

## Anticandidal in vitro test of crude ethanol extract of pliek u on the growth of *Candida albicans*

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**Abstract** This research was conducted to know the activity of crude ethanol extract of pliek u (E) through in vitro assays. Pliek u obtained from households industry in the Reudep village Aceh Besar, Aceh province. Yeasts used in clinical trials is *Candida albicans* isolates obtained from the Mycology Laboratory of the Faculty of Veterinary Medicine, Bogor Agricultural University. Anticandidal activity was determined on a paper disc, whereas in the broth dilution method is used to set a killing time activity of the E. The concentration of E that is used to determine the killing time activity is the value of LC50. Crude ethanol extract of pliek u (E) showed anticandidal activity with inhibitory zone diameters of  $10.67 \pm 0.47$  mm. Ethanol extract of pliek u at a concentration of 3.36 mg / ml can reduce the number of *C. albicans* within two hours at  $2.57 \log$  CFU / ml compared to controls at  $7.42 \log$  CFU / ml. Based on this study can be concluded that ethanol extract of pliek u (E) can be considered an alternative drug for treating candidiasis, so it needs further research to veterinary clinics in vivo applications.

**Key words:** anticandidal, pliek u, fermented meat coconut, *Candida albicans*

### Introduction

Pliek u is coconut meat fermented involuntary during few days naturally without the addition of any microbes. It is the last product of fermentation after separation of the oil during the first, second and third steps of fermentation (Nurliana et al., 2008). Pliek u and pliek u oil have extended use traditionally as spice in Aceh. Pliek u oil is used for generations by the people of Aceh as cooking oil and to reduce a number of health problem such as skin diseases, fever, headache, injuries and stomache.

Traditional fermented foods have an increasing nutritional value by reduction of toxic compounds, better digestibility and production of vitamins and antimicrobes (Campbell-Platt 2000). Fermented foods containing organic acids, bacteriocins, alcohol, fatty acid, enzymes are resistant to deterioration by spoilage microorganisms. More recently, it has been established that some natural compounds produced by fermenting cultures can be extracted and purified, and they use as preservative foods or antimicrobial agents (Hoover 2000). The traditional fermented food such as tempeh possessed powerful antimicrobial activity (Gandjar 2000).

Based on many reports, coconut meat and its oil contain various substances with therapeutic effects. The components of lipid such as fatty acids and their derivatives in coconut are very useful as functional components or antimicrobial compounds (Kabara 2000). Free fatty acids and their monoglycerides were proven as antimicrobial activity against various microorganisms such as bacteria, fungi and virus, and also did not generate resistance of microbes (Wang et al. 1993; Kabara 2000; Nair et al. 2005). Crude ethanol extract of pliek u has an excellent antibacterial activity compared to hexane extract of pliek u and oil pliek u (Nurliana et al. 2008; 2010).

Using plant extract as alternative antimicrobial therapy re-grow since the 1990's with increased disease caused by microbial infections. Microbes resistance against certain antimicrobial occurs due to uncontrolled use of antimicrobials that are broad spectrum (Reimer et al., 1997; Pfaller et al., 1998; Pappas, 2006), causing increased incidence of diseases caused by bacterial and fungal infections (Pfaller et al. 2000; Petersen et al. 2004). Mycosis or thrush or candidiasis is an infection caused by *Candida* sp, particularly by *Candida albicans* and is zoonotic (PAHO, 2003). Candidiasis can occur due to the use of prophylactic antifungal such as broad-spectrum antibacterial (Samaranayake and Samaranayake, 2001; Pappas, 2006). Candidiasis in animals have shown similar symptoms to humans. Lesions in the human digestive tractus caused by *C. albicans* also showed similar lesions in the digestive tractus of swine (Andrutis et al., 2000). Factors that cause colonization, invasion and spread of *Candida* sp in the digestive tract of animals is also caused by antibiotic therapy, antifungal and immune deficiencies.

In Indonesia the case of animals candidiasis less attention, since there are many diseases caused by bacteria and viruses that also cause morbidity and mortality in animals. Levels of toxicity, resistance of microbes (reemerging infection) and the expensive the prices of antifungal causing many researches are conducted to discovery the new anti-candida to treat mycosis, candidiasis. Therefore, to support the benefits of pliek u as a source of antimicrobial, it is necessary to detect the activity of ethanol extract of pliek u against *C. albicans*.

## **Materials and Methods**

### ***Pliek u***

Pliek u was obtained from home industry, in Redeup village at the Aceh Besar, Aceh, which gave antibacterial activity. Oil pliek u consisted of minyeuk simplah (M) and minyeuk Brok (MB).

### **Preparation of ethanol extrac from *pliek u***

*Pliek u* 20 g was macerated in 200 ml of ethanol 96% and stirred in incubator shaker (Innova 4230, New Branswick scientific, Edison, USA) at 28°C and 130 rpm for 24 hours. This extraction procedure was repeated three times. Extracts were filtered using fritted glass filter which is connected with vacum pump. The extracts were pooled and concentrated using rotary evaporator (Büchi, Switzerland) with waterbath temperature of 40-50°C and under pressure of 175 mBAR. The crude ethanol extract was concentrated with air from air compressor. Extraction procedure was done according Sudirman (2005).

### ***Candida albicans* and Culture Media**

Clinical isolate of *Candida albicans* was used as a test microbe, obtained from Mycology Laboratory of Veterinary Medicine Faculty, Bogor Agricultural University. The activity of E, M, and MB were determined using Potato Dextrose semi-solid agar and Broth.

### **Detection of anti-candida Activity**

Anti-candida activity test detected using the paper disc method, dropped 100 mL (99.0-100.5 mg) of E and Oil pliek u (M and MB) on paper discs (13 mm diameter) respectively, then dried with a hair dryer (temperature  $\pm 40^\circ\text{C}$ ), sterilized with UV light (254 nm) for 30 minutes. All paper disks placed on PDA semisolid containing *C. albicans* ( $10^6$  CFU/ml), pre-incubated at temperature  $10^\circ\text{C}$  for 3 hours, then incubated at optimal growth temperature of *C. albicans* at room temperature (26-28°C) for 48-72 hours. Candistin used as controls (Pharos) of 100 mL containing 10,000 IU nystatin per paper disc. Each test performed three replicates. Anti-candida activity measured by clear zone formed using a millimeter scale ruler.

### **Detection killing time activity of crude ethanol extract of pliek u**

Testing was done by adding crude ethanol extract of pliek u (E) at concentration of 3.36 mg/ml (LC50 value) in Potato Dextrose broth containing *C. albicans* CFU  $10^6$ /ml. LC50 values obtained from the results of previous studies based on toxicity tests using *Artemia salina* L (Nurliana et al., 2010). Observations were made at 0, 2, 4, 6, 8, 10 and 12 hours incubation. Mount of *C. albicans* on the initial number (0 \*) and control (0 \*\*) were observed every time incubation were calculated based on plate count method (Swanson et al., 1992).

### **Analysis of data**

The results of anti-candida activity were analyzed descriptively and statistical using analysis of varians, data were shown in form of tables and figures.

## **Results and Discussion**

### ***Anti-candidal activity***

The tests of some antimicrobial against *C. albicans* resulted various clear zone inhibition. Based on paper disc test showed that the anti-candida activity (clear zone diameter) of ethanol extract of pliek u (E) have greater activity (11.67 mm) than MB (8.00 mm), but smaller than anti-candida/candistin (control) (13.67 mm), whereas M showed very little activity (1.00 mm) (Table 1; Figure 1). Although the activity of E was smaller than commercial anticandida/candistin agents (nystatin), but E has a very active anti-candida.

Level criteria of antimicrobial activity was tested (high, moderate and inactive activity) was determined by Elgayyar et al., (2001). Ethanol extract of pliek u (E) is an antimicrobial classified as very active (diameter clear zone  $> 8$  mm) , while the MB is

classified as moderate and inactive for MS. Types of antimicrobials and microbes and also test methods are greatly affect antimicrobial activity (Maguire 2000). Antimicrobial activity was tested using paper disk is strongly influenced by the type and size of paper discs, concentration of media properties and the ability of antimicrobials in diffuse media. Addition of other materials carried by the compound and the type of microbes that are used also affects the antimicrobial activity.

Table 1. Anti-candida activity against *C. albicans*

Type of anti-candidal	Mean± SD of diameter clear zone (mm)
MS	2,00±0
MB	8,00±0,80
E	10,67±0,47
Candistin*	13,67±1,24

\* nystatin 10.000 IU/100 µl



Figure 1. Growth inhibition of *C. albicans* (Ca) caused by M (minyeuk simplah), MB (minyeuk Brok), E (ethanol extract of pliek u) and Cd (candistin).

#### **Killing time activity of Ethanol Extract of pliek u**

The affect of ethanol extract of pliek u (E) and incubation time on the number of *C. albicans* shown on Figure 2. Addition of 3,36 mg / ml of E and incubation times were very significantly ( $P < 0.05$ ) affect against the number of *C. albicans*. Ethanol extract of pliek u (E) at a dose of 3,36 mg / ml decreased the number of *C. albicans* within 2-12 hours of storage than control, but decreasing of *C. albicans* growth was no difference between the in incubation time started 2-12 hours. In previous studies on reducing the number of bacteria (*Staphylococcus aureus* and *Escherichia coli*) after being given the same doses of E showed a decrease after incubation time of 2-4 hours (Nurliana et al. 2008).

Sensitivity of *C. albicans* against ethanol extract of pliek u probably influenced by the growth phase of the test microbes. The growth of cells in log phase or exponential phase is more sensitive and more easily killed than in the stationary phase (Corre et al. 1990 cited by Carson et al. 2002), where the number *C. albicans* on the observation at 12 hours equal to of observations at 8 hours. There was a little antimicrobial effect at the time of the synthesis process of cells during the static phase, it can be caused componens of antimicrobial compounds and its interaction with cell wall and membrane of micobes.

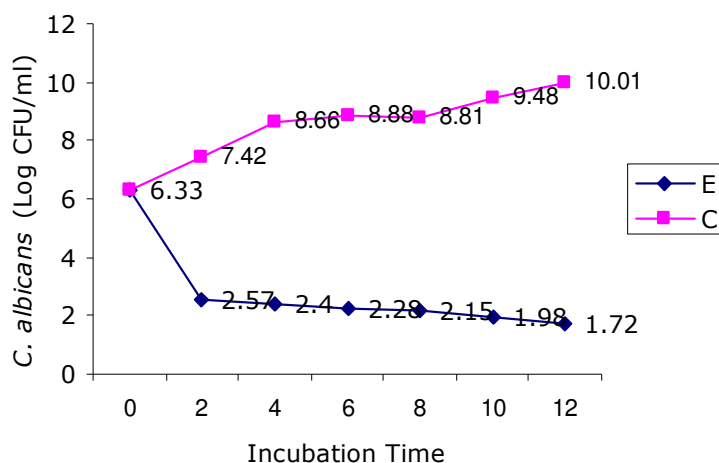


Figure 2. Effect of E at 3,36 mg/ml against *C. albicans* growth in 12 hours incubation; Control (C); Ethanol extract of pliek u (E)

### Conclusions

Ethanol extract of pliek u (E) has anti-candida activity. ethanol extract of pliek u (E) can be considered an alternative drug for treating candidiasis, so it needs further research to veterinary clinics in vivo applications.

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