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# POTENCY OF *Areca catechu* FLESH EXTRACT IN INHIBITING SOFT ROT FUNGI OF MELONS AND BANANAS

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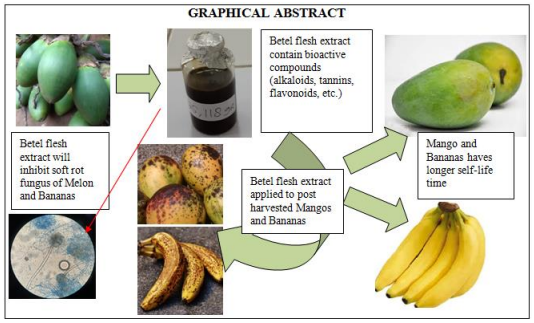
## Graphical abstract




## Abstract

Areca fruit (*Areca catechu*) has traditionally been used as a tooth strengthening and whitener through the habit of "chewing," in Indonesian culture. In Madura, the use of young areca nuts is an herbal medicine for male virility. Wider utilization has not been performed, even though betel nuts contain bioactive compounds, potentially as antimicrobials. The aim of this research was to obtain evidence for the use of watery extract of betel nuts as a natural ingredient to extend fruits' shelf life from fungal attacks. The fungus isolated from melon fruit was *Meyerozyma* sp., and the isolate from banana fruit was *Aspergillus* sp. Extraction of betel nuts using water as a solvent produces a dark brown concentrated solution. The in vitro antifungal test result showed that the inhibitory activity of areca flesh water extract against fungal isolates was still lower than that of 0.2% ketoconazole. The in vivo test results on "Arumanis" mangoes and "Ambon" bananas with *Aspergillus* sp. from bananas showed that hyphae had not appeared on days 4 and 5 of storage at room temperature 25 °C, respectively. Areca fruit extract can extend the shelf life of the fruit at room temperature.

Keywords: Areca catechu, flesh extract, antifungal, soft rot fungus, decayed fruits



## 1.0 INTRODUCTION

The potency of areca nut in traditional medicine is supported by the chemical compounds contained in it. A previous study on areca nut content provided information that areca seeds contain chemical compounds from the saponin, flavonoid, tannin, phenol, steroid, and alkaloid groups [1]. These various chemical compounds are thought to be present in the flesh of the areca nut. These chemical compounds support the potential of areca nut as an antibacterial, antifungal, and antioxidant agent.

The antifungal potential test of areca seed extract has been widely studied, including hexane, ethyl acetate, and water extracts on the fungus *Candida albicans* [2]. Testing of the antifungal activity of water extracts via the betel nut maceration method was conducted on fungi that cause rot, for example, mucormycosis agent for banana, melon, or papaya; potato late blight fungus for strawberries; or alternaria leaf spot for tomatoes. The activity test of areca seed extract has been applied to various fungi; however, the antifungal test of areca nut water extract has not been performed on the fungus that causes fruit rot, through both in vitro and in vivo testing.

The short-term goal is to obtain scientific evidence of the antifungal activity of betel nut water extract against the growth of fungi that cause fruit rot, which

can be achieved through the following stages: 1) isolating fungi that cause fruit rot from several fruits and performing macroscopic, microscopic, and molecular identification; 2) extracting betel nuts using distilled water solvent via the maceration method; and 3) assessing the antifungal activity of betel nut extract against rotting fungi in vitro and in vivo. The long-term goal of this research is to obtain natural antifungal products that have been tested for their effectiveness and safety for application in the food and non-food fields.

## 2.0 METHODOLOGY

### Areca Nut Flesh Preparation

Areca nuts were used in the form of mature fruits that are still green. Furthermore, the areca fruits were released from the seed. The flesh was beated with a stone pestle for easier drying. The drying process was performed in an open space without direct sunlight until it was dry (approximately 2 weeks). Furthermore, the dried areca flesh was used for its fibers. The dried fibers were then cut into pieces with scissors and ground into powder using a grain chopper.

### Extraction

Five grams of areca flesh powder was added with 50 mL of solvent (distilled water). The mixture of areca flesh powder and distilled water was left for 1 h in a water bath at 70 °C/hot water maceration [3] and then filtered with a filter paper. The residue was extracted again in the same way, repeated three times. The extract was evaporated in an oven at a temperature of 50 °C to make it concentrated.

### Isolation of the Fungus that Causes Fruit Rot

Fruits that will be used for fungal isolation (melons and bananas) were kept at room temperature until they were ripe. The fruits were cut with a sterile cutter, then washed with 70% alcohol, and drained. Fruit pieces were placed on Potato Dextrose Agar (PDA) media in a Petri dish and incubated for up to 5 days. Subsequently, subcultures were performed for the dominant colonies until pure cultures were obtained. Pure cultures were incubated for 7 days for spore production.

### Identification of Isolates

Identification was conducted on pure cultures produced macroscopically, microscopically, and molecularly on isolates obtained from melons and bananas.

### In Vitro Antifungal Activity Testing

In vitro antifungal activity was tested to observe the effect of areca water extract on the growth of fungi isolated in seeded PDA medium. The extract was spread on the agar surface and was inoculated with isolate in the center of the media by using the tip of a toothpick. All Petri dishes were incubated in an incubator at 27 °C for 4 days.

The growth of fungi was observed through the diameter of the colony. The length of the diameter was compared for every treatment, which were negative control, positive control, and each value of extract concentration (1:0 and 1:1). The smaller the diameter, the higher the antifungal activity. The antifungal activity was compared with the negative control without extract (sterile distilled water) and the positive control with fungicide of 0.2% ketoconazole.

### In Vivo Antifungal Activity Testing

In vivo antifungal activity tests were performed on mango (*Mangifera indica* var. Arumanis) and banana (*Musa paradisiaca* var. Ambon). The Arumanis mangoes with uniform size and level of maturity were selected. They were washed with soap, drained, wiped with 70% alcohol, left for 30 min for the alcohol to evaporate, and then ready to be used as test fruit for antifungal tests. The Ambon bananas are removed from the base, and the former base is covered with wrap film. They were then sterilized in the same way with mangoes.

Both types of fruit were artificially injured [4] using a sterile cutter with a wound size of 2 × 2 cm2. Furthermore, the surface of the wound was smeared with alcohol, and the alcohol was allowed to evaporate. Then, the artificial wound surface was applied with four types of treatment. The four treatments were smeared with concentrated areca flesh extract without dilution (hereinafter referred to as 1:0 concentration), fruit extract with 50% dilution (hereinafter referred to as 1:1 concentration), negative control (sterile distilled water), and positive control (0.2% ketoconazole), respectively. The number of each fruit was 12 pieces for 4 treatments × 3 replications.

The whole fruit was placed in a closed plastic container that has been sterilized with 70% alcohol, incubated at room temperature, and observed every day.

## 3.0 RESULTS AND DISCUSSION

### Isolation and Identification of Soft Rot Fungus of Melons and Bananas

In the isolation stage of the fungus causing fruit rot, fungal isolates were obtained from melons and bananas as shown in Figure 1. It can be seen from Figure 1 that the fungi isolated from each fruit were not of the same type. Fungi isolated from melons are less white in color with a thread-like structure. The last colony comes from bananas, which look like threads with very dense black spores. The difference in the

types of fungi obtained was probably caused by differences in physical (fruit skin and fruit texture hardness) and chemical properties (chemical composition). Another influencing factor is the difference in the place where the fruit is grown or the environment in which the fruit is produced, that is, melons grow above the ground, whereas bananas hang on trees far from the ground.

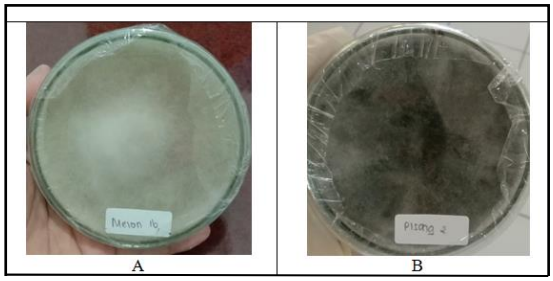



**Figure 1** Fungi isolated from rotted (A. Melon and B. Banana)

The fungus that grows on each of the different types of fruit will be further microscopically and molecularly observed to determine the type with more certainty. The results of microscopic identification can be seen in Figure 2.

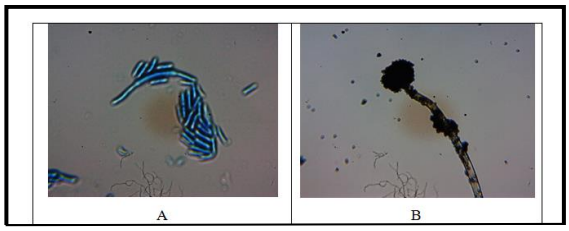



**Figure 2** Fungal isolates from melons (A) and bananas (B) on microscopic identification

In Figure 2, it can be seen that the fungal isolates from melons lead to unicellular fungi (yeast/yeasts), whereas the isolates from bananas lead to multicellular fungi (thread/mold/molds) from the genus Aspergillus. Molecular identification showed that the isolate from melon fruit was *Meyerozyma guilliermondii* from the Ascomycota family, whereas the isolate obtained from bananas was *Aspergillus foetidus*, based on the phylogenetic features in Figures 3 and 4. The presence of *Aspergillus* sp. on bananas is in accordance with the results obtained by a previous study [5] that has isolated molds that are pathogenic for banana plants in Iran.

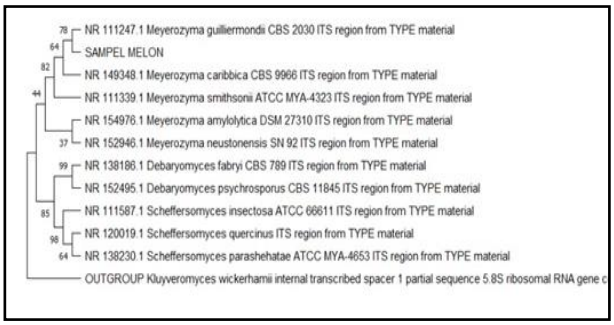



**Figure 3** Phylogenetic tree of fungal isolate from rotted melons

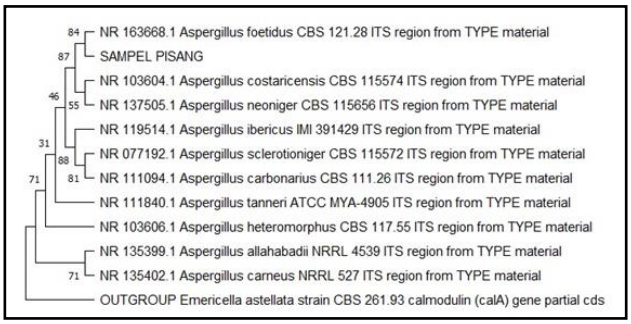



**Figure 4** Phylogenetic tree of fungal isolate s from rotted bananas

## In Vitro Antifungal Testing of Areca Flesh Extracts

The in vitro antifungal test results from the aqueous extract of areca flesh (aqueous extract of betel flesh, AEBF) on PDA media can be seen in Figure 5 for isolates from melons and Figure 6 for isolates from bananas.

In Figures 5 and 6, it can be seen that the water extract of areca flesh generally had antifungal activity, especially against fungal isolates from melons, whereas isolates from bananas showed less inhibitory activity. Different types of fungi, namely, unicellular and multicellular, have different levels of susceptibility to antifungal compounds. This difference is caused by the nature of the structure and different growth rates. Multicellular fungi, also called thread fungi, have a thread-like structure that can spread quickly through the growth medium. Unicellular fungi do not have a mycelium structure, so they have the ability to grow smaller than multicellular fungi.

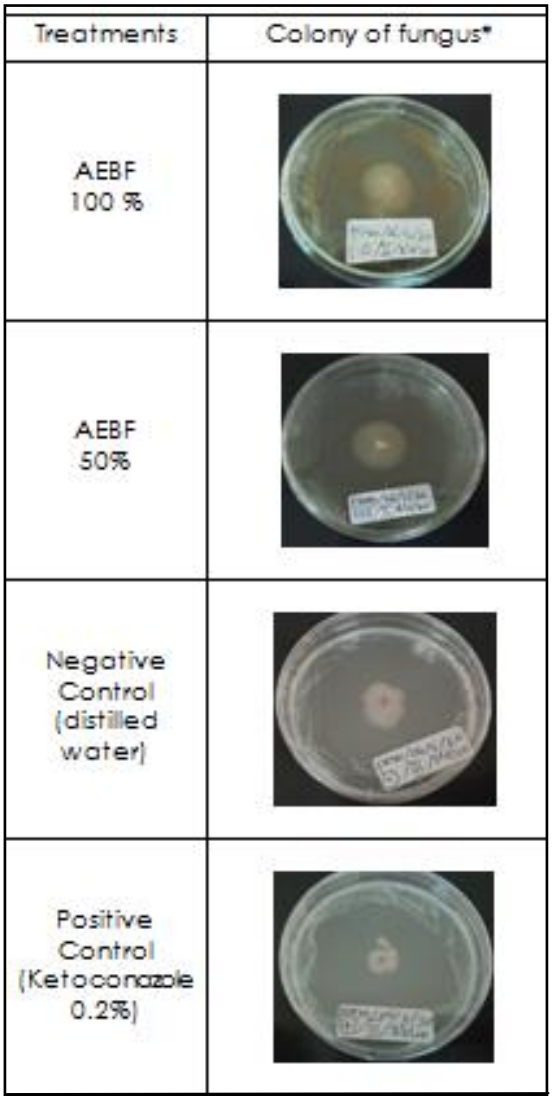



**Figure 5** Antifungal activity testing of betel flesh water extract against fungal isolates from melon (*Best result from 3 replications)

Growth inhibition against yeast from both types of extract concentrations (100% and 50%) showed almost the same effectiveness This means that yeast growth can also be inhibited, even though the extract is diluted up to 50%. These results are in accordance with those obtained by previous studies [6, 7] that betel nut extract has the ability to inhibit the growth of *C. albicans* that is a unicellular fungus. *Meyerozyma* sp. isolated from melon fruit is a unicellular fungus and has similar characteristics with *C. albicans*, so that AEBF can also inhibit its growth.

The effectiveness of AEBF diluted up to 50% (1:1 concentration) against mold isolated from banana fruit that is a multicellular fungus is very low. This is based on the results of colonies growing on media added with an AEBF concentration of 1:1 almost the same in diameter compared to media added with distilled water (negative control). AEBF dilution to 50% so that it reaches a concentration of 1:1 contains very low bioactive compounds inhibiting fungal growth, such as aquadest that is a treatment without extract.

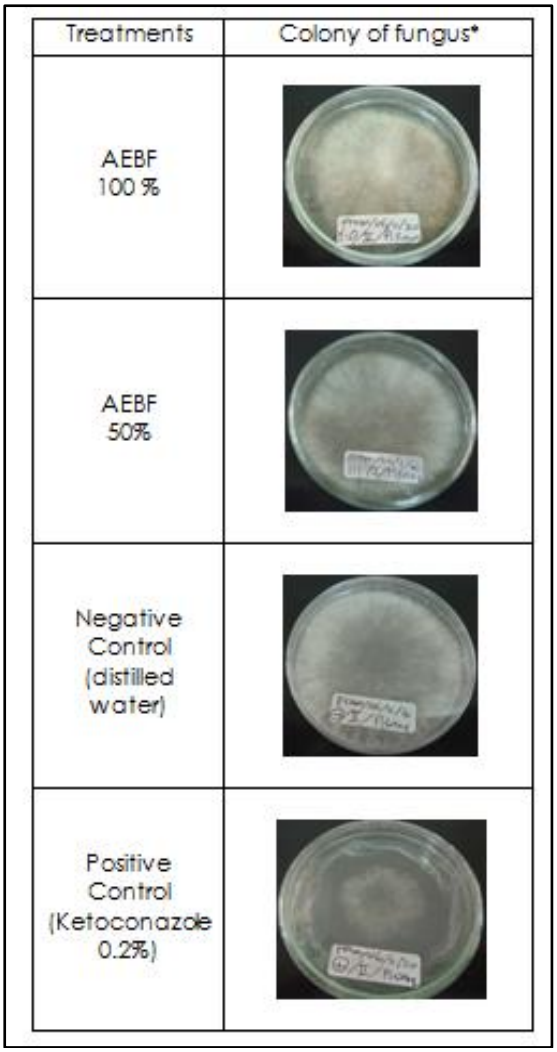



**Figure 6** Antifungal activity testing of betel flesh water extract against fungal isolates from banana (*Best result from 3 replications)

The difference in the effectiveness of AEBF against yeasts and molds indicates that yeast growth is more easily inhibited, because with a concentration diluted with up to 50% aquadest, it is able to provide equivalent inhibition to AEBF without dilution. Multicellular fungi (molds) showed a lower growth inhibition response than yeasts, which were the results of the 50% AEBF concentration treatment equivalent to the addition of distilled water. This result contradicts with the results of a previous study [8] that showed that the unicellular fungus, *C. albicans,* was more difficult to inhibit than the molds *Aspergillus niger*, *Aspergillus flavus*, and *Penicillium notatum* using ethanol extract of areca root. A previous study [8] stated that *C. albicans* was difficult to inhibit because of its resistance to various commercial antifungals. In the study using AEBF, the yeast used

was not *C. albicans*, thus allowing for differences in responses.

The results of the potential test of AEBF as an antifungal agent against two different types of fungi showed that AEBF has potential as an antifungal agent, with unequal effectiveness for unicellular (yeast) and multicellular (mold) fungi. The yeast *Meyerozyma* sp. has higher growth inhibition response than the mold *Aspergillus* sp. to the water extract of areca nut. This result is better than the result shown by a previous study [9]. Areca nut extract did not inhibit filamentous fungi *Mucor*, *A. niger*, and *Cladosporium*. The effectiveness of the aqueous extract of areca nut could be increased by substituting the solvent used, for example, with alcohol, because according to a previous study [10], water extract showed smaller inhibition than alcoholic extract against *C. albicans*. The solvent used in this research was distilled water, because extracts obtained will not give side effect or toxicity [11].

Water extract able to give antifungal activity because its qualitatively contain phenol [12, 13] which has a bioactivity as an antioxidant and an anti-antimicrobial. AEBF is also effective against fungi including *A. niger*, but it has to identify intensively the compounds liable as antifungal agent [14].

## In Vivo Antifungal Test Results

The results of an in vivo antifungal test on "Arumanis" mango and "Ambon" banana were performed by comparing the growth of the fungus in the form of mycelium on the surface of the artificial wound, compared to the negative control (artificial wound smeared with distilled water), and positive control (artificial wound smeared with 0,2% ketoconazole).

The results of observations of the application of AEBF on fruit preservation can be seen in Tables 1 and 2 for mango and banana, respectively.

In testing the effectiveness of AEBF in inhibiting the growth of Aspergillus sp. on mangoes, molds appeared to start growing on mango samples on day 4 of observation for the negative control treatment. The negative control treatment was the addition of sterile distilled water on the surface of the artificial wound on the fruit samples. Mango fruit samples from other treatments did not appear to be overgrown with mold on day 4. This means that the use of AEBF can delay mold growth until day 4, or it can be said that AEBF can preserve mangoes for 4 days longer than without the addition of AEBF. A previous study [15] also stated that the chloroform extract of *Areca catechu* that contained alkaloid and phenolic compounds is able to inhibit mycelium growth and spore germination of *Colletotrichum gloeosporioides*, a fungus causing anthracnose of mangoes.

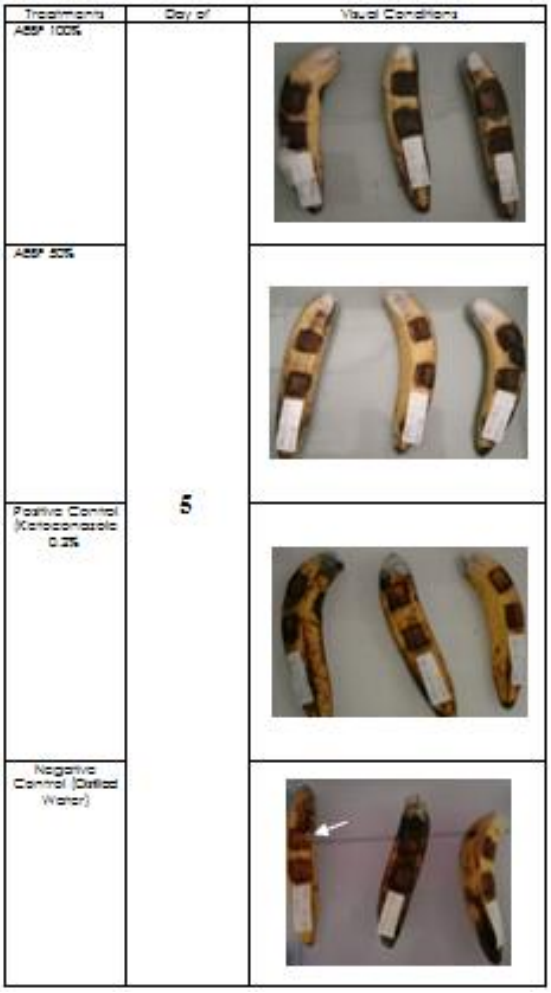
**Table 1** Results of AEBF antifungal potential test on mango fruit preservation

| Treatments | Day of | Visual |
|---|---|---|
| AEBF 100% | 4 |  |
| AEBF 50% | |  |
| Positive Control (Ketoconazole 0.2% | |  |
| Negative Control (Distilled Water) | |  |

In the application of AEBF to the prevention of the growth of the test fungus from bananas (Aspergillus sp.), the banana samples showed different results (Table 2) with those produced from mango samples (Table 1). In the banana fruit samples, fungal growth did not appear until day 5 on the bananas given AEBF and in the samples added with commercial antifungal agent (0.2% ketoconazole), but on day 5, fungal growth was visible on the fruit samples without the addition of AEBF. These results indicate that the shelf life of bananas at room temperature can be extended to 5 days with the addition of AEBF, a day longer than that of mangoes. *Aspergillus* sp. produces cell wall degrading enzymes that cause fruit soft rot [16]. A. niger is also identified as the fruit rotting fungus of mangoes [17].

The difference in yield of mango and banana is caused by differences in the content of bioactive compounds. Bioactive compounds that act as antioxidants can inhibit fungal growth through the mechanism of disruption of the metabolic pathway for the synthesis of cell wall components, namely, ergosterol, glucan, chitin, protein, and glucosamine [18].

**Table 2** Results of AEBF antifungal potential test on banana fruit preservation




A previous study [19] stated that the bioactive compounds in banana fruit flesh and skin are ferulic acid hexoside, rutin, flavonoids (quercetin, myricetin, kaempferol, and cyanidin), carotene (yellow pigment), acids, organics, and tannins. Banana peel also contains high total phenol, which is 29 mg/g as GAE. The main bioactive compounds in mango peel according to a previous study [20] are flavanols (epicatechin-gallate and epigallocatechin-gallate), flavonols (quercetin-3-O-glucopyranoside and rutin), and phenolic acids (gallic acid, o-coumaric acid, and syringic acid). In the present study, the fruit samples were sliced as thinly as possible using a cutter, so that the injured part was then inoculated with AEBF and the fungal isolate.

Based on in vitro and in vivo tests for the antifungal potential of the AEBF, AEBF has potential as an antifungal agent and can be applied to the post-harvest fruit preservation process. AEBF is proven to be used as a natural ingredient that can extend the shelf life of mangoes and bananas at room temperature, that are 4 days for mangoes and 5 days for bananas.

## 4.0 CONCLUSION

Areca flesh water extract has antifungal activity against fungi isolated from melons and bananas in vitro. Its application to fruit preservation through in vivo tests showed that areca flesh water extract is able to extend the life span of mangoes for 4 days and bananas for 5 days.

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