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Antifungal activities of the rhizome extract of five member Zingiberaceae against *Candida albicans* and *Trichophyton rubrum*

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Abstract. Prastiyanto ME, Rohmah N, Efendi L, Arifin R, Wardoyo FA, Wilson W, Mukaromah AH, Dewi SS, Darmawati S. 2021. Antifungal activities of the rhizome extract of five member Zingiberaceae against *Candida albicans* and *Trichophyton rubrum*. *Biodiversitas* 22: 1509-1513. Fungal infections have now become serious health issues. One of the strategies to avoid the problems of fungal infections is by using natural product from plants that are effective against many human pathogenic fungi. The study portrayed the use of the extracts of plant rhizomes as the alternatives to fight against number of human pathogenic fungi. This research aimed to investigate the antifungal activities of crude ethanol extract of five member of the family Zingiberaceae (*Curcuma longa*, *Alpinia galanga*, *Zingiber officinale* var. *rubrum*, *Zingiber officinale* var. *officinarium* and *Zingiber officinale* var. *amarum*), which are widely used as folk medicines against *Candida albicans* and *Trichophyton rubrum*. Crude ethanol extracts of five members of Zingiberaceae were evaluated for their antifungal activities and the results were calculated based on the zones of inhibition using the diffusion method. The extract showed antifungal activity against *Candida albicans* in the agar well diffusion assay (10.2-27.1 mm inhibition diameter) and against *T. rubrum* (27.3-44.3 mm inhibition diameter). The data have revealed that all rhizomes have the potential to be developed as antifungal agents, particularly against *C. albicans* and *T. rubrum*. Studies on the antifungal activity against yeast-like (*C. albicans*) and filamentous (*T. rubrum*) can provide new information about the benefits of members Zingiberaceae as a source of natural antifungal. Researchers can select the type of rhizome that has more potential for further extraction to obtain pure compounds that can be used as antifungals.

Keywords: Antifungal activity, *Candida albicans*, *Trichophyton rubrum*, Zingiberaceae, zones of inhibition

INTRODUCTION

Fungal infections have become a serious threat to human health and they are associated with at least 1.5 million deaths worldwide each year (Brown et al. 2012). Fungal infection is a common problem occurs at least 20 - 25% of the world's population. Since 1980, the prevalence of fungal infection has been increased in many patient groups (Soetojo and Astari 2016). Human infections those involving fungal, constitute a serious problem, especially in tropical and subtropical developing countries including Indonesia.

Indonesia is one of the countries with tropical climate, that has climate characteristics such as high temperature and humidity. The characteristics of the climate in Indonesia cause the skin to become sweaty and moist. Based on the climatic conditions, lack of knowledge about fungal infections, many people who do not maintain personal hygiene and get exposed to the risk factors of candidiasis in Indonesia. Candidiasis also being the third leading cause of fungal infection in the United States and Europe (Puspitasari et al. 2019).

Dermatophytes and *Candida* spp. are the main infection causing pathogens. Infections caused by the genera *Candida* and *Trichophyton* are responsible for more than 300 million acute or chronic infections worldwide (Boral et

al. 2017). Dermatophytes are a group of fungi responsible for the most common fungal infections in humans. *Arthrodermataceae*, *Onygenales* infect keratin tissues such as hair, nails, and skin. Dermatophytosis is known as "ringworm" or "tinea" with circular lesions on the skin of an infected person (Reiss et al. 2012). *Trichophyton rubrum* is the fungal pathogen causing tinea infection on nails and skin (Ghannoum and Isham 2014; Pathania et al. 2018; Rudramurthy et al. 2018; Singh et al. 2018; Zhan and Liu 2017), and is responsible for 9.5% of all *Trichophyton* infections (Hube et al. 2015). *T. rubrum* is the main cause of chronic superficial infections that affect human psychology and social life (Kong et al. 2015).

While candidiasis is one of the most common infections, widely recognized as major cause of mortality, morbidity and considerable health expenditure (Ashley et al. 2012; Calandra et al. 2016; Kollef et al. 2012; Lortholary et al. 2014). The incidence of *Candida albicans* in Asia from several epidemiological studies suggested that *C. albicans* is the most frequently identified species with an average of 56% of cases of candidiasis (Lewis and Williams 2017; Muadcheingka et al. 2015). *Candida albicans* is still the leading cause of *Candida bloodstream infection* by percentage 33.3% in Singapore, 55.5% in Taiwan 55.6%, and 41% in Japan (Puspitasari et al. 2019). The genus *Candida* consists of 377 species which are

members of *Saccharomycetales*. *C. albicans* is the cause of opportunistic and nosocomial infections (McManus and Coleman 2014). *C. albicans* is ranked among the common five infectious agents causing sepsis and infection of mucosal surfaces of the gut (Vincent et al. 2009). *Candida* can cause severe and invasive infections because it can manifest as candidaemia, disseminated candidiasis, endocarditis, meningitis, endophthalmitis, and infections of other internal organs (Pappas et al. 2016).

The conventional treatments for fungal infection are the use of antifungal agents, such as Azole (ketoconazole, miconazole, and imidazoles), Polyene (nystatin, pimaricin, and amphotericin B), Allylamine (naftifine and terbinafine), Morpholine (amorolfine), and Antimetabolite such as 5-Fluorocytosine. However, the uncontrolled use of antibiotics contributes to the emergence of multidrug resistance fungal strains (Cretton et al. 2017; Piras et al. 2018). So novel antifungal agents are needed from natural biological sources. Biological antifungal can be obtained from the *Actinomycetes* (Sipriyadi et al. 2016), Honey (Oro et al. 2015), saliva (Conti et al. 2013), Venoms of the snakes (Siigur et al. 2019), and Plant (Espino et al. 2019).

Utilization of plants as medicine has been done since ancient times, (Al-dhabi and Arasu 2016; Elango et al. 2016; Manh et al. 2017). Many plants are used as traditional medicines having anticancer (Adnan and Ahmed 2019), antioxidant (Diniyah et al. 2020), antibacterial (Prastiyanto et al. 2020a; 2020b) and antifungal (Kader et al. 2011) properties. The members in Zingiberaceae have been recognized in uses of medicine such as antibacterial (Wibowo et al. 2020), antifungal (Akter et al. 2018), anticancer (Mahomoodally et al. 2019), anti-arthritis (Murugesan et al. 2020) and antioxidant (Ghafoor et al. 2020).

The extraction process is carried out with the aim of taking the active compound present in the plant. Solvents commonly used in the extraction of plants and herbs such as ethanol, methanol and acetone. Apart from the solvent in the extraction process, the method that used in the extraction process is also important to be considered. Extraction methods that are often used such as maceration, soxhletation, infusion, and percolation. The easiest and simplest extraction method to do is maceration method. The maceration method does not require heat, so it does not have the potential to damage the active compounds in the Zingiberaceae.

This research was conducted to investigate the potential of members Zingiberaceae (*Curcuma longa*, *Alpinia galanga*, *Zingiber officinale* var. *rubrum*, *Zingiber officinale* var. *officinatum* and *Zingiber officinale* var. *amarum*) as antifungal agent against *C. albicans* and *T. rubrum*. The data of study are useful for researchers in the fields of medicine, chemistry, biology and pharmacy. By incorporating the data, the researchers can select the types of rhizomes that are more potential for further extraction to obtain pure compounds than can be used as antifungals. The data are insightful to be incorporated for further developments or experiments because researchers can choose the types of rhizomes that are prospective for

further extraction to attain pure compounds that can be used as antifungals

MATERIALS AND METHODS

Plants collection and fungal preparation

The rhizomes of five member Zingiberaceae (*Curcuma longa*, *Alpinia galanga*, *Zingiber officinale* var. *rubrum*, *Zingiber officinale* var. *officinatum* and *Zingiber officinale* var. *amarum*) were obtained from a home garden in Kedungmundu, Semarang, Indonesia. The Rhizomes were washed with tap water to remove all unwanted materials, cut off, rinsed with sterilized distilled water, and then sun dried for three days. The dried rhizome was then ground into a fine powder using a grinding machine and stored in a sterile air-tight container until further process. Fungal isolates (*T. rubrum* and *C. albicans*) were isolated from patients of the Dr. Kariadi Hospital, Semarang, Indonesia. The Fungal were subcultured for 48 h at 35 ± 2 °C on Sabouraud Dextrose Agar (SDA) medium.

Preparations of extracts

The extracts of rhizomes were prepared with a maceration method using ethanol solvent. 100 g of powdered rhizomes of five members of Zingiberaceae (*Curcuma longa*, *Alpinia galanga*, *Zingiber officinale* var. *rubrum*, *Zingiber officinale* var. *officinatum* and *Zingiber officinale* var. *amarum*) was soaked in 300 mL of ethanol for 24 hours under normal room temperature and protected from light in a rotary shaker. The replacement of the solvent was carried out until the solution became clear with the assumption that there was not any active compound contained in the dry powder. The supernatant was filtered on Whatman filter paper grade 1. The solution was concentrated under reduced pressure using a rotary evaporator at 50°C. The crude extracts were collected and allowed to dry at room temperature.

Antifungal susceptibility test

The antifungal activities of the rhizomes of five plants were evaluated using a well-diffusion assay that was modified (Sales et al. 2016). In this method, 100 μ L of each microorganism test equivalent to a 1 McFarland standard was inoculated on the SDA. It was later spreaded on the surface of medium by using a sterilized glass spreader. After 10 minutes of inoculation, the wells were prepared using sterilized steel corkborer (1cm diameter). Wells were made in each plate, out of which five wells were loaded with the various concentrations of five plants, Cl, Ag (0.25-1.00 mg/mL) and Zr, Zo and Za (0.05-0.2 mg/mL). All plates of *C. albicans* were then incubated aerobically at 35 ± 2 °C for 24 hours and plates of *T. rubrum* were incubated aerobically at 35 ± 2 °C for five days. The antifungal activities of the extracts were determined by measuring the diameter of the inhibition zone in mm against the tested fungi.

Phytochemical screening

All extracts were screened for the presence of different classes of secondary metabolites, including alkaloids and flavonoids using previously described methods (Wadood et al. 2013).

RESULTS AND DISCUSSION

Yield of plant extracts

The data provided in this article pinpointed the potential of antifungal activities of ethanolic extracts of *Curcuma longa* (Cl), *Alpinia galanga* (Ag), *Zingiber officinale*. var. *rubrum* (Zr), *Zingiber officinale* var. *officinatum* (Zo), and *Zingiber officinale* var. *amarum* (Za) against *C. albicans* and *T. rubrum*.

We presented the data acquired from extraction, antifungal susceptibility test, and phytochemical screening. The ethanolic extracts of five rhizomes were calculated for

the crude extract (Table 1). All the extracts indicated that the constituents were relatively polar.

Antifungal activities

The agar well diffusion method is a method that is routinely used in clinical laboratories. The antifungal activities of the extracts were determined by measuring the diameter of the inhibition zone in mm against the tested fungi. The antifungal activities of the five extracts were assayed in vitro by agar diffusion method against *C. albicans* and *T. rubrum*. The zones of inhibition are presented in Figure 1. All plants demonstrated the zones of inhibition against *C. albicans* and *T. rubrum*.

Phytochemical

The screening of the phytochemical composition was conducted for all plants. The secondary metabolites are shown in Table 2.

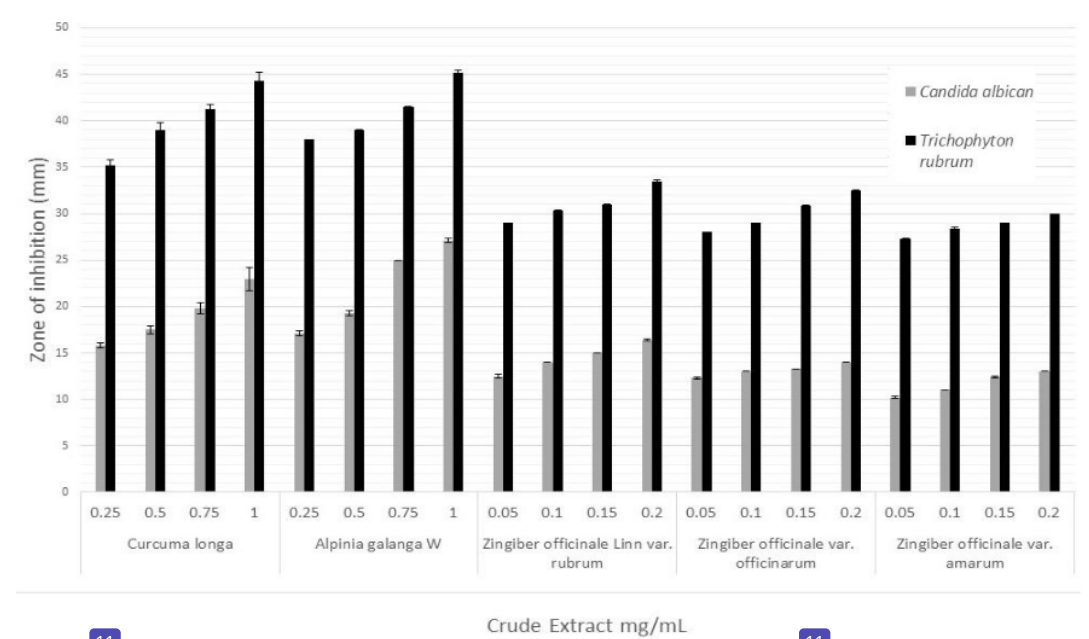


Figure 1. The diameters of the inhibition zones of ethanol extracts from five member Zingiberaceae against *C. albicans* and *T. rubrum*

Table 1. The crude extract

Plants	Part of plants	Solvent (96%)	Crude extract (%)
<i>Curcuma longa</i>	Rhizome	Ethanol	19.7
<i>Alpinia galanga</i>	Rhizome	Ethanol	13.3
<i>Zingiber officinale</i> var. <i>rubrum</i>	Rhizome	Ethanol	15.1
<i>Zingiber officinale</i> var. <i>officinatum</i>	Rhizome	Ethanol	14.1
<i>Zingiber officinale</i> var. <i>amarum</i>	Rhizome	Ethanol	11.7

33

Table 2. The results of the phytochemical analysis of rhizome extracts of the selected medicinal plants

Plant extracts	Secondary metabolites	
	Alkaloid	Flavonoid
<i>Curcuma longa</i>	-	+
<i>Alpinia galanga</i>	-	+
<i>Zingiber officinale</i> var. <i>rubrum</i>	-	+
<i>Zingiber officinale</i> var. <i>officinatum</i>	-	+
<i>Zingiber officinale</i> var. <i>amarum</i>	-	+

Note: +: Present; -: Absent.

Research for antifungal agents from natural ingredients has become an important effort, given the higher and many levels of antibiotic resistance among pathogenic fungi. One effort in this study focused on the use of Zingiberaceae members (*Curcuma longa*, *Alpinia galanga* W, *Zingiber officinale* var. *rubrum*, *Zingiber officinale* var. *officinatum* and *Zingiber officinale* var. *amarum*), which are widely available, and less expensive. Thus, the research of alternative medicines and natural from plants against microbial pathogenic has become an important concern all over the world (Gechev et al. 2014).

The antifungal activities of the five extracts were assayed *in vitro* by agar diffusion method against *C. albicans* and *T. rubrum*. All plants demonstrated the zones of inhibition against *C. albicans* and *T. rubrum*. The diameters of the zones of inhibition with various concentrations Cl, Ag (0.25-1.00 mg/mL) and Zr, Zo and Za (0.05-0.2 mg/mL) are presented in Figure 1. The extract showed antifungal activity against *C. albicans* in the agar well diffusion assay (10.2-27.1 mm inhibition diameter) and against *T. rubrum* (27.3-44.3 mm inhibition diameter). These results are in accordance with (Muruges et al. (2019) wherethey studied potent antifungal action of *C. longa* (alcoholic extract) against *C. albicans*. *A. galanga* has antifungal activity against filamentous fungi (Handajani and Purwoko 2008), *Z. officinale* (ginger) had pronounced antifungal activity, including strains that were highly resistant to amphotericin B and ketoconazole (Ficker et al. 2003).

Flavonoids were present in all rhizomes of tested plants. These bioactive compounds have been reported to be used by plants for protection against bacterial and are responsible for antimicrobial activity (Khalid et al. 2019; Kumar and Pandey 2013). The mechanism of antifungal activity flavonoids compound in the study still unknown but according to Herrera et al. (2010); Paula et al. (2012); Yousefbeyk et al. (2014), flavonoids inhibit the human fungal pathogens. Flavonoids isolated from *Helichrysum chasmolyticum* were able to inhibit *C. albicans* (Süzgeçselçuk and Birteksöz 2011), whereas Flavonoids isolated from *Camellia sinensis* were able to inhibit *T. rubrum* (Buzzini et al. 2009). Flavonoids inhibits efflux pump in fungi (Serpa et al. 2012).

In conclusion, the extract showed antifungal activity against *C. albicans* in the agar well diffusion assay (10.2-27.1 mm inhibition diameter) and against *T. rubrum* (27.3-

44.3 mm inhibition diameter). The data have revealed that all rhizomes have the potential to be developed as antifungal agents, particularly against *C. albicans* and *T. rubrum*.

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