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In vitro Antifungal Activity of The Orange Jasmine (*Murraya paniculata* [L.] Jack.) Leaves Ethanol Extract From Indonesia Against *Candida albicans*

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Abstract
Candida albicans can cause infections that range from superficial infections of the skin to life-threatening systemic infections. The use of antifungal drugs in the therapy of fungal diseases can lead to the development of antifungal resistance. The availability of natural materials can be used as a source of traditional medicine that can answer the challenge of fungal resistance. Empirically, Orange jasmine (*Murraya paniculata* [L.] Jack.) leaves have been used to treat vaginal discharge caused by *C. albicans*. Therefore, this research study was aimed to evaluate the antifungal activity of orange Jasmine leaves extract against *C. albicans* in vitro. The research methods included: extraction, phytochemical screening, antifungal activity test and determination of minimum inhibitory concentration (MIC). The simplisia of Orange jasmine leaves was extracted using a maceration method. The phytochemical screening was conducted using standard method. Antifungal activity was tested using the agar diffusion method. The extract concentrations used in the antifungal activity test were 20%, 40%, 60%, and 80% w/v with metronidazole in concentration of 40% w/v as comparative agent. Furthermore, the MIC test was done by a macrodilution method and following by subculturing the overnight result on to the surface of agar media to determine the minimum fungicidal concentration. The results showed that the ethanol extract of orange jasmine leaves had antibacterial activity against *C. albicans* with MIC/MFC ranged at 0,3125-0,625 %w/v.

Keywords: *Murraya paniculata,* metronidazole, orange jasmine, *Candida albicans,* antifungal

Introduction

In most individuals, *Candida albicans* resides as a lifelong, harmless commensal. Under certain circumstances, however, *C. albicans* can cause infections that range from superficial infections of the skin to life-threatening systemic infections [1]. *Candida albicans* can cause two major types of infections in humans: superficial infections, such as oral or vaginal candidiasis, and life-threatening systemic infections [2]. The pathogenicity of Candida species is attributed to certain virulence factors, such as the ability to evade host defences, adherence, biofilm formation (on host tissue and on medical devices) and the production of tissue-damaging hydrolytic enzymes such as proteases, phospholipases and haemolysin [3]. *Candida albicans* and to a lesser extent other Candida species are present in the oral cavity of up to 75% of the population [4]. In healthy individuals this colonization generally remains benign. However, mildly immunocompromised individuals can frequently suffer from recalcitrant infections of the oral cavity. These oral infections with Candida species are termed oral candidiasis (OC) [4]. Such infections are predominantly

caused by *C. albicans* and can affect the oropharynx and/or the esophagus of persons with dysfunctions of the adaptive immune system. Indeed, HIV is a major risk factor for developing OC. Further risk factors for developing OC include the wearing of dentures and extremes of age [5]. It is estimated that approximately 75% of all women suffer at least once in their lifetime from vulvovaginal candidiasis (VVC), with 40–50% experiencing at least one additional episode of infection [6]. A small percentage of women (5–8%) suffer from at least four recurrent VVC per year [7]. Predisposing factors for VVC are less well defined than for OC and include diabetes mellitus, use of antibiotics, oral contraception, pregnancy and hormone therapy [8]. Despite their frequency and associated morbidity, superficial *C. albicans* infections are non-lethal. In stark contrast, systemic candidiasis is associated with a high crude mortality rate, even with first line antifungal therapy [9-11].

The incidence of fungal infections has increased significantly, so contributing to morbidity and mortality. This is caused by an increase in antimicrobial resistance and the restricted number of antifungal drugs, which retain many side effects. The study of plants as an alternative to other forms of drug discovery has attracted great attention because, according to the World Health Organization, these would be the best sources for obtaining a wide variety of drugs and could benefit a large population [12]. For many years, *Murraya paniculata* has been used as an ornamental and a medicinal plant [13]. But due to its hardiness and wide range of soil tolerance, orange jasmine is commonly used as a hedge. Eventhough *M. paniculata* leaves extract were reported to contain coumarins [14,15] and flavonoids [16-18]. Coumarin (2H-1-benzopyran-2-one) is a plant-derived natural product known for its pharmacological properties such as anti-inflammatory, anticoagulant, antibacterial, antifungal, antiviral, anticancer, antihypertensive, antitubercular, anticonvulsant, antiadipogenic, antihyperglycemic, antioxidant, and neuroprotective properties [19]. Although distributed throughout all parts of the plant, the coumarins occur at the highest levels in the leaves of *M. paniculata* [20]. Therefore, there is a need to evaluate whether leaf extracts obtained from the Indonesian orange jasmine may possess the antifungal potential against pathogenic *C. albicans.*

Materials and Methods

Material

Mature leaves of the *M. paniculata* were obtained from Bogor, West Java, Indonesia. Only healthy green leaves without any visible damage were sampled. Plant sample was identified in Plant Taxonomy Laboratory of Biology Major, Faculty of Mathematics and Natural Science Padjadjaran University.

The culture media that were used are *Sabouraud Dextrosa Agar* (SDA-Oxoid), and *Sabouraud Dextrosa Broth* (SDB-Oxoid). In this study, metronidazole was used as a comparison substance. The chemicals used are distilled water, normal saline solution, barium chloride solution (Merck), sulfuric acid solution (Merck), n-butanol, ferric chloride reagent (Merck), Dragendorf reagents, Lieberman - Burchard reagent, Mayer reagent, technical toluene (Brataco), and vanillin (Merck).

Methods

Preparation of Simplisia

The leaves were washed using clean water to remove impurities and reduce the microbes attached to the material. The washing process should be done with the shortest time to avoid the dissolving and wasting of substances contained in the material. Then

the leaves are drained, after which the leaves were cut into small pieces about 1 cm in wide. This process was done to speed up the drying process, then the pieces of leaves were dried in such place that was not exposed to direct sunlight. The good result of the drying process is a simplicia containing water content of ± 8- 10%. The drying of the simplicia was carried out until the weight of the simplicia was constant and when it were squeezed, the sound of dry leaves would be heard.

Preparation of Extracts

The extraction process of *M. paniculata* simplisia was conducted using a maceration method. Of 4.5 Kg wet weights, were gained 1.6 Kg of dried simplisia. Then the dried leaves were extracted for 3x24 h using ethanol 70% as the solvent. The extracts were evaporated using a rotary evaporator at 40-50 ^{O}C, then continued to evaporate on a water bath until dried extract with a constant weight was obtained. From 1.6 Kg dried simplisia, can obtain 403.4 g viscous extracts.

Phytochemical Screening of Secondary Metabolites

Phytochemical screening of secondary metabolites was using a standard method to determine the contains alkaloids, flavonoids, tannins, quinones, saponins, steroids, and triterpenoids, in both simplisia and ethanol extracts of *M. paniculata* leaf [21].

Preparation of The Fungal Suspension

Candida albicans colonies from an agar plate culture were suspended in 0.95% sterile saline to obtain a turbidity optically comparable to that of the 0.3 McFarland standard. This results in a suspension containing approximately 1 x 10^6 CFU/ml.

Antifungal Activity Test

The antifungal activity of the extracts was done using the agar diffusion methods. The leaf extract was diluted with dimethylsulfoxide (DMSO) in several test concentration variations ie 20%, 40%, 60%, and 80% w/v and metronidazole with concentration of 40% w / v. Incorporated 20 mL of sterile SDA at 40-45 ^{0}C to a sterile petri dish and leaved it to become solid. A total of 20 µL of *C. albicans* suspension was added on solid medium. The fungal suspension was flattened using a spreader. After that, the test medium was perforated and a total of 50 µL of extract at each test concentration were inserted into each of the holes on the test medium. All the test media were then incubated at 37 ° C for 48 hours. The diameter zones of inhibition were measured using a caliper. The tests were carried out in duplicate.

Determination of MIC and MFC Value

MIC value of the *M. paniculata* leaf extract was determined using macrodillution broth. The concentration of extract ranges should be prepared one step higher than the final dilution range required. In this study, the concentrations of extract were as follows: 10%, 5%, 2.5%, 1.25%, 0.625%, 0.3125%, 0.1562%, 0.0781%, 0.039%, and 0.0195 % w/v. Then a loopfull Ose of fungal suspension was added to every tube. The liquid media, then were incubated at temperature 37ºC for 48 h. The MIC value was the lowest concentration of an extract that inhibits the growth of *C. albicans*. As MFC determination, the loop was dipped into the overnight incubation of MIC tube, then streaked it on to the agar surface. After that, the plates were incubated at temperature 37ºC for 48 h.

Results and Discussion

Phytochemical Screening Result

The results of the phytochemical analysis revealed varying constituents of these extracts, as follows: tannins, steroids, and saponins**.** The result of phytochemical screening can be seen in Table 1.

Table 1: Phytochemical screening

Secondary metabolites	Results	
	Simplisia	Extract
Alkaloids	-	-
Quinones	-	-
Tannins	+	+
Flavonoids	-	-
Saponins	+	+
Steroids/Triterpenoids	+	+

Note: (+) presence; (-) absence

Among those detected secondary metabolites, another research showed that saponin from *Sapindus saponaria* fruits extracts were reported has strong activity against *C. albicans* [22].

Antifungal Activity Result

In vitro study had demonstrated that the leaf extracts of *M. paniculata* had antifungal activity against *C. albicans*. The diameter data can be seen in table 2.

Table 2: Antifungal activity results

Concentration (% w/v)	Diameter of Inhibition (mm)
20	11.590±0.0200
40	11.750±0.1186
60	12.073±0.0205
80	12.123±0.0339

Note: Perforator diameter = 6 mm

MIC and MFC Determination Result

Minimum inhibitory concentrations (MIC) refer to the lowest concentration of an antimicrobial that will inhibit the visible growth of fungal cell. The result of MIC determination can be seen in Table 3.

Table 3: MIC Results

Extract concentration (%^{w}/v)	Fungal growth
0.0195	+
0.0390	+
0.0781	+
0.1562	+
0.3125	+
0.625	-
1.25	-
2.5	-
5	-
10	-

Note: (+) = colony absence; (-) = colony presence

The extracts showed the value of MIC/MFC ranged between 0,3125-0,625 %w/v. These results indicated that the extract of *M. paniculata* leaves is a potent candidate for antifungal infection caused by *C. albicans*.

Conclusion

Our results demonstrated that ethanol extracts of *M. paniculata* leaves have active antifungal activity against *C. albicans*.

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