>0.05$); thus, H0 was accepted. It can be concluded that there was no significant difference between the total colony of *Candida albicans* in the treatment group of black cumin extract of 0.25% concentration and the treatment group of the green betel leave extract of 50% concentration.

Table 2. The results of the Mann Whitney test

	The Number of *Candida albicans*
Asymp. Sig. (2-tailed)	.659
Exact Sig. [2*(1-tailed Sig.)]	.666 [b]

DISCUSSION

The study shows no significant mean difference between the total colony of *Candida albicans* in the treatment group of black cumin extract and the green betel leave extract ($p>0.05$). Therefore, it is not in line with the hypothesis revealing that black cumin extract more effectively hinders the growth of *Candida albicans* on acrylic resin denture base compared to green betel leave extract.

Less effectiveness of the black cumin extract of 0.25% in hindering the growth of *Candida albicans* colony is influenced by the low-level concentration from the black cumin extract. It is in line with Ornay et al., [8,] denoting that substance concentration influences the effectiveness of the antifungal agent. Low extract concentration results in low active ingredients working as an antifungal that can cause low antifungal ability.

This statement is in line with research conducted by Rahmawati et al.,[9] revealing that black cumin extract with 10% concentration contains more fungal agents than black cumin extract with 20% concentration. Furthermore, another study conducted by Dharma and Subaryanti[10] showed that on the examination of the diameter of inhibition activity, the black cumin extract with a concentration of 20% and 30% has no inhibition zone compared to the black cumin extract with concentrations of 40%, 50%, and 60%. Therefore, it can be concluded that low-level concentration of the black cumin extract results in low active ingredient as an antifungal agent.

The result of this study is not in line with research conducted by Nagham Adil Ghani[11,] revealing that the black cumin extract with 0.25% concentration showed the most effective ability in hindering the growth of the *Candida albicans* colony due to the different areas of the black cumin seeds. The difference in the effectiveness of the plants could be influenced by the origin of the plants, agro-climate factors, and phytochemical contents in the used extract.[12] The research used black cumin seeds earned from Iraq, while this research used black cumin seeds from Indonesia.
Furthermore, according to Dharma and Subaryanti,[10] the difference in climate and land nutrition could influence active ingredient level from simplicia. Furthermore, according to Suryadi et al., [13] black cumin can grow in Jordan with a sub-tropical climate and Indonesia with a tropical climate. Therefore, the different environments will influence the plant response in nutrient absorption in the land as well as the bioactive production. Both statements align with research conducted by Mahfur[14], revealing that black cumin originating from Indonesia did not contain essential oil, while those originating from Habasyah and India contained essential oil. In addition, this research also used green betel leaf originating from Indonesia.

The betel leaves are mostly distributed in tropical and subtropical countries worldwide, including Indonesia.[15] As all of the plants originated from Indonesia, they did not influence nutrient absorption and bioactive production response. Thus, it can be concluded that the different environments influence the active ingredient level of the plants.

The black cumin and green betel leave extract contain chemical compounds with the same antifungal agent, namely essential oil. The essential oil for the black cumin contains derivative compounds such as *thymoquinone, thymohydroquinone, dithymoquinone, p-cymene, carvacrol, 4-terpineol, t-anethol, sesquiterpene longifolene, α-pinene,* and *thymol*.[16] Out of the 10 compounds, three compounds showed good antifungal properties, especially in hindering the growth of *Candida albicans*, namely *thymoquinone, thymohydroquinone* and *thymol*.[5] It is in line with the study conducted by Halamova et al., [17] evaluating the antifungal activity of *thymoquinone, thymohydroquinone* and *dithymoquinone* in vitro used the microdilution method at 6 species of rotting milk yeast. It revealed that *thymoquinone* and *thymohydroquinone* showed antifungal properties that could hinder the development of *Candida albicans*.

Thymoquinone is known to have an action mechanism as an antifungal activity by hindering the cell formation, destroying membrane cytoplasm, and changing the nucleus to amorphous (no shape).[18] Furthermore, *thymol,* also known to have antifungal activity, also has an action

mechanism in hindering the growth of *Candida albicans* by poisoning protoplasm, destroying and penetrating the cell wall, as well as precipitating cell proteins. [9] Besides essential oil, saponin also can help hinder the growth of *Candida albicans*. The action mechanism of saponins works by conducting protein denaturation and breaking down the membrane cell that leads to the dead cell.[2]

Green betel leave is also known to contain an essential oil that has a derivative compound in the form of *hydroxychavicol, p-cymene, α-terpinol, terpinyl acetate, methyl eugenol, caryophyllenes, chavibetol, allylpyrocatechol diacetate, stearaldehyde, anethole, eugenol, safrole, ursolic acid, 3β-acetyl ursolic*, and β-*sitosterol*. *Hydroxychavicol* is the only derivative compound of essential oil that plays a role as an antifungal agent. [7]

Ali et al., [19] stated that *hydroxychavicol* has an action mechanism in resisting *Candida albicans* by damaging the cell membrane structure that obstructs the permeability barrier. Furthermore, Singburaudom[20] added that *hydroxychavicol* also has an action mechanism in hindering biofilm growth and reducing biofilm formation carried out by *Candida albicans* as well as preventing the formation of glucan, which is not water-soluble.

Other contents that can hinder the growth of *Candida albicans* include alkaloids and flavonoids. Alkaloids could hinder the growth of *Candida albicans* by obstructing the biosynthesis of nucleic acid. Meanwhile, flavonoids have an action mechanism by impeding pseudohyphae formation during pathogenesis.

CONCLUSION

Based on the results of this study, it can be concluded that the black cumin extract was not more effective in hindering the growth of *Candida albicans* colony on the acrylic resin denture base than the green betel leave extract.

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