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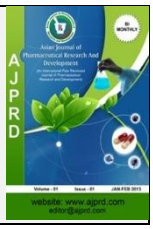

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Research Article

Antifungal Active Fraction of Peria Pantai(*Colubrina Asiatica* (L.) Brong) Leaf Against Fluconazole-Resistance *Candida Albicans*

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A B S T R A C T

Objective: A novel antifungal needs to be found to overcome fungal resistance to antifungal drugs. Medicinal plants contain biologically active compounds as antifungal agents. Peria Pantai (*Colubrinaasiatica* L. Brong) has been used as a folk medicine by the Acehnese community to treat skin disease. This research aim was to evaluate antifungal activity of *C. asiatica* leaf fraction against *Candidaalbicans* resistant fluconazole.

Methode: The extraction was accomplished consecutively as solvent multistep using *n*-hexane, ethyl acetate, and methanol. Phytochemical investigation has done to each extract/fraction. The antifungal activity has been evaluated by *Kirby-Bauer* method.

Result: *The result* of phytochemical investigation of *n*-hexane fraction contains steroid compound, ethyl acetate fraction contains saponin and steroid compounds, and the methanol fraction contains flavonoid, saponin, tannin, and steroid compounds. n-Hexane and ethyl acetate fraction of Peria Pantai leaf have not shown antifungal activity, while it has been shown on methanol fraction with inhibition zone 5,6; 7,17; 8,25; and 11,4 mm at 10%, 20%, 30% and 40%concentration.

Keywords: *Colubrina asiatica, Antifungal agent, Fluconazole-Resiatance Candida albicans, Kirby-Bauer method, secondary metabolite.*

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INTRODUCTION

The epidemiological data propose that the incidence and prevalence of great mycoses continues to be a community health problem . The increased use of antifungal agents has derived in the development of resistance to these drugs. Candida albicans is one of the fungals that can cause progressive systemic disease in patients who have low immunity. Fluconazole is the most commonly prescribed antifungal drugs for Candida infections. But now, fluconazole has resistant to C. albicans. The Center for Disease Control and Prevention USA (2013) estimates of fluconazole-resistance C. albicans infection is 3,400/ year[2,3]. The deployment of multidrug-resistant strains of fungus and therefore the reduced number of medicine available make it needed to get new classes of antifungals from natural products including medicinal plants.

Historically, herbs and spices have enjoyed an upscale tradition of use for their medicinal properties and supply unlimited opportunities for brand spanking

new drug leads due to the large chemical diversity[1]. *Colubrina asiatica* L. Brong or 'Peria Pantai', a medicinal plant from the Rhamnaceae family is found in East Africa to India, Southeast Asia, Sumatra, Tropical Australia, Tropical America, and the Pacific Islands[10].The people in Acehnese (Sumatra-Indonesia) has used*C. asiatica* leaves as soap substitute when they are suffering from measles and chickenpox to reduce itching on the skin. In India, people have used this leaves for food, medicine, fish poison, chewstick and tooth cleaners. The leaves contain secondarymetabolite such as flavonoid, tanin, saponin, danterpenoid that potential as antifungal agent[4]. Several studies have been reported the bioactivity of this medicinal plant such as antifungal, antibacterial and antioxidant properties from leaves and stem[4], antimalarial activity against *Plasmodium falciparum*[13] and CNS effect from the leaves[15].

Since no previous study about the activity of *C. asiatica* against fluconazole-resistance *C. albicans*, it is needed further study to explore it. In this research, we use different polarity of organic solvents to extract the leaves, then subjected various concentration of the extractsagainst fluconazole-resistance *C. albicans.*Hopefully this plant can be develop as antifungal agent.

MATERIALS AND METHODS

This research was conducted at the research laboratory of Pharmacy Department, Microbiology Laboratory of BiologyDepartment, Faculty of Mathematics and Natural Sciences, Syiah Kuala University. This study was conducted in December 2016.

Plant material

The leaves of Peria Pantai were collected from the village of Lam Trieng, Aceh Besar District in July, 2016. Peria Pantai leaf taken is with fresh characteristics, light green to dark green. Peria Pantai plant was authenticated by the Herbarium Bogoriense in the Botanical Field of the Biology Research Center-LIPI Bogor. The leaves weighed as much as5 kg and then washed using clean running water. The leaves were dried at room temperature for 30 days without being exposed to direct sunlight. The dried leaves were ground into powder using an electric grinder.

Preparation of leaf extract/fraction

The leaf powder was extracted using maceration tehnique for 7 days. Eight hundred g ofleaf powder was macerated consecutively as solvent multistep using *n*-hexane, ethyl acetate, and methanol with the ratioleaf powder to solvent was 1:10. The extracts/fractions were filtered through whatman No. 1 filter paper and concentrated with rotary evaporator at 50 °C. The semisolid extract/fraction than store in refrigerator for further analyis.

Determination of extraction yield (% yield)

The yield (%, w/w) from all the dried fractions was calculated as:

Yield (%) = (W1∗100)/W2

W1 is the weight of the fraction after evaporate the solvent

W2 is the weight of the plant powder.

Preliminary Phytochemical test

Phytochemical screening in this study according to Harborne (1996) with a litle modification.

Characterization of fractions

The characterization of fraction was conducted based on methods developed by WHO**(17)**. This methods include water and total ash content, water soluble and ethanol soluble fraction content

Fungal isolate.

The clinical isolates used for this investigation was fluconazole-resistance *Candida albicans*. The possitive control was Ketocenazole. This clinical isolate and possitive control collected from the Laboratory of Microbiology, Faculty of Medicine, University of Indonesia. The isolate

cultivated in Sabouraud Dextrose Agar (SDA) medium and inkubated at 37 °C for 24 hours. The concentration of fungal suspensions were adjusted to10^6 CFU/mL.

Screening for antifungal activities

The investigation of antifungal activity was carried out by agar diffusion method (Kirby-Bauer) using disc paper. All of the fractions dissolve in its extraction solvent. Twenty five mL of Sabouraud Dextrose Agar was poured into each Petri dish. Fungal were grown in SDA medium at 37°C for 24h. Each petri dish was divided into 7 parts. As much as 0,5 ml fungal suspension containing approximately 10^6 CFU/mL dispersed over agar on petri dish. 20 µl n-hexane, ethyl acetate, methanol fractions was absorbed by steril paper disc respectively were placed on agar. Ketoconazole was used for possitive control and n-hexane, ethyl acetate, methanol as negative control. Inhibition zones were determined after incubation for 48 h at 37°C .All tests were done for each extracts with 10%, 20%, 30% and 40% concentration in triplicate.

Table: 1. The Yield of Fractions

Weight of C.asiatica Dried leaf (g)	*C. asiatica* leaf fraction	Weight of Fraction (g)	Yield (%)
800	*n*-Hexana extract	25,33	3,16
	Ethyl Acetateextract	30,18	3,77
	Methanol extract	83,29	10,41

RESULT AND DISCUSSION

Plant authenticated

The Bogoriense Herbarium in the Botany Field of the Biology Research Center-LIPI Bogor was authenticated that plant used in this study were species of *Colubrina asiatica* (L.) Brong from the Rhamnaceae family. The classification was described below.

Domain : Eukaryota

Kingdom : Planta

Phylum : Spermatophyta

Subphylum : Angiospermae

Class : Dicotyledona

Order : Rhamnales

Family : Rhamnaceae

Genus : Colubrina

Species : Colubrina asiatica

The Yield and Phytochemical Constituent of Each Fractions

The result of leaf extracs/fractions preparation using maceration methode is presented in Table 1.

The percentage yield offractions were 3,16%, 3,77% and 10,41% respectively for n-hexane, ethyl acetate and methanol fraction.

The extraction *C. asiatica* leaf using maceration method consecutively assolventmultistepusing *n*-hexane,ethyl acetate,andmethanol which extracted the compound into its polarity group due to the solvent.From Table 1, we conclude that disparity of this yield is due to the soluble phytochemical constituentfrom *C. asiatica* leaf which different solubility due to the solvent in use.

The result of phytochemical screening show in Table 2. This data show the secondary metabolites soluble in *C. asiatica*

leaf extracts. n-Hexane extract contains steroid, ethyl acetate extract contains saponin and steroid, methanol extract contains flavonoid, tannin, saponin and steroid compounds. This result directly proportional to the percentage yield of the extracts where the methanol extract has the highest yield. However this dataslightly different with previous report that C. asiatica contain alkaloid as well (4). The differences in the location of growth can affect the content of secondary metabolites of a plant.

Table: 2 The Phytohemical Screening of Fraction

C. asiatica leaf extracts	Secondary Metabolite					
	Alkaloid	**Flavonoid**	***Saponin***	***Tannin***	***Steroid***	***Triterpenoid***
n-Heksana extract	-	-	-	-	+	-
Ethyl Acetateextract	-	-	+	-	+	-
Methanol extract	-	+	+	+	+	-
Not: (+) : presents the secondary metabolite from the extract (-) : not presents the secondary metabolite from the extract						

Characteristic of Fraction

The characteristic of water content from n-hexane, ethyl acetate and methanol fraction were 11,86%, 16,23%, and 26,18% respectively. This data meet the standard requirement that water content from the fraction doesn't exceed than 30% to avoid the growth of fungi and microorganisms. The total ash value from n-hexane, ethyl acetate and methanol fraction were 6,4%, 5,02%, and 1,64% respectively. These values show the total amount of material remaining after ignition which is derived from plant tissue itself and derived from the residue of the extraneous matter (like sand and soil) adhering to the plant surface[17]. The soluble water content from n-hexane, ethyl acetate and methanol fraction were 26%, 49% and 74,6% respectively. This value increases simultaneously

as the polarity as the solvent increase. The ethanol soluble content from n-hexane, ethyl acetate and methanol fraction were 39%, 80% and 78,6% respectively. These data showed that ethyl acetate fraction more soluble to ethanol than others.

Antifungal Activity

The antifungal activity of *C. asiatica* leaffractions were shown on the Table 3 and Figure 1. n-Hexane and methanol fractions of each concentration didn't show inhibition zone while methanol fraction at 10%, 20%, 30%, 40% concentration showed antifungal activity with inhibition zone diammeter 5,6 mm, 7,17 mm, 8,25 mm, 11,4 mm respectively.Antifungal activity of *C. asiatica* leaffractions on this reasearch was obtained by measuring inhibition zone from each extract concentration (10, 20, 30 and 40%). Table 3 presents Ketocenazole as possitive control showed very strong activity with inhibition zone (46,48-48,01 mm). n-Hexane and ethyl acetate fractions of *C.asiatica* leaf didn't show antifungal activity with 0 mm inhibition zone but methanol fraction showed moderate until strong (5,6-11,4 mm) antifungal activity against fluconazole-resistance *Candida albicans*. The methanolic fraction concentration is directly proportional to the antifungal activity. The greater the fraction concentration, the higher the antifungal activity produced.Based on the exploration from previous researchs, we didn't find the antifungal activities of *C. asiatica* leaf against fluconazole-resistance *C. albicans*. However, it has been reported the antifungal activity from methanol and aceton extracts against *C. albicans* with inhibition zone diameter less than 20 mm [8]. Flavonoid and jujubogenin glycoside responsible for antifungal activity of *C. asiatica* leaf[10].

The antifungal natural products belonging to allmajor classes of secondary metabolites suchasphenolics, alkaloids, terpenoids, saponins, flavonoids, proteins, andpeptides [1]. In line with previous study[8], this study showed that flavonoid, saponin, tannin and steroid compounds from themethanol fraction have responsibility for the antifungal properties. Flavonoids often inhibit fungal growth with various underlying mechanisms, including cell wall disruption, the induction of mitochondrial dysfunction, and inhibiting the following: cell membrane formation, cellular division , RNA and protein synthesis, and the efflux mediated pumping system [2]. The major mechanism of antifungal activity of saponins, triterpenoids, steroids or steroidal alkaloids glycosylated is apparently due to their ability to complex with sterols in fungal membranes and to cause loss of membrane integrity. Aggregation of the saponin-sterol complexes within the membrane could also be mediated by interactions between the sugar residues of the saponin molecules. Removal of the sugar residue which attached to C-3 leads to loss of biological activity[12].

Nevertheless, we need to develop this research in the futureto isolate the compounds of methanolicfraction and subjected to antifungal activity to find out which compounds are the most responsible for their antifungal properties.

Table: 3 The Antifungal Activity of *C. asiatica* Leaf Fractions

***C. asiatica* leaf fractions**	**Average of inhibition zone diammeter (mm)±SD**			
Concentration (%)	**10%**	**20%**	**30%**	**40%**
n-Heksana extract	0 ±0	0 ±0	0 ±0	0 ±0
Ethyl Acetateextract	0 ±0	0 ±0	0 ±0	0 ±0
Methanol extract	5,6±0,89	7,17±0,25		11,4±1,2
Ketoconazole (+C)	46,48±3,72	46,42±0,71	46,9	48,01±0,
Solvent	**Average of inhibition zone diammeter (mm)±SD**			
* *n*-heksana	0 ±0			
* etil asetat	0 ±0			
* methanol	0 ±0			

CONCLUSION

n-Hexane and ethyl acetate fraction didn't show antifungal activity with 0 mm inhibition zone at any test concentration while methanolic fraction of *C. asiatica*at 40% concentration has the highest antifungal activity against Fluconazole-resistance*C.Albicans* with diameter inhibition zone 11,4 mm. Flavonoid, tannin, steroid and saponin compounds are responsible for antifungal properties of *C. asiatica* methanolic fraction.

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