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ANTICANDIDA ACTIVITY OF ETHANOL EXTRACT JERINGAU (*Acorus calamus L*) RHIZOME AGAINST *Candida albicans* ISOLATE REMOVABLE FULL DENTURE ACRYLIC

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ABSTRACT

Background: Maintaining denture hygiene by brushing and soaking it in disinfectant can prevent Candida infections. Jeringau (*Acorus calamus L*) is an herbal plant that its rhizome extract has antifungal activity against *Candida albicans*.

Aim of study: This study aimed to measure whether the value of minimum inhibitory concentration (MIC_{50}) of ethanol extract rhizome of Jeringau against *C.albicans* isolates the maxillary removable full denture acrylic.

Materials and methods: Rhizome of jeringau was prepared. In vitro, study was conducted laboratory experimentally with ten samples tested by serial dilution ethanol extract rhizome of jeringau method with eight different concentrations. They were dropped to 0,1 ml *C. albicans* and incubated at 37 °C for 24 hours and repeated twice. They were grown on Sabouraud Dextro Agar medium that were incubated and seen its growth.

Results: Inhibition zone extract of rhizome jeringau concentration 100% on plate I (9,4 mm) and plate II (8,85 mm). MIC_{50} of ethanol extract rhizome of jeringau is 2,5 mg/ml.

Conclusion: Rhizome of jeringau containing β-asarone which is the major active component of antifungal against *C. albicans*. It was terpenoid group. Terpenoid caused porin damage. When porin was broken it would reduce the permeability of fungal cell wall resulting in fungal cells would lack of nutrients, thus the growth of fungi was inhibited

Keywords: *Candida albicans*, ethanol extract, Jeringau

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1 Introduction

Oral candidiasis is the most common opportunistic infection that attacks the oral mucosa. In most cases, lesions are caused by yeast *Candida albicans* [1]. A variaty of predisposing systemic and local factors lead to the transition from commensal to pathogenic *Candida.* Systemic factors include immunosuppressed, (such as HIV, leukimia, malnutrition), endocrine dysfunction, and use of systemic corticosteroids. Local factors include xerostomia, corticosteroid inhalers, and the use of dentures [2].

Denture stomatitis is a benign and common disease that affects denture wearers. It may be described as a chronic inflammation, with erythema of the oral mucosal tissues supporting a removable prosthesis. The condition is usually asymptomatic, but can give rise to bleeding of the affected areas of mucosa, a burning sensation, halitosis, a bad taste and xerostomia [3].

Management will tend to diminish the need of denture which should reduce the incidence of oral candidiasis. Daily hygienization and disinfection of removable dental prostheses is necessary to promote health and conservation of the oral tissues. The maintenance of healthy mucosa is related to the degree of cleanliness of the prostheses in contact with oral tissue [4].

The most commonly used commercial chemical denture cleansers for use with immersion techniques include alkaline peroxides and hypochlorite. Immersion of denture acrylic in antifungal materials such as sodium hypochlorite and chlorhexidine effectively reduces the attachment of *C. albicans* to tissues [5]. Immersion of denture in chemical or antiseptic materials can have side effects on denture. The long time immersion in sodium hypochlorite and chlorhexidine causes the pigment color of the acrylic plate to fade [6].

Currently herbal medicine is being developed and is an alternative choice. The advantages of using herbal medicine are natural, relatively affordable and safe prices, and relatively no side effects, so many researchers try to find alternative ingredients from herbal medicines. Herbal medicines that have been widely used as raw material for medicine include betel, aloe vera, jeringau rhizome, and others. Jeringau (*Acorus calamus* L), very popularly known as “sweet flag,” is native to Central Asia, North America, and Eastern Europe. *A. calamus* L is an herbal plant that its rhizome extract has antifungal activity against *C.albicans* [7].

The differences in the location of plant growth, climate, and geographical differences can result in differences in the content of the active substance so that research is needed to obtain the minimum inhibitory concentration of jeringau rhizome extract in Indonesia against *C. albicans* isolates of removable full dentures acrylic. The purpose of this study was to obtain the minimum inhibitory concentration value of the ethanol extract of the jeringau rhizome on *C.albicans* isolates the maxillary removable full denture acrylic.

2 Materials and methods

2.1 Collection of plant material

The plant material was collected from field at Babakan Baru Village, West Java Indonesia. It was thoroughly washed and shade dried at room temperature (37^0C) for about a week (Fig.1). Then It was cut pieces. 100 gram of the pieces plant material were used for extraction.

2.2 Preparation of ethanolic plant extract

Making of jeringau rhizome extract using 70% ethanol by maceration for 3 x 24 hours and every 24 hours macerate is collected and remacerated with a new 70% ethanol solution. The maserate obtained is thickened by a rotary evaporator until a thicker extract is obtained. The extract was then poured into an evaporator so that a thick extract was obtained which was considered to have a pure concentration.

2.3 Microorganism

Examination of culture colonies characteristic on CCA is carried out after the incubation process. CCA is a medium for detecting and isolating

Candida spp. *C. albicans* colonies will show green, blue (*C. tropicalis*), bluish green (*C. dubliniensis*), brown young (*C. galabrata*), pink (*C. krusei*). Colonies that showed the characteristics of *C. albicans* were isolated from oese then planted in SDA (Sabouraud Dextro Agar) then incubated at 37^0C for 18-24 hours to obtain pure isolates of *C. albicans*. *C. albicans* suspension is made equivalent to the turbidity of Mc Farland 0.5 using glucose bulyon as a solvent.

Fig. 1: Jeringau (*Acorus calamus L*)

2.4 Agar well diffusion assay

Determination of antifungal activity was carried out by agar diffusion method. First the SDA plate is prepared, then the suspension of *C. albicans* which is equivalent to the turbidity of Mc Fahrland 0.5 is taken as much as 0.1 ml planted on the SDA plate. Next heats the hole maker which serves as a perforator which of jeringau rhizome extract with a concentration of 100% and sterile distilled water as negative control. Then incubated at 37^0C for 24 hours facultative anaerobe and observed the inhibition area formed around the holes. The area of inhibition formed is measured by calculating the diameter of the inhibition area minus the diameter of the hole, then the result is divided in two [8].

2.5 Serial dilution assay

In vitro study method was conducted laboratory experimentaly with ten samples tested by the method of serial dilution ethanol extract rhizome of jeringau with eight different concentrations. The examination material was carried out by applying a sterile cotton bud to the base plate of maxillary removable full denture acrylic. The results of the smear were inserted into the transport medium in the test tube containing glucose bulyon. The examination material was planted in Candida Chromogenic Agar (CCA) and then incubated at 37^OC for 24-48 hours.

Making a standard solution by diluting jeringau rhizome extract by dissolving 1 gram of jeringau rhizome extract into 12.5 ml glucose bulyon so that the concentration of 80 mg/ml was obtained. Determination of the MIC of jeringau rhizoma extract on the growth of *C. albicans* was carried out by serial dilution of eight different concentrations as well as positive control and negative control. The MIC of the test material is

determined based on the 2x dilution method in 10 tubes.

A total of 2 ml of bulyon was filled into tubes 2 to 8 and 10. The first tube was filled with 4 ml of the standard solution, then pipette as much as 2 ml into tube 2, then shake until homogeneous. After that, from the tube 2, 2 ml of the solution was taken and then put into tube 3, and shaken until homogeneous, and so on until tube 9. The suspension of *C. albicans* in a bulyon was made equal to the turbidity of Mc. Fahrland 0.5 in pipettes of 0.1 ml each to tubes 1 to 8 and 10. Tube 9 only contains a bulyon and jeringau rhizome extract solution was a negative control and tube 10 containing a solution of bulyon and *C. albicans* was a positive control. After that all the tubes were incubated at 37^0 C for 18-24 hours.

The turbidity of each tube was recorded and then 1 oese was taken from each tube to be planted in SDA then the culture was incubated at the same temperature and time. The culture of the sector was observed in the number of colonies growing. MIC was shown in sectors that show the least growth of colonies.

3 Result

The examination material consisted of a swab of five the maxillary full dentures using a sterile cotton bud which was then inserted into a test tube containing glucose bulyon. The result of culture 0.1 ml of test material in CCA to show the growth of the Candida colony. Candida which shows green colonies is *C. albicans,* blue (*C. tropicalis*), bluish green (*C. dubliniensis*), brown young (*C. galabrata*), pink (*C. krusei*) (Fig.2).

Colonies that show the characteristics of *C. albicans* in Candida Chromogenic Agar (CCA), which are light green, taken with a sterile needle and then cultured at SDA. The results of culture in SDA show round colonies, slippery, convex, yellowish white, distinctive smell like yeast (Fig. 3a). The results of microscopic examination of suspect colonies as Candida with Gram staining showed that the yeast showed a round, oval or oval shape with cell size of 3µm x 5 µm, purple and Gram positive (Fig. 3b).

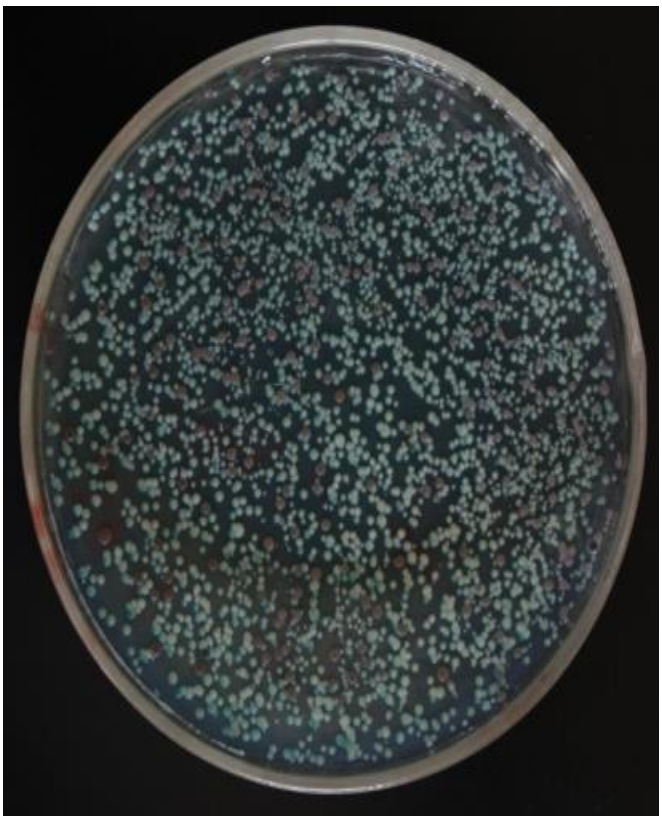

Fig. 2: Culture of candida on candida chromogenic agar.

Fig. 3a: *C. albicans* on saboraud dextro agar (SDA).

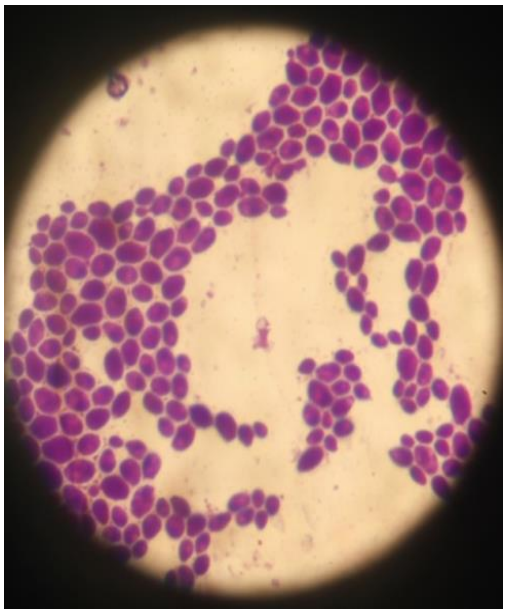

Fig. 3b: Gram staining *C.albicans.*

Inhibition test extract of rhizome jeringau. Planting 0.1 ml of suspension of *C. albicans* equivalent to 0.5 Mc. Fahrland at SDA. Then made 2 holes, the first hole was dripped with 100% dingo rhizome extract and the second hole was stained with sterile distilled water as negative control. Inhibition zone extract of rhizome jeringau concentration 100% on plate I (9,4 mm) and plate II (8,85 mm) (Fig. 4).

Fig. 4: Inhibition zone extract of rhizome jeringau against *C.albicans*

The dilution results of *A. calamus* L rhizome extract ethanol extracted 0.1 ml *C. albicans* with Mc Fahrland 0.5 turbidity were then incubated 37^{o}C for 18-24 hours showing different levels of color turbidity in each tube. The solution in the first to fourth tubes is dark brown to brownish yellow while fifth tube has begun to show a clearer color. This is because the ethanol extract of the jeringau rhizome is dark brown (Fig. 5).

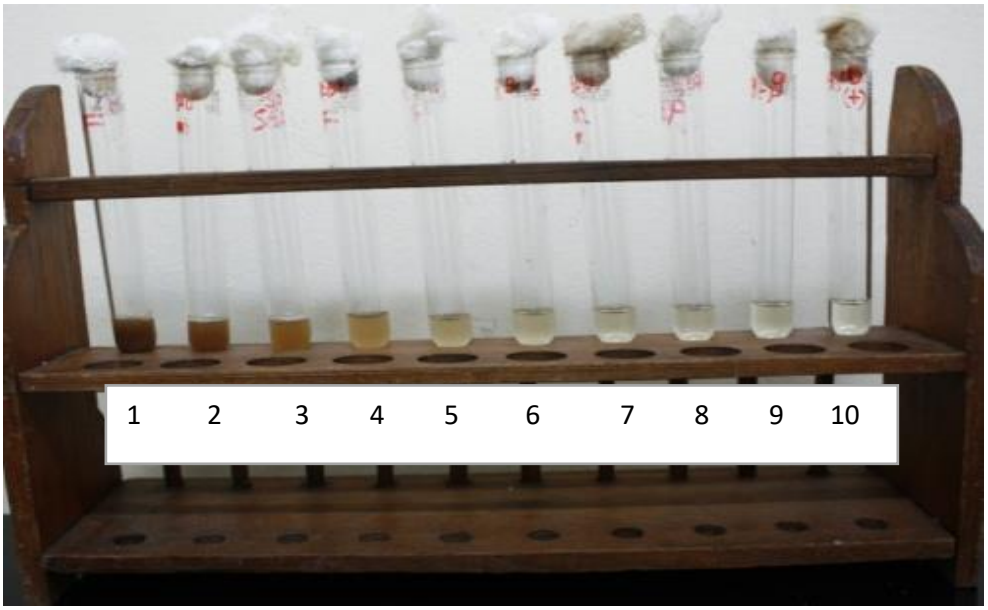

Fig. 5: Results culture of *C. albicans* in MIC test in tubes reaction. Concentration of jeringau rhizome ethanol extract on tubes 1-8 (80,40,20,10,5, 2.5, 1.25, 0.625 mg/ml), tube 9 negative control, tube 10 positive control.

Fig.6: MIC determination with growth of *C.albicans* on SDA. Concentration of jeringau rhizome ethanol extract on sector SDA 1-8 (80,40,20,10,5,2.5,1.25,0.625) mg/ml, sector 9 and 10 (negative and positive control)

The results of sector culture in the SDA show the first to fourth sectors that there is little growth in the *C. albicans* colonies. Colony growth increased in the fifth to eighth sectors (Fig.6).

Table 1: Mean of colonies *C.albicans*

Group of Test		N	Mean of Colonies
Tube	Concentration mg/ml		
9	Control (-)	10	0
1	80	10	31,60
2	40	10	69,45
3	20	10	6694,80
4	10	10	13350,15
5	5	10	23335,85
6	2,5	10	26668,05
7	1,25	10	56672,10
8	0,625	10	73335,30
10	Control (+)	10	100000

The first tube with a concentration of 80 mg/ml had the highest inhibition activity of 99,968 colonies. The eighth tube with a concentration of 0.625 has the smallest inhibition activity of 26,665 colonies. The sixth tube with a concentration of 2.5 mg/ml have a inhibitory power exceeding 50% of the initial colonies, namely 73,332 colonies. The first to sixth tubes have a resistance of more than 50% of the initial colony (Table 1).

The results of the planting sector show the number of colonies of *C. albicans* that are different for each different concentration. Concentrations that can inhibit the growth of *C. albicans* more than 50% of the initial colonies are in tubes 6,5,4,3,2,1. The smallest concentration of them is the sixth tube that MIC_{50} of the jeringau rhizome ethanol extract against *C. albicans* is 2.5 mg/ml.

4 Discuccion

The results of this study differ from the hypothesis that MIC of jeringau rhizome ethanol extract against *C. albicans* isolates removable full denture acrylic is about 4-5 mg/ml. This can be caused by differences in jeringau rhizome extract solvents between these studies using ethanol solvent while previous studies used ethyl acetate solvent.

Study potential jeringau rhizome and its active principle, β-asarone, was evaluated against *C. albicans.* β-asarone exhibited promising growth inhibitory activity at 0,5 mg/ml. The growth inhibitory activity of β-asarone might be through inhibition of ergosterol biosynthesis [9]. The lyophilized aqueous extract fungicidal property had been observed with an MIC/ MFC value for *C. albicans* at 1,56 µg/ml for *C. albicans* ATCC 90029 and 1,56 µg/ml for clinical [10]. The results of this study are different from the results of research conducted before because differences in the location of plant growth, climate, and geographical differences can result in differences amount the compouds active.

The effect of β-asarone on fungal hyphae and conidia shows drastic changes with shrinking and destroyed morphological forms. This can be caused by a leak in the cell wall or perhaps a change in membrane permeability.

Essential oils (alfa asarone, beta asarone) were a mixture of compounds which were terpenoid group consisting of monoterpenes and sesquiterpenes. Terpenoids have ability to inhibit the growth of fungi by damaging the cell membrane so that the fungal growth was inhibited. Terpenoids reacted with porin on outer membrane of fungal cell wall to form a strong

polymer bond so that it caused porin damage. When porin was broken it would reduce the permeability of fungal cell wall resulting in fungal cells would lack of nutrients, thus the growth of fungi was inhibited or die [11].

5 Conclusion

Based on the results of this study, it can be concluded that the minimum inhibitory concentration 50 of the jeringau rhizome ethanol extract against *C. albicans* of removable full denture acrylic isolates was 2.5 mg/ml.

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