

Original Research Report**ANTIFUNGAL ACTIVITY OF KINAR (*Kleinhovia hospita* L.) LEAF ETHANOL EXTRACT AGAINST *Malassezia furfur***

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ABSTRACT

Pityriasis versicolor is a superficial dermatomycosis that can decrease human self-confidence. This infection is caused by the fungus *Malassezia furfur*. Eighty percent of recurrence cases after treatment and resistance to antifungal agents were found. Therefore, alternative medicine is needed. Kinar (*Kleinhovia hospita* Linn.) is a tropical plant that have bioactive compounds, such as alkaloids, flavonoids, tannins, and saponins. The purpose of this study was to determine the effectiveness of kinar leaf extract against the growth of *Malassezia furfur*. This research was a laboratory experimental study using paper disc diffusion method. Kinar leaves (green and yellow leaves) were macerated using 96% ethanol and made into concentrations of 10%, 20%, 40%, 60%, 80%, and 100%. As much as 200 mg of ketoconazole was used as a positive control and distilled water as a negative control then tested on *Malassezia furfur* using Sabouraud dextrose agar (SDA). The parameter observed was the clear zone formed around the paper disc. The tests and observations showed that there was a clear zone formed around the paper disc. It means that the kinar leaf extract cannot inhibit the growth of *Malassezia furfur*.

Keywords: Antifungal; *Kleinhovia hospita* L.; *Malassezia furfur*; tropical disease; human and medicine

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1. Alternative medicine is needed for pityriasis versicolor because there are recurrence cases and resistance to antifungal agents.
2. Kinar leaf ethanol extract cannot inhibit the growth of *Malassezia furfur*.
3. Further studies are suggested on the type of solvent and appropriate concentration to attract more bioactive compounds.

INTRODUCTION

Dermatomycosis or superficial mycosis is a disease of the skin, nails, and hair caused by dermatophyte and non dermatophyte fungi. The incidence of superficial fungal infections worldwide is common, with a progressive increase, and has affected about 20-25% of the global population. Generally, dermatomycoses occur in tropical countries. Skin fungal infections are estimated to have a high prevalence in Indonesia, such as pityriasis versicolor (PV) or commonly known as *panu* in Indonesian (World Health Organization 2005, Yusuf et al. 2017, Araya et al. 2021).

In places with high humidity and an average temperature of 28-33°C, it is common to sweat easily and it can cause skin conditions. Other factors that can increase the risk of fungal infections are poor sanitation, lack of health knowledge, and densely populated or low socio-economic environment. Under

these conditions, *Malassezia furfur* can easily infect humans, even though this fungus is actually a normal flora (Yusuf et al. 2017, Ariana 2018).

Researches conducted by public medical teaching hospitals in Indonesia often find the cases of pityriasis versicolor. In the Outpatient Unit of the Department of Dermatology and Venereology, Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, the number of superficial mycoses cases found was 502 (5.47%) in 2011, 312 (4.91%) in 2012, and 322 (5.90%) in 2013. The examination in 2011-2013 using 20% potassium hydroxide (KOH) and Parker's ink resulted in the most Dermatology and Venereology, Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, the number of superficial mycoses cases found was 502 (5.47%) in 2011, 312 (4.91%) in 2012, and 322 (5.90%) in 2013. The examination in 2011-2013 using 20% potassium hydroxide (KOH) and Parker's ink resulted in the most commonly found diagnosis which was pityriasis

versicolor in 284 patients (Rosida & Ervianti 2017). In the period 2013-2015, the Outpatient Unit of the Pediatric Dermatology, Department of Dermatology and Venereology, Dr. Soetomo Hospital treated 43,073 patients, 94 (28.3%) of which were diagnosed with pityriasis versicolor and 1 (0.3%) with tinea pedis (Sheiladji & Zulkarnain 2016). The Outpatient Clinic of Dermatology and Venereology of Prof. Dr. R.D. Kandou Central General Hospital, Manado, Indonesia, found 36 (0.87%) pityriasis versicolor diagnosed cases in January-December (Isa et al. 2016).

Previous research conducted by Gupta & Foley (2015) revealed that pityriasis versicolor is still difficult to cure because 80% of relapses occur after treatment within two years. The infections caused by *Malassezia furfur* can be treated with antifungals given via systemic or topical routes. The first treatment for pityriasis versicolor is the topical antifungals. However, *Malassezia furfur* strains were found to be resistant to antifungals, such as the azole group. Kalyani et al. (2014) conducted a study on 100 samples of pityriasis versicolor patients, with 41 tested positive for KOH and 32 positive for culture. Of these 32 patients, 23 (69%) were found to have recurrent infections. From the isolates of *Malassezia furfur* in the study, 75% isolates were found to be sensitive to ketoconazole and 100% isolates were susceptible to fluconazole and clotrimazole. Circulars issued by the National Agency of Drug and Food Control of the Republic of Indonesia (*Badan Pengawas Obat dan Makanan/BPOM RI*) and the United States Food and Drug Administration (U.S. FDA) revealed that ketoconazole causes hepatotoxicity (Kang et al. 2019). In addition, pityriasis versicolor has a bad stigma in society and can affect the quality of life and self-confidence, so most patients who come for treatment complain about their appearance (Gupta et al. 2002, Radila 2022). Currently, there is no treatment to provide satisfactory results in treating the signs and symptoms of pityriasis versicolor and especially its recurrence (Mahmoud et al. 2014). It is necessary to look for the latest alternative treatment with antifungal activity that is certainly better and has very little toxicity to prevent the recurrence of pityriasis versicolor. The treatment mentioned is the use of tropical plants as herbal medicines because until now there are still many people using plants as alternative medicines (Sears & Schwartz 2017).

Tropical plants can produce natural chemical compounds, such as pesticides, insecticides, antifungals, and cytotoxics (Valli et al. 2012, Vleminckx et al. 2018). Plant secondary metabolites also play an important role in determining the biological activity in the use of medicinal plants. Therefore, identification and isolation of secondary metabolites is important for standardization and improvement of plant quality.

Kinar plants (*Kleinhovia hospita* Linn.) have been widely used by Indonesian people as medicine, starting from the trunk, bark, until the leaves. A study has isolated more than 24,000 secondary metabolic structures of *Kleinhovia hospita* Linn. and evaluated their biological activities (Gaffar & Mamahit 2010). All parts of this tree produce natural chemical compounds, such as cyanogens, alkaloids, proanthocyanins, cyanidins, flavanols, kaempferols and quercetins and saponins (Hasanuddin & Andini 2017). In Negeri Latu, Amalatu District, West Seram Regency, Maluku, Indonesia, yellow leaves of this plant are commonly used by the local people as a topical medicine mixed with whitening pounded in a coconut shell.

MATERIALS AND METHODS

Pure culture of *Malassezia furfur* was obtained from Indi Laboratorium, then tested and identified in the Microbiology Laboratory of Faculty of Agriculture, Universitas Mulawarman, Indonesia. The culture was grown on Sabouraud dextrose agar (SDA) supplemented with 1% (v/v) pure olive oil, followed by an incubation at 37°C for 2-7 days. The *Malassezia* strains were maintained on the same medium.



Figure 1. Sample and extract preparation: (A) the drying stage of green and yellow kinar leaves; (B) green and yellow kinar leaves in powder form; (C) green and yellow kinar leaf ethanol extracts in various concentrations

The culture was examined under the microscope using 10% KOH and methylene blue, and then the characters were recorded (Figure 1). Two types of kinar leaves (green leaves and yellow leaves) collected were washed, shade dried, and ground into a fine powder. The powder was weighed into 200 g portions, then each of them was soaked in 1,100 mL 96% ethanol for a day with intermittent stirring. The extracts were filtered and concentrated using rotary evaporator. The concentrated extracts were subjected to determination of weight per milliliter. The extracts were made into six concentrations, i.e. 10%, 20%, 40%, 60%, 80%, and 100%.

The culture of *Malassezia furfur* was swabbed over the Sabouraud dextrose agar using sterile cotton buds. Blank paper discs dipped in various concentration of kinar leaf extracts were placed equidistantly around the margin of the plates. The positive and negative control were 200 mg ketoconazole and maintained with filter paper discs dipped in distilled water. Two replicates were maintained. The plates were incubated at 37 °C and the inhibition zone was observed after 7 days.

RESULTS

This study used samples of green and yellow kinar leaves that were extracted with maceration method using 96% ethanol solvent and divided into several concentrations of 10%, 20%, 40%, 60%, 80%, and 100%. The ethanol extract of kinar leaves was then tested for the inhibitory activity against *Malassezia furfur* by disc diffusion method.

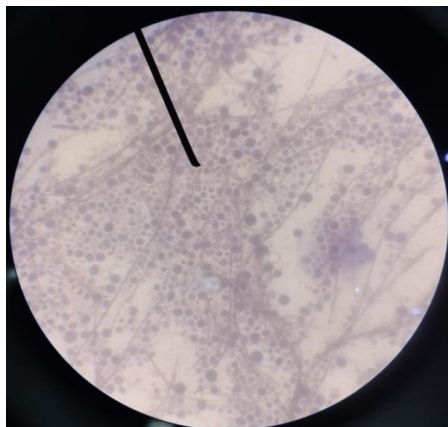


Figure 2. Morphological character of *M. furfur* (40x magnification). The presence of hyphae and spores generally exhibits the characteristic appearance of “spaghetti and meatballs”

Table 1 shows the results of the inhibition zone measurement on the green and yellow kinar leaf extracts against the growth of *Malassezia furfur*. The green and yellow kinar leaf extracts in all concentrations showed that the diameter of the inhibition zone was 0 and no clear zone was formed

Table 1 shows the results of the inhibition zone measurement on the green and yellow kinar leaf extracts against the growth of *Malassezia furfur*. The green and yellow kinar leaf extracts in all concentrations showed that the diameter of the inhibition zone was 0 and no clear zone was formed (Figure 3), indicating that the kinar leaf ethanol extracts were not able to inhibit the growth of *Malassezia furfur*.

Pathogenic fungi	Concentration %	Green leaf extracts		Yellow leaf extracts	
		Zone of inhibition (mm)	Zone of inhibition category	Zone of inhibition on (mm)	Zone of inhibition category
<i>Malassezia furfur</i>	10%	0	No inhibition zone	0	No inhibition zone
	20%	0	No inhibition zone	0	No inhibition zone
	40%	0	No inhibition zone	0	No inhibition zone
	60%	0	No inhibition zone	0	No inhibition zone
	80%	0	No inhibition zone	0	No inhibition zone
	100%	0	No inhibition zone	0	No inhibition zone
	+	0	No inhibition zone	0	No inhibition zone
	-	0	No inhibition zone	0	No inhibition zone

Table 1. Antifungal activities of *Kleinhovia hospita L.* leaf extracts against *M. furfur*

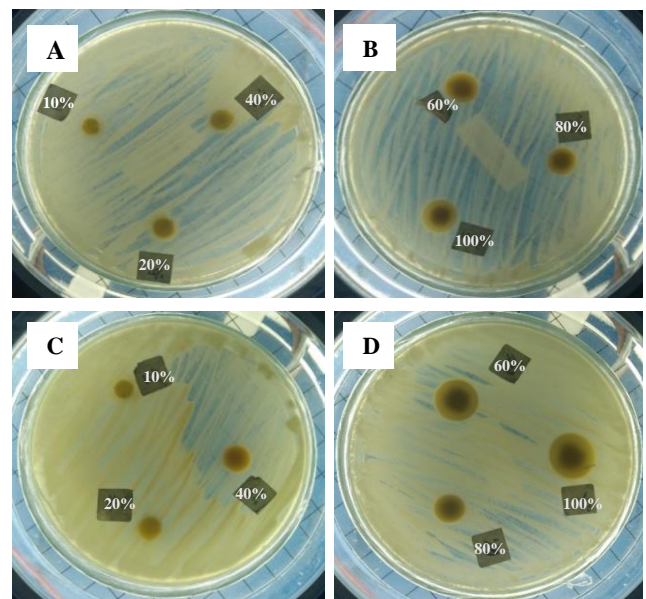


Figure 3. Antifungal assay: (A & B) inhibition zone of green kinar leaf extracts against *M. furfur*; (C & D) inhibition zone of yellow kinar leaf extract against *M. furfur*

DISCUSSION

Malassezia plays a pathogenic role in various skin diseases like pityriasis versicolor, seborrheic dermatitis, folliculitis, atopic dermatitis, and dandruff (Prohic et al. 2016). Generally, the treatment of these diseases consisted of azole drugs, such as fluconazole and ketoconazole. However, the increasing use of antifungal agents has led to adverse effects including severe toxicity in mammalian cells and urticaria (Kyriakidis et al. 2016). *Malassezia furfur* related diseases are often refractory to therapy and require extensive use of antifungal and anti-inflammatory drugs that can cause drug resistance. Therefore, it is important to find safe and effective treatment without side effects (Kyriakidis et al. 2016, Sivasankar et al. 2017, Kulkarni et al. 2020). There are reports concerning the sensitivity of *Malassezia* to natural antifungals or anti-*Malassezia* agents. The development of antifungal agents derived from plants produces the anti-*Malassezia* agents that are increasingly efficient. This current study showed that the 96% ethanol extract of the green and yellow kinar (*Kleinhovia hospita* Linn.) leaves did not show any inhibition zones.

Sanjaya et al. (2021) explained the antifungal activity of the plant *Melastoma malabathricum*, which found that the absence of an inhibition zone indicates the inability of the metabolite compounds of the plant in penetrating the cell walls of *Malassezia furfur*. The cell wall matrix of the genus *Malassezia*, such as *Malassezia furfur*, consists of various components in the form of polysaccharides and proteins. It constructs the cell wall of *Malassezia* to be relatively thick and contain a multilaminar ultrastructure and fat so that it is difficult to penetrate (Shibata et al. 2009). *Malassezia furfur* is able to form biofilms to provide protection against other microbes, create safe conditions for the proliferation, act as a barrier from secondary metabolites, such as antifungals, and protect itself from human immune system (Sanjaya et al. 2021). The biofilm consists of a matrix of extracellular polysaccharides, amyloids, DNA, and adhesive fibers that provide permanent adhesion to healthy skin. The area of the biofilms provides a low pH and high concentration of metallic ions which can prevent kinar leaf bioactive compounds from reaching fungal cells (Allen et al. 2015). Yunus & Malik (2019) tested the inhibition of kinar leaf extracts against enteropathogenic bacteria and the results showed the ability of kinar leaf extracts in inhibiting the growth of *Escherichia coli* at a 35% concentration and *Salmonella typhi* at 55% concentration. Kinar leaf extracts are only able to inhibit the growth of bacteria (Stalhberger et al. 2014). The influential factor that might be the cause is the type of solvent used. The solvent used in this study was 96% ethanol, while some previous studies used

70% ethanol to obtain the bioactive compounds needed to inhibit the *Malassezia furfur* because it is more effective than 90% and 96% ethanols in attracting more polar compounds and producing a higher amount of active ingredients (Azis et al. 2014, Kusuma et al. 2018). Stevani (2021) used 70% ethanol as a solvent in the extraction process of kenikir (*Cosmos caudatus* Kunth.) leaves and obtained desirable compounds, such as alkaloids, flavonoids, phenolics, saponins and steroids. Those compounds are known to be able to inhibit the growth of *Malassezia furfur* (Triana et al. 2016, Dirga et al. 2022).

Strength and limitation

In order to treat pityriasis versicolor, it will be important to conduct more study in the area of herbal medicine to extract more beneficial chemicals, and an appropriate breakdown type and concentration.

CONCLUSION

Due to its activity against the *Malassezia furfur*, kinar (*Kleinhovia hospita* Linn.) leaf ethanol extracts cannot be developed as herbal medicine for the treatment of pityriasis versicolor. It is recommended to conduct further studies on the type of solvent and the appropriate concentration to attract more bioactive compounds.

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Conflict of interest

The authors declare that there was no conflict of interest.

Funding disclosure

The authors received no specific funding for this research.

Author contribution

All the authors contributed to the experimental design of the research, as well as to the acquisition, analysis, and interpretation of the obtained results. Moreover, all the authors contributed to the writing and critical revision of the manuscript.

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