

Inhibitory activity of *Lagerstreomia speciosa* extract against *Candida albicans*, *Aspergillus fumigatus*, and *Aspergillus flavus*

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ABSTRACT

Resistance of antifungal therapeutics has been reported, for instance, azole antifungal against *Candida* and *Aspergillus*. The discovery of new antifungal agent is thus warranted. *Lagerstreomia speciosa* is a notable Asian medicinal plant with profound antidiabetic activity. We previously demonstrated that the plant extract and one of the active substances, corosolic acid, have potential inhibitory effects against methicillin-resistant *Staphylococcus aureus* and can potentiate cefotaxime. The current study investigated the antifungal activity of bark and leaf extracts against three clinical fungal isolates. Well diffusion and broth microdilution methods were employed to assess the antifungal activity of *L. speciosa* leaf and bark extracts against *Candida albicans*, *Aspergillus fumigatus*, and *Aspergillus flavus* isolated from patients in Jakarta. *Candida albicans* was susceptible to the leaf and bark extracts as evidenced by the inhibitory zones > 20 mm and minimum inhibitory concentration (MIC) of the leaf extract was categorized as susceptible-dose-dependent. *Aspergillus fumigatus* and *A. flavus* were also relatively susceptible to the leaf extract, while the MIC of the bark extract categorized as susceptible-dose-dependent against both fungus. *Lagerstreomia speciosa* is a potential antifungal agent with comparable activity to fluconazole; therefore, further bioassay-guided fractionation of both extracts is warranted to identify the antifungal compound.

INTRODUCTION

Fungal infections are prevalent, particularly in the tropics, and are often associated with other health problems such as asthma, AIDS, cancer, organ transplantation, and also corticosteroid therapies [1]. *Candida albicans* is the most prevalent fungal species causing mucosal disease candidiasis, which can be divided into mouth, throat, and esophagus, vaginal candidiasis, and invasive candidiasis [2]. Invasive candidiasis is associated with infection of the blood, heart, brain, eye, bone, and other organs. Nearly 95% of invasive candidiasis is caused due to the presence of *C. albicans*, *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis*, and *Candida krusei* [1]. Besides candidiasis, aspergillosis is an infectious disease caused by *Aspergillus*, such as allergic bronchopulmonary aspergillosis, allergic *Aspergillus*

sinusitis, aspergilloma, chronic pulmonary aspergillosis, invasive aspergillosis, and cutaneous (skin) aspergillosis. A recent study reported that *Aspergillus fumigatus* caused around 60% of infections, followed by *Aspergillus flavus* [3].

Antifungal therapeutics have long been used to manage fungal infections, although some resistance has been reported, for instance, azole antifungal against *Candida* and *Aspergillus* species and echinocandin multidrug against *C. glabrata* [4]. Therefore, the attempts to identify new antifungal therapeutics are constantly increasing, such as natural products such as well-known antifungal drugs, polyenes, and echinocandins [5].

Lagerstromia speciosa is a medicinal plant widely distributed in Asia, including China, India, the Philippines, Malaysia, and Indonesia. The plant is commonly used as an ingredient, spice, or traditional medicine [6,7]. Various parts of the plant, such as the bark, flower, and leaves are commonly used for disease prevention and treatment, especially diabetes mellitus [8,9]. *Lagerstreomia speciosa* contains corosolic acid as a key marker compound [10], ellagitannin (lagerstannins A, B, and C), monomeric and dimeric ellagitannins (flosins A and B and reginins A, B, C, and D), ellagic acid, quercetin, isoquercetin, coumarin,

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β -sitosterol, neolignan, and ursolic acid, all of which are responsible for the biological and pharmacological activity [7,9,11]. Antifungal activities, especially from the fruit and flower of *L. speciosa*, have been reported [12,13]. However, little is known about how the leaf and bark extracts may also inhibit fungal growth.

Previously, our group reported that corosolic acid, methanolic leaf, and bark extracts of *L. speciosa* exhibited profound antibacterial activity and showed that corosolic acid enhanced the antibacterial activity of cefotaxime. This study was extended to investigate whether corosolic acid, methanolic bark, and leaf extracts of *L. speciosa* possess antifungal activity against three different clinical fungal isolates that commonly caused candidiasis and aspergillosis.

MATERIAL AND METHODS

Preparation of drug

The positive control fluconazole (Pharmaceutical product, Indonesia) was diluted in sterile aquadest and medium according to Clinical and Laboratory Standards Institute (CLSI) M27-A2 to obtain a working concentration of 2 mg/ml for the antifungal assay [14].

Plant extraction

The plant material was a kind gift from Professor Fitmawati, Department of Biology, University of Riau, and had been previously identified using *ITS* coding region [8]. The voucher specimen was deposited in the Department of Pharmacy, Atma Jaya Catholic University of Indonesia, for the current study. The leaves and bark were separately cleaned and ground before methanolic maceration. Excess methanol solvent was evaporated using a rotary evaporator to obtain dry extracts, which were then diluted in dimethyl sulfoxide (PAN-Biotech, Aidenbach, Germany) to obtain a working concentration of 80 mg/ml. Determination of the test concentration was previously described in several studies [15,16].

Antifungal activity

Microorganisms and culture

The three microorganisms used were *C. albicans*, *A. fumigatus*, and *A. flavus* obtained as a clinical isolate from the Department of Parasitology, Faculty of Medicine, University of Indonesia. The fungal isolates were confirmed using HiCHROM Agar for *C. albicans* with a blue/green appearance (Fig. S1) and the slide culture technique for *A. fumigatus* and *A. flavus* (Fig. S2-S3). For the assay, one inoculating loop of 24 hours *C. albicans* was suspended in 10 ml NaCl (0.9%) and homogenized using a bench vortex for 15 s. The absorbance of the homogenized *C. albicans* suspension was measured at 530 nm to reach the turbidity of McFarland 0.5 (Absorbance of 0.5–0.6), equivalent to $0.5\text{--}2.5 \times 10^3$ CFU/ml. One inoculating loop of 72 hours *A. fumigatus* or *A. flavus* was suspended in 10 ml NaCl (0.9%) to obtain *A. fumigatus* suspension according to the previously mentioned procedure.

Well-diffusion antifungal assay

Well-diffusion antifungal assay was performed according to CLSI guidelines and previous studies [14,17].

Briefly, 20 ml of Mueller Hinton Agar (MHA, Oxoid, United Kingdom) was poured onto a 20 cm diameter Petri dish and allowed to dry for 30 minutes. The *C. albicans* suspension was then evenly spread on the top of the MHA before 8 mm diameter wells were made in the agar at a distance of approximately 2.5 cm between wells. Then, 50 μ l of 2 mg/ml fluconazole, 80 mg/ml, and 40 mg/ml of extract were placed in each well. The same procedure was applied to *A. fumigatus* and *A. flavus*. The petri dishes with *C. albicans* were incubated at 35°C for 24 hours and 48 hours for *A. fumigatus* or *A. flavus*. The inhibitory zone was then determined based on the diameter of the inhibitory zone against *C. albicans* as susceptible when the inhibitory zone \geq 19 mm, dose-dependently susceptible if the inhibitory zone was 18–13 mm, and resistant if the inhibitory zone \leq 12 mm [18].

Broth microdilution antifungal assay

The method was adopted from CLSI guidelines and previous studies [14,17]. Twofold serial dilutions of fluconazole with 100 μ l media RPMI 1640 (PAN Biotech, Germany) were performed in a 96-well plate to obtain serial dilutions in a range of 0.0155–32 μ g/ml [14]. Plant extracts were prepared similarly to obtain serial dilutions in a range of 0.020–40 mg/ml. Afterward, 100 μ l of *C. albicans* suspension was added to each well and incubated at 35°C for 24 hours. Similar procedures were applied for *A. fumigatus* and *A. flavus*, but the incubation time was 48–72 hours. The minimum inhibitory concentration (MIC) was determined for each tested drug and extract. The percentage of fungal growth was calculated based on the absorbance reading at 600 nm before and after incubation according to the formula:

$$\% \text{ Fungal growth} = \frac{\text{OD Tested drug after incubation} - \text{OD Tested drug before incubation}}{\text{OD Growth control after incubation} - \text{OD Growth control before incubation}}$$

$$\% \text{ Inhibitory fungal growth} = 100\% - \% \text{ fungal growth}$$

Statistical analysis

The number of replications was determined according to Federer ($t(r-1) > 15$), $n = 3$. The data were analyzed using Microsoft Office Excel and presented as the average inhibitory zone \pm SD and the average MIC \pm SD for each tested strain.

RESULTS AND DISCUSSION

Lagerstreomia speciosa, as a medicinal plant, contains some important bioactive compounds, including corosolic acid [10]. Previously, we showed that *L. speciosa* methanolic leaves extract and corosolic acid profoundly exhibits antibacterial activity against *Staphylococcus aureus*, including the methicillin-resistant *S. aureus*. The current study investigated how *L. speciosa* extracts inhibit fungal growth. Methanol has been considered the universal organic solvent that can extract various phytochemicals, often with high antioxidant and anti-inflammatory activities, resulting in higher extraction efficiency. It is, therefore, appropriate to screen for bioactivity before further fractionation guided by a bioassay of active compounds [19,20]. In our current study, besides the extract,

Table 1. Diameter of the inhibitory zone of the leaf and bark extracts of *L. speciosa* against *C. albicans* and *Aspergillus*.

Microorganism	Extract	Extract concentration (mg/ml)	Diameter of inhibitory Zone (mm)
<i>Candida albicans</i>	Bark	2	17.67 ± 0.29
		4	22.67 ± 0.29
	Leaves	2	21.00 ± 0.50
		4	24.17 ± 0.58
<i>Aspergillus fumigatus</i>	Bark	2	n.i.
		4	n.i.
	Leaves	2	12.67 ± 0.29
		4	15.50 ± 1.00
<i>Aspergillus flavus</i>	Bark	2	n.i.
		4	n.i.
	Leaves	2	10.83 ± 1.04
		4	14.33 ± 0.29

n.i.: no inhibitory zone was observed.

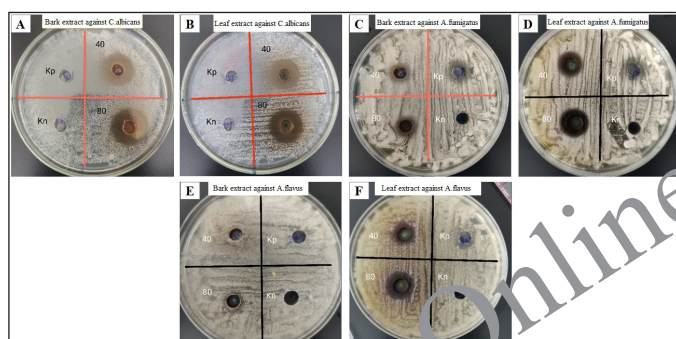


Figure 1. Inhibitory zone of the bark (A, C, E) and leaf (B, D, F) extracts of *L. speciosa* against *C. albicans* (A, B), *Aspergillus fumigatus* (C, D), and *A. flavus* (E, F) tested using the well diffusion method. Kp: positive control (Fluconazole 0,1 mg/ml), Kn: negative control (DMSO 2.5%), 40: concentration of extract in test (2 mg/ml), 80: concentration of extract in test (4 mg/ml).

we also tested 0.125 mg/ml of corosolic acid against all fungal strains; however, no inhibitory fungal growth was observed (data not shown). In the well-diffusion method, *C. albicans* showed sensitivity to leaf and bark *L. speciosa* extracts at concentrations of 2 and 4 mg/ml. In contrast, *A. flavus* and *A. fumigatus* resisted the bark extract and were dose-dependently susceptible to the leaf extract (Table 1, Fig. 1). A previous study by [12] showed that *C. albicans* was susceptible dose-dependent to *L. speciosa* flower extract (2 mg/ml) [12], whereas [13] reported that the extract was inactive against *A. niger*.

Using broth microdilution, *C. albicans*, *A. flavus*, and *A. fumigatus* were dose-dependently susceptible to the positive control fluconazole [21]. Fluconazole (16 µg/ml) inhibited 54.15% ± 0.007% of *C. albicans* growth, 36.86% ± 0.003% of *A. fumigatus*, and 27.60% ± 0.003% of *A. flavus* at 32 µg/ml. No study was conducted previously on the antifungal activity of leaf and bark extracts of *L. speciosa* using the microdilution method. In the current study, the MIC of *L. speciosa* bark extract

Table 2. MIC of the bark and leaf extracts of *L. speciosa* against *C. albicans* and *Aspergillus*.

Microorganism	Extract	MIC (mg/ml)
<i>Candida albicans</i>	Bark	10.87 ± 0.82
	Leaves	4.69 ± 0.03
<i>Aspergillus fumigatus</i>	Bark	7.75 ± 0.32
	Leaves	9.46 ± 0.23
<i>Aspergillus flavus</i>	Bark	12.65 ± 0.32
	Leaves	23.87 ± 0.34

against *C. albicans* was 10.867 ± 0.822 mg/ml, against *A. fumigatus* was 7.747 ± 0.322 mg/ml, and against *A. flavus* was 12.65 ± 0.32 mg/ml, whereas the MIC of the *L. speciosa* leaf extract against *C. albicans* was 4.69 ± 0.034 mg/ml, against *A. fumigatus* was 9.462 ± 0.231 mg/ml, and against *A. flavus* was 23.872 ± 0.338 mg/ml (Table 2). Based on the microdilution test, the bark extract was more potent than the leaf extract in inhibiting the growth of the *Aspergillus* strains, while the leaf extract was more potent against *C. albicans* than *Aspergillus*.

Nasrin and Ahmad [22] reported that the antifungal activity of *L. speciosa* bark extract was slightly more potent than the leaf extract based on the disc diffusion method containing 500 µg extract/disc against clinical *C. albicans* and *A. niger* cultures. In contrast, low fungal inhibitory activity was observed from *L. speciosa* flowers against *A. niger* using the disc diffusion method [13]. A review reported that corosolic acid possesses antifungal activity, although the mechanism of action remains unclear [6]. It was also not in accordance with the current study. Polyphenol compounds contained in *L. speciosa*, such as tannin, ellagitannin, flavonoid, and flavonols, may also play important roles in the antifungal activity of the extract, as exhibited in several studies [23–25]. The possible mechanism of action of polyphenols against fungi includes plasma membrane disruption, mitochondrial dysfunction, inhibition of cell wall formation, and inhibition of RNA and DNA or protein synthesis [26].

Besides polyphenols, the *L. speciosa* extract also contains steroids and triterpenoids with phenolic –OH group that may bind to the sulfhydryl group via non-specific interaction with protein and inhibit fungal biochemical process [27] as well as disturb the membrane integrity [28]. Those chemical compounds can work synergistically to inhibit microbial growth compared to the single active compound, corosolic acid, with no fungal inhibitory activity. The *L. speciosa* extract (2,000 mg/ml) showed no toxicity/mortality or morbidity in the acute toxicity study using the Sprague-Dawley rat based on movement, physical parameters, behavior, and overall appearance [29], indicating that the extract is safe as a novel antifungal candidate.

CONCLUSION

The leaf and bark extracts of *L. speciosa* demonstrated inhibitory activity against *C. albicans*, *A. fumigatus*, and *A. flavus* based on two antifungal test methods, thus are potential novel antifungals. Bioassay-guided fractionation of both

extracts is warranted to identify the novel antifungal fractions and/or compounds.

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AUTHOR CONTRIBUTION

All authors made intellectual and substantial contributions to the conception and design of the work; acquisition, analysis, and interpretation of data; drafting the manuscript and revising it critically; and all authors have approved the final version.

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CONFLICT OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

This study does not involve direct experiments on animals or human subjects.

DATA AVAILABILITY

All data generated and analyzed are included in this research article.

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