# The Synergistic Effects of Areca Nut Extract and Chitosan toward *Candida albicans* in Vitro

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***Abstract*—Areca nut (*Areca catechu L.*) as one of traditional medicines is bactericidal and fungicidal. The anti-fungal compounds of areca nut are saponins, phenolics, flavonoids, terpenoids, steroids and alkaloids. Chitosan is a biopolymer having fairly extensive benefits in biomedical and pharmaceutical fields, i.e. chitosan fibers can be used as surgical sutures, wound dressings, and drugs carriers. Chitosan is also biodegradable, non-toxic, non-immunogenic and biocompatible with the body tissue of mammals. Vulvovaginal candidiasis is a yeast infection caused by *Candida albicans*. This research aims to determine the synergistic effects of areca nut extract and chitosan toward *Candida albicans*. The research methods include the extraction of areca nut; the synthesis of chitosan 2% and the effectiveness test of in vitro using diffusion method. The result shows the synergistic effects of areca nut extract and chitosan toward *Candidia albicans.* The extracts of 5% of areca nut and 1.25% of chitosan are effective as fungicides against *Candidia albicans*. A mixture of 5% of areca nut and 1.25% of chitosan with the ratio of 1:1 shows optimum effectiveness as fungicides against *Candida albicans*, whereas 1.25% of chitosan is a more effective fungicide than positive control. The results are expected to be applied in the field of obstetrics, especially in postpartum care for the prevention of candidiasis or *fluor albus* by using effective and safe natural ingredients.**

***Index Terms*—areca nut extract, *Candida albicans, chitosan*, in vitro, synergistic**

## I. Introduction

Areca palm has been widely used by Indonesians since long ago as the mixture of chewing betel leaves. The cooking water of the areca palm can be used as mouthwash, also as wound and scabies medicines. It is also believed that this plant is advantageous for strengthening teeth. The phenolic chemical contents of areca fruits are bactericidal and fungicidal. The anti-fungal compounds of areca nut are saponins, phenolics, flavonoids, terpenoids, steroids and alkaloids [1].

Chitosan has a wide range of benefits in biomedical and pharmaceutical fields, i.e. chitosan fibers can be used as surgical sutures that can be absorbed by human body, wound dressings, and drugs carriers. Chitosan also affects the blood clotting process that it is used as hemostatic. Chitosan is biodegradable, non-toxic, non-immunogenic and biologically fit with animals body tissues [2], [3]. In the pharmaceutical industry, chitosan can be used as materials for membrane but its mechanical strength is low. Therefore, to improve its mechanical strength, chitosan is cross-linked with 25% of glycerol so that it can be used as hemodialysis membranes [4].

*Candida* is a group of about 150 yeast species. *C. albicans* is responsible for about 70 to 80% of all candidal infections. Other significant species include *C. glabrata*, *C. tropicalis*, *C. krusei*, and *C. dubliniensis*. Candidiasis is skin infection with *Candida sp*, most commonly *Candida albicans*. Candidiasis occurs when there is an overgrowth of *Candida*. Causes may include taking certain drugs especially antibiotics, corticosteroids, and some birth control pills, pregnancy, being overweight, having a bacterial infection, or several different health conditions, for example, a weakened immune system, diabetes, and psoriasis. Infections can occur anywhere and are most common in skinfolds, digital web spaces, genitals, cuticles, and oral mucosa. Symptoms and signs vary by site. Diagnosis is by clinical appearance and potassium hydroxide wet mount of skin scrapings. Treatment is with drying agents and antifungals.

Research on the effectiveness of chitosan that is integrated with areca nut extract as an antiseptic soap has not been conducted. It is expected that the results of this research can be applied in the field of obstetrics, especially in postpartum care to prevent the infection of candidiasis or *fluor albus* by utilizing effective and safe natural ingredients.

## II. Material and Methods

### A. Material and Samples

The research was conducted at the Microbiology and Parasitology Laboratory of Medical Faculty of Sebelas Maret University and the Science Laboratory of Kusuma Husada Surakarta School of Health for 6 months.

The samples were areca nut extracts; chitosan produced from shrimp shells and crab (*Portunus pelagicus*) shells wastes, isolate: *Candida albicans*, Sabaraud Glucose Agar (SGA) culture media, Standards

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Brown II solution, methylene blue dye, and physiological sterile NaCl, chitosan manufactured by Biotechsurindo Cirebon.

### B. Isolation of Chitosan

The samples of shrimp or crab shells were synthesized through deacetylation, demineralization and chitin deproteination stages [5], [6]. The manufactured-chitosan were obtained from PT. Biotech Surindo Cirebon Indonesia. 1,5% of chitosan were dissolved to be 10% of acetic acid solution as Fig. 1.

Figure 1. Crab shells wastes and the products of chitosan synthesis

### C. The Making of Areca Nuts Extract

The extraction of fresh areca nuts was conducted using maceration method [7]. A total of 100 grams of fresh areca nuts were extracted in 1 liter of methanol with magnetic stirrer stirring (150 rpm) at room temperature for 3 hours. After that, the mixture was filtered twice using Whatman filter paper No. 4 and No. 1. The filtrate obtained from the extraction I and II were collected, then the solvent was evaporated with rotary vacuum evaporator at the temperature of 45 ℃, until the solvent condensation did not occur in the condenser, to obtain 100% thick extract. The extract of areca nuts methanol was made with the concentration of 5%, 10%, 15%.

### D. The Making of Candida Albicans Suspension

Pure cultures of *Candida albicans* ATCC 10231 that had been incubated for 48 hours, at the temperature of 37 ℃ were inoculated and suspended in physiological sterile NaCl to obtain a turbidity level according to the standard solution of $10^8$ Mc Farland or containing $10^8$ CFU/ml cells. This suspension was used for inoculation in the test medium of diffusion method [8].

### E. The Test of Antifungal Activity with Diffusion Method

The effectiveness of ethanolic extract of areca nuts and chitosan were tested using diffusion method, using SGA media (Sabaraud Glucose Agar) which were inoculated with leveling suspension *Candida albicans* using sterile cotton sticks. Then, wells were made using boor proof and each of them was filled with the test compound, negative and positive controls. Galenic ethanolic extract 96% with the concentration of 10%, 15%, 20% were dropped into the wells, each of them with the volume of 50 µl, then they were incubated for 48 hours at the temperature of 37 ℃. Next, the formation of the clear area around the wells was observed and the diameter of the constraints area was measured as Fig. 2 and Table I.

TABLE I. THE ACTIVITY TEST RESULTS OF THE MIXTURE OF ARECA NUTS AND CHITOSAN ANTIFUNGAL TOWARD *CANDIDA ALBICANS*

| NO | Description | I(cm) | II(cm) |
|---|---|---|---|
| 1 | Biji Pinang 5% | 0.1 | 0.2 |
| 2 | Biji pinang : Kitosan 1:1 | 0.2 | 0.3 |
| 3 | Biji Pinang : Kitosan1:2 | 0.1 | 0.1 |
| 4 | Kitosan 1.25% | 0.2 | 0.3 |
| 5 | Biji Pinang : Kitosan 2:1 | 0.1 | 0.2 |
| 6 | Paten | 0 | 0 |
| 7 | Asam Asetat | 0 | 0 |

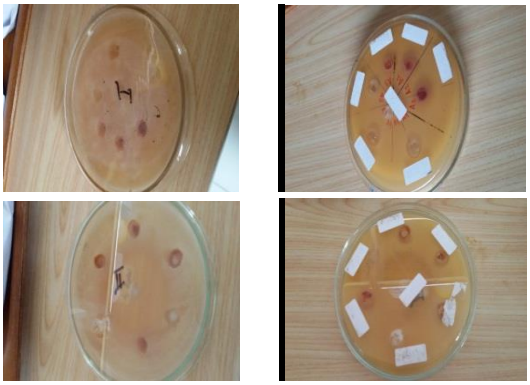

Figure 2. The effectiveness of snail slime and chitosan in wound healing rate

## III. RESULTS AND DISCUSION

### A. The Effectiveness Test of Agensia toward Candida albicans in Vitro

The experimental results and statistical analysis indicates that there is synergistic effect of areca nut extracts and chitosan on *Candidia albicans* (Table I). The extracts of 5% areca nut and 1.25% chitosan are effective as fungicides against *Candidia albicans*. A mixture of 5% areca nut and 1.25% chitosan with the ratio of 1:1 showed optimum effectiveness as fungicides against *Candida albicans*, while 1.25% chitosans are more effective fungicides than positive control and acetic acid.

Areca nut extract contains phenolic active substances which are bactericidal and fungicidal. Anti-fungal compounds in areca nut belong to compound group of saponin, phenolics, flavonoids, terpenoids, steroids and alkaloids [9]. Areca seed also contains alkaloids, including arecoline, arekolidine, arekain, guvakolin, guvasine and isoguvasine. Ethanolic extract of areca nut contains condensed tannins, hydrolyzed tannins, flavonoids, and phenolic compounds, gallic acid, gum, lignin, volatile and non-volatile oils, and salt [9]. The effectiveness of phenolic in areca nut is able to denature the protein binding in cell membrane, so that the cell wall lysis and can penetrate nuclei of candida albicans, the fungus so it can not be breed. Betel nut is widely used as a traditional medicine since antiquity both in Java as a cure wounds also in Borneo (Kalimantan Island in Indonesia) as a remedy scabies. Water boiled betel nut is also used cope with the menstrual blood excess, nosebleeds.

Ethanolic extract of areca nut is potential to have antimicrobial power against *Staphyllococcus aureus, Staphylococcus epidermidis, Salmonella typhi, Escherichia coli, Pseudomonas sp, Bacillus cereus,* and *Candida albicans* [10].

Chitosan's fungicidal effect is caused by the chitosan's good chemical reactivity because of the chains of a number of hydroxyl (OH) and amine groups (NH2). Most polysaccharides found in nature are neutral and acidic such as cellulose, dextran, peptin, alginic acid and agar, while chitosan is an example of polysaccharide which is alkaline, and chitosan is included in heteropolimer [11]. Important property of chitosan is having a positive charge in acidic solutions that are antifungal stronger than chitin. In addition, chitosan is polycationic so it can be used as a clotting agent. Activation of antimicrobial of chitosan is influenced by several intrinsic and extrinsic factors. A low molecular weight chitosan has better activity. Deacetylated chitosan is more perfect, and therefore it is more anti-microbial compared to chitosan which has a proportion of more acetylated amino group because of greater increased solubility and density of payload [12]-[14]. Chitooligosakarida can serve as a natural prebiotic antihypercholesterolemia [15], [12]. The mechanism of COS as anti hypercholesterolemia associated Chitooligosacharida role in the metabolism of carbohydrates and bioadsorbent very effective especially as microparticles with high porosity properties so as to bind macromolecular compounds such as fats [16]-[20].

Candidiasis is a fungal infection caused by yeasts that belong to the genus *Candida*. There are over 20 species of *Candida* yeasts that can cause infection in humans and the most common of which is *Candida albicans*. *Candida* yeasts normally live on the skin and mucous membranes without causing infection; however, overgrowth of these organisms can cause symptoms to develop. Symptoms of candidiasis vary depending on the area of the body that is infected. Genital / vulvovaginal candidiasis (VVC) is also sometimes called a "yeast infection." It is a common infection that occurs when there is overgrowth of the yeast called Candida. Candida is always present in and on the body in small amounts. However, when an imbalance occurs, such as when the normal acidity of the vagina changes or when hormonal balance changes, *Candida* can multiply. When that happens, symptoms of candidiasis may appear. Women with VVC usually experience genital itching, burning, and sometimes a "cottage cheese-like" vaginal discharge. Men with genital candidiasis may experience an itchy rash on the penis. Nearly 75% of all adult women have had at least one "yeast infection" in their lifetime. On rare occasions, men can also get genital candidiasis. VVC occurs more frequently and more severely in people with weakened immune systems. Other conditions that may put a woman at risk for genital candidiasis include: pregnancy, diabetes, long-term use of broad-spectrum antibiotics and use of corticosteroid medications [21], [22].

Differences in the effectiveness of antifungal areca nut and chitosan are due to the different quality and quantity of the active substances of different chitosans and areca nut. Moreover the effectiveness of a substance is strongly influenced by the mechanism or mode of action of active compounds against *Candida albicans*' physiological properties. *Candida albicans*' cell wall is built up with chitin compound so that the effectiveness of a material as a fungicidal substance should be able to degrade the cell wall of *Candida albican*s [23], [24]. Areca nut is more effective as an antifungal than chitosan; it is likely due to the phenolic compounds contained in areca seed that is more fungicidal than chitosan and chitosan is a derivative of chitin and fungal cell wall is contrived of chitin.

A mixture of 5% areca nut and 1.25% chitosan (1:2 and 2:1) is more effective as fungicide compared to patent drug. It indicates that areca nut and chitosan are potential and provide a synergistic effect as fungicides against *Candida albicans*. To determine the MIC (Minimum Inhibitory Concentration) or MBC (Minimum Kill Concentration) of areca nut and chitosan against *Candida albicans*, it is necessary to do further research using dilution method.

## IV. Conclusion

Extracts of 15% areca nut and 1.25% chitosan are effective as fungicides against *Candida albicans.*

A mixture of 15% areca nut and 1.25% chitosan with ratio of 1:1 shows optimum effectivity as fungicides *Candida albicans.*

The presence of synergistic effect of ethanol extract mixture of areca nut and chitosan is utilyzed to inhibit the growth of *Candida albicans.*

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