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Research Report

The effectiveness of *Nigella sativa* seed extract in inhibiting *Candida albicans* on heat cured acrylic resin

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ABSTRACT

***Background**: Indonesia has a variety of plants that can be used for medicines. One of the medicinal plants is Nigella sativa. Nigella sativa has been used for medicinal purposes, both as medicinal herb and as medicinal oil. It contains saponin and atsiri oils that have antifungal, antimicrobial and antibacterial effects. Nigella sativa has been suggested as denture cleansers since it can inhibit the growth of Candida albicans (C. albicans) on heat cured acrylic resin. **Purpose**: The aim of this research is to know the effectiveness of Nigella sativa seed extract in inhibiting the growth of C. albicans on heat cured acrylic resin. **Methods**: Eighteen acrylic samples were divided into three groups. Group I was control group, only contaminated with C. albicans without immersing in any solution. Group II was acrylic sample immersed in sterile aquades for one hour. Group III was acrylic sample immersed in Nigella sativa seed extract for one hour. **Results**: There were significant differences of C. albicans ($p < 0.05$) among the three groups. The number of Candida albicans was significantly higher in Group I, while that in group II was lower than that in group I, and that in group III was the lowest. **Conclusion**: Nigella sativa seed extract was effective in inhibiting the growth of C. albicans on heat cured acrylic resin.*

***Key words**: Nigella sativa, Candida albicans, heat cured acrylic resin*

ABSTRAK

***Latar belakang**: Indonesia memiliki berbagai tanaman yang dapat dipakai sebagai obat, salah satu tanaman tersebut adalah jinten hitam (Nigella sativa). Pada beberapa negara jinten hitam telah digunakan untuk berbagai tujuan, baik sebagai obat herbal maupun sebagai minyak. kandungan jinten hitam adalah saponin dan minyak atsiri yang mempunyai efek anti jamur dan anti mikroba. Jinten hitam disarankan sebagai pilihan pembersih gigi tiruan yang dapat menghambat pertumbuhan Candida albicans (C. albicans) pada resin akrilik heat cured. **Tujuan**: Tujuan penelitian ini adalah mengetahui efektivitas dari ekstrak biji jinten hitam dalam menghambat pertumbuhan C. albicans pada resin akrilik heat cured. **Metode**: Delapan belas sampel akrilik heat cured dibagi dalam tiga kelompok, kelompok I sebagai kelompok kontrol, lempeng akrilik tanpa direndam dalam bahan apapun selama 1 jam. Kelompok perlakuan II, lempeng akrilik direndam dalam aquades steril selama 1 jam. Kelompok perlakuan III, lempeng akrilik direndam dalam ekstrak biji jinten hitam selama 1 jam. **Hasil**: Ada perbedaan yang bermakna dari C. albicans ($p < 0,05$) pada ketiga kelompok tersebut. Jumlah C. albicans lebih banyak secara bermakna pada kelompok I, pada kelompok II lebih sedikit daripada kelompok I dan kelompok III paling sedikit. **Kesimpulan**: Ekstrak biji jinten efektif dalam menghambat pertumbuhan C. albicans pada resin akrilik heat cured.*

***Kata kunci**: Jinten hitam, Candida albicans, resin akrilik heat cured*

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INTRODUCTION

Acrylic resin is an option for making removable denture base because it is relatively cheap, easy to repair, easy to make denture by using simple equipments, color stable, and easy to be polished.[1] Acrylic resin material has been known since 1937, and used by dentists since 1946 until now since the materials are well received in the dentistry.[2] Materials often used as removable denture base is polymethyl metacrylate (PMMA) acrylic resin, heat cured type which need heat during polymerization.[3]

One of acrylic resin properties is absorbing the water when contacting with saliva it will absorb saliva. When inserted, it will be coated with protein-rich saliva so that pellicle is formed. Pellicle is able to attach with microorganisms, such as *Candida albicans (C. albicans)*.[4] After two hours, pellicle will formed plaque, ie a collection of microorganisms, glycoproteins matrix and polysaccharides which attached to the tooth surface. The process of plaque formation is the same while happened on the denture surface.[5]

Plaque and food accumulation then will cause the frequency and density of *C. albicans* increased. *C. albicans* can contribute to the occurrence of denture stomatitis. Denture stomatitis is an inflammation that occurs on the oral mucosa resulted from using denture.[6]

Removable denture cleansing can be done in two ways: mechanical and chemical ways. One effective way to clean acrylic resin denture is by immersing removable denture in antiseptic liquid/solution or denture cleanser.[7]

Denture cleanser should not cause abrasion or denture dimension changing, therefore, denture cleanser must not contain alcohol.[5] Denture cleanser with buffer containing alcohol actually can make the surface of the acrylic resin acrylic microcrazed. Crazing is the separation of polymer molecular chain caused by mechanical pressure or solvents. Crazing consists of small cracks that make acrylic become brittle, known as an early symptom of acrylic fracture. Denture cleanser materials on market today are quite expensive for the user, therefore, there is a need to find for an alternative material.

Indonesia has a rich natural biodiversity. These diverse plants have already been used by Indonesian people for a long time, either as food or medicines.[9] Since ancient times, Indonesian ancestors have come to know and used medicinal plants to maintain their health and to treat their diseases. The general term of medicinal herbs is traditional herbal medicine. This is suitable with the recommendation of World Health Organization (WHO) that in order to improve and distribute equitable health services for all communities, thus, these effort must be nurtured, and developed in order to be more efficient and effective.[8] In Indonesia many traditional plants are available to be used as antiseptic materials, one of which is *Nigella sativa* that has been used since thousands of years ago and has been examined by experts as a very useful herbs for health.[9]

Nigella sativa also has antibacterial and antifungal effects of aqueous, methanol and chloroform extracts which is able to fight *C. albicans,* compared with standard medicines, such as clotrimazole, cloxacillin and gentamicin. It contains saponin that has antimicrobial effect and atsiri oil used as antiseptic, antioxidant, also have activities against several gram-positive, gram-negative bacteria, and anti-fungal properties. Scientists in Europe have recently stated that *Habbatus sauda* (the black seed) works as an anti-bacterial and anti fungi.[10]

This research is aimed to examine the effectiveness of *Nigella sativa* in inhibiting the growth of *C. albicans* on heat cured acrylic resin. Therefore, this research is expected to provide information to health professionals and public about the benefits of black *Nigella sativa* especially for health, as an alternative denture cleanser.

MATERIALS AND METHODS

The samples in this research is 18 heat cured acrylic resin plate. The dimension of the samples is 10 mm x 10 mm x 1 mm. The samples were devided into three groups, six samples each. Group I was the control group in which the acrylic resin plate was not immersed in any material. Group II was the treatment group in which the acrylic resin plate was immersed in sterile aquades for 1 hour. Group III was the treatment group in which acrylic resin plate was immersed in 20 ml *Nigella sativa* seed extract for 1 hour. The criteria of the sample size: not porous, not polished, not changing in shape, and flat for the surface of the sample.

C. albicans was incubated for 2 × 24 hours at temperature of 37° C. A colony was taken and grown on blood serum for 2 hours at 37° C. Then, *C. albicans* as much as a loop was put into Sabouraud's broth 5 ml, and incubated for 48 hours at 37° C. After incubated, it was adjusted to the standard of Mc.Farland 3 which is identical with 900×10^6 *C. albicans*. This suspension later was used to immerse the acrylic resin.

Saliva taken from one person as much as 50 ml was put in test tube with certain criteria. Saliva was taken from 22 year old health male students who were not smoking, not taking anti-fungal medications, and not taking antibiotics. The saliva was centrifuged for 15–20 minutes at 1000 rpm at 4° C to obtain supernatant which was then filtered and put in sterile test tube for the preparation of pellicle formation on heat-cured acrylic resin base. Sterilization of heat-cured acrylic resin base was conducted by using an autoclave at 121° C for 18 minutes. The heat-cured acrylic resin base was put into sterile saliva for 1 hour at room temperature to form pelikel. The heat cured acrylic resin was removed and rinsed with PBS solution 2 times, was put into Sabouraud's broth media containing a suspension of *C. albicans.* Afterwards, it was incubated 37° C for 24 hours. Each sample was put in a test tube, immersed in 20 cc sterile aquades and 20 cc *Nigella sativa* seed extract until all parts of acrylic plate was immersed for 1 hour. For control group sample was not immersed in any materials. All of the samples were put in Sabouraod's Broth 10 ml, and vibrated with a vortex for 30 seconds to release

C. albicans attaching to the samples. 0.1 ml of *C. albicans* suspension was taken using 1 ml of tuberculin syringe, dripped on Sabouraud's dextrose agar, made spreading, and then incubated for 48 hours at temperature of 37° C. The measurement of *C. albicans* colonies was conducted by using colony counter with unit of cfu/ml. The data was analyzed using ANOVA test.

RESULTS

It was found that the number of *C. albicans* colonies on heat cured acrylic resin after immersing in 20 cc *Nigella sativa* was low compare after immersing in sterile aquadest or without immersion.

Table 1. The mean and standard deviation of the number of *C. albicans* colonies on the heat cured acrylic resins

Group	Number	Mean	Standard Deviation
I	6	164.5000	46.16384
II	6	62.6667	41.12258
III	6	25.0000	16.26038

Prior to statistical calculations, normality test (Kolmogorov-Smirnov) was conducted. Furthermore, to determine whether there are differences among the three groups, ANOVA test was then conducted with significance $p = 0.05$. To know the difference among each treatment group, Least Significant Difference (LSD) test was conducted. There was significant differences between the number of *C. albicans* colonies on acrylic resins immersed in *Nigella sativa* seed extract and without immersed ($p < 0.05$). There was no significant difference of the number of *C. albicans* colonies on between acrylic resin immersed in *Nigella sativa* seed extract and that immersed in sterile aquades ($p > 0.05$) (Table 2).

DISCUSSION

The colony numbers of *C. albicans* on heat cured acrylic resin immersed in *Nigella sativa* seed extract, sterile aquades, and without immersed can be detected. The number of *C. albicans* on heat cured acrylic resin immersed in *Nigella sativa* seed extract, sterile aquades, and without immersed was different among them. This difference was caused by the differences of those materials used to immerse the heat cured acrylic resin.

Table 2. The results of LSD test on the number of *C. albicans* colonies on heat cured acrylic resins

Group	I	II	III
I	-	p = 0.001 *	p = 0.001 *
II	p = 0.001 *	-	p = 0.097
III	p = 0.001 *	p = 0.097	-

*: Significant differences

C. albicans can actually be classified into *Candida* species, the most common and most widely found fungus in oral cavity. *C. albicans* can be found in the entire of oral mucosal surface, especially palatal mucosa and tongue. *C. albicans* is a pathogenic, opportunistic, dimorphic fungus normally found in oral cavity. In oral cavity, there are actually many different strains of *C. albicans* with particular phenotype characteristics that determine its character as commensal or pathogenic.[6]

C. albicans is dangerous, however, if the body's defense is weak, or especially got decreased immune system, then the commensal character of *C. albicans* can be turned into pathogenic one that can cause infections.[10] *C. albicans* can be found in 66% of denture users who have healthy oral cavity, but the prevalence of *C. albicans* can be increased on denture users.[11] Meanwhile, *C. albicans* in oral cavity of non- denture users will only be considered as normal flora with the prevalence of 45%, whereas that in denture users will be increased to 47.5–55.6%.[6] *Candida* is often found on the surface of the maxillary denture because of continuous pressure under the maxillary denture so that saliva antibodies of the area are reduced and the fungus will be able to breed well in between the denture and mucosa.[6]

As noted in the introductory part, it is already known that one of the characters of acrylic resin is absorbing water when contacting with saliva, and then the acrylic resin will absorb saliva.[5] On the other hand, denture is a good place for gathering the remnants of food. Thus, people who have poor hygiene and long wearing dentures will possibly

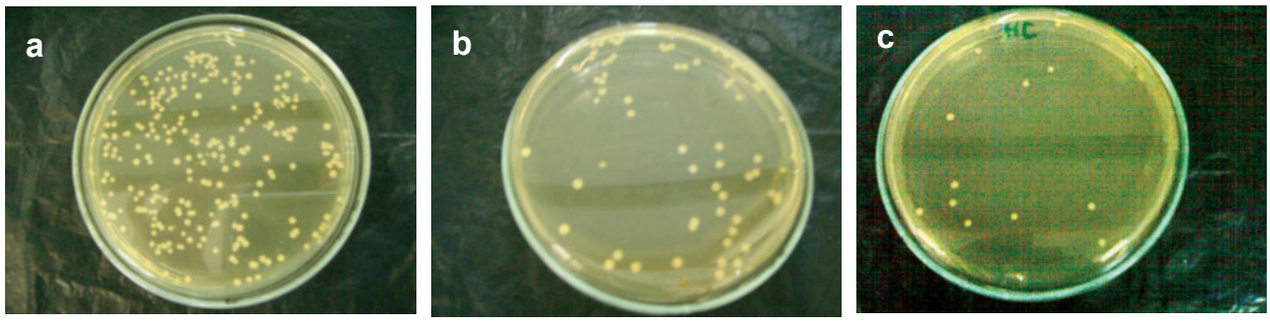

Figure 1. The number of *C. albicans* colonies on control group (a), group I (b), and group II (c).

get plaques on their denture. The buildup of plaque and food scraps then will cause the frequency and density of *C. albicans* increased. *C. albicans* will contribute greatly to the occurrence of denture stomatitis. Denture stomatitis is an inflammation that occurs in oral mucosa resulted from using denture.[6]

To decrease the number of *C. albicans* and prevent denture stomatitis, removable denture cleaning must be conducted. One effective way to clean acrylic resins is by immersing the removable denture in antiseptic liquid/ solution or denture cleanser. Research conducted by the Faisol in 1993 tries to analyze the use of cleaning material soap to maintain the cleanliness of removable denture in which the denture must be brushed with soap and then immersed in antiseptic solution. Faisol then conducted a research on the effect of cleaning material soaps on removable denture base. The result of the research shows that the effective prevention of denture stomatitis can be conducted by using chemical materials, ie immersing the denture in antiseptic solution for 15 minutes, 30 minutes, 1 hour, or all night depended on chemical cleaning materials.[7] Similarly, Zarb *et al*.,[12] also argued that dentures should be cleaned by immersing it in denture cleansers at least 15 minutes at night since 15 minutes is sufficient to kill microorganisms. Based on the above literatures, this research then determined 1 hour immersing time for examining the effectiveness of the materials used as denture cleansers in inhibiting the growth of *C. albicans*.

In this research, moreover, *Nigella sativa* seed extract was used as antiseptic solution or denture cleanser. From the data obtained, it is known that the average of *C. albicans* in Group III (with the immersion in *Nigella sativa* seed extract) was lower than the average of *C. albicans* in Group I and Group II (by immersion in sterile aquades). Based on the data and data analysis, it then can also be known that use of *Nigella sativa* seed extract had a very significant effect on the inhibition of growth of *C. albicans* on heat cured acrylic resin since there was an active compound containing antimicrobial power in *Nigella sativa* seed. Thus, the ability of *Nigella sativa* seed in inhibiting the growth of *C. albicans* was caused by the active compound contained in *Nigella sativa* seed extract.

Nigella sativa seed actually contains atsiri oils, nigelion and arganin crystals, fatty acid, carotene, and 15 kinds of amino acids, proteins, and carbohydrates. Furthermore, it also contains various minerals, like calcium, sodium, potassium, magnesium, selenium, iron, vitamins A, B1, B2, B6, C, E, and niacin. Besides that, it contains chemical forms, such as fats and vegetable oils (35%), carbohydrates (32%), protein (21%), water (5%), saponins, nigellin.[11] Among the contents of *Nigella sativa* seed, the ability of *Nigella sativa* seed as an antimicrobial is caused by saponins and atsiri oils. Saponin is a glycoside forming base in water that exist on many kinds of plants. Therefore, saponin is considered as a surface active chemical compound that can be detected based on their ability to form base. Saponin can be used as an antimicrobial.

Destruction of microbes by antimicrobials, moreover, is considered as bacteriostatic since it still depends on the immune reaction ability of hosts. The action mechanism of antimicrobial can actually be classified into four main methods, namely the inhibition of cell wall synthesis, the inhibition of cell membrane function, the inhibition of protein synthesis, and the inhibition of the nucleat acid synthesis. Saponin then will broke cytoplasmic membrane and kill cells. Therefore, saponin can be used as antimicrobial.

The mechanism of saponin, furthermore, is by denaturing bacteria and breaking cell membranes that can not be repaired again. Since saponin is considered as antimicrobial, saponin then can inhibit the growth of fungi, such as *C. albicans* which atsiri oils can serve as antiseptics, antioxidants, anti fungi and can be able to attack several gram-positive and Gram-negative bacteria. On the immersion in sterile aquades, it is even known that the number of *C. albicans* was decreased because of sterile aquades, but the decreasing was still less than on the immersion in *Nigella sativa* seed extract. This is probably because *Nigella sativa* seed extract contains active compounds that have antimicrobial power, ie saponins and atsiri oil, while sterile aquades only contains sterile solution. It can be concluded that *Nigella sativa* seed extract effective in inhibiting the growth of *C. albicans* on heat cured acrylic resin.

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