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No announcements have been published.

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JMSB is a Journal published online twice a year in the middle of the year (June) and the end of the year (December).

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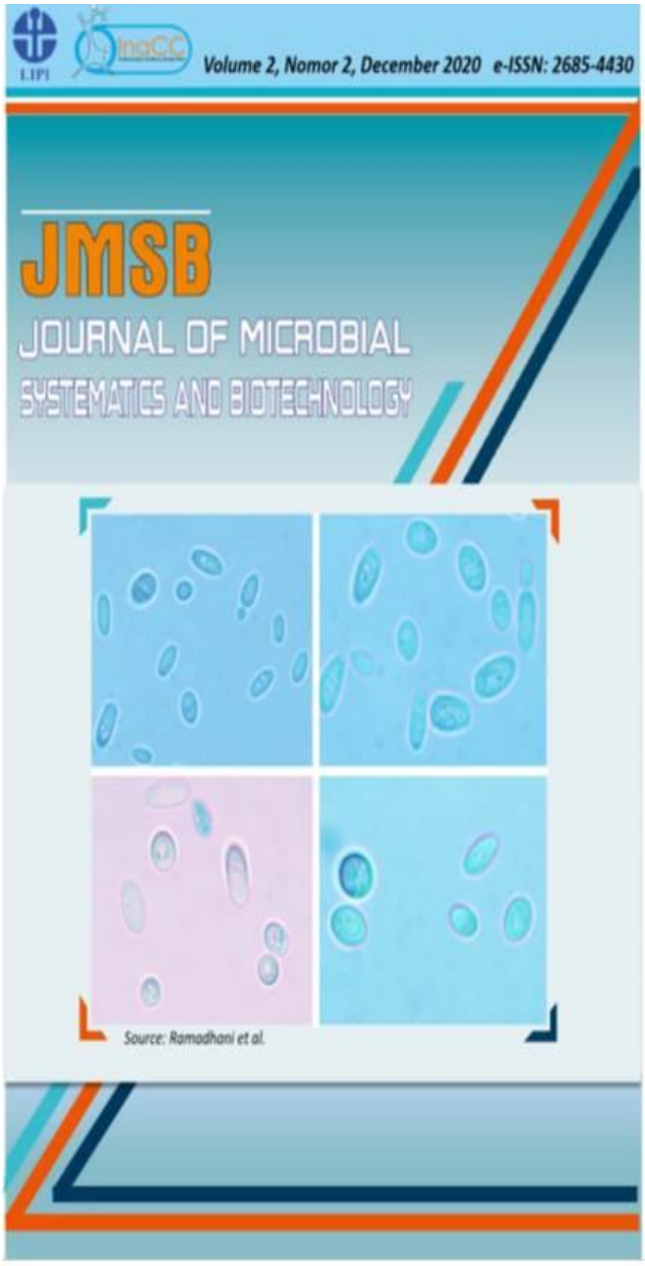

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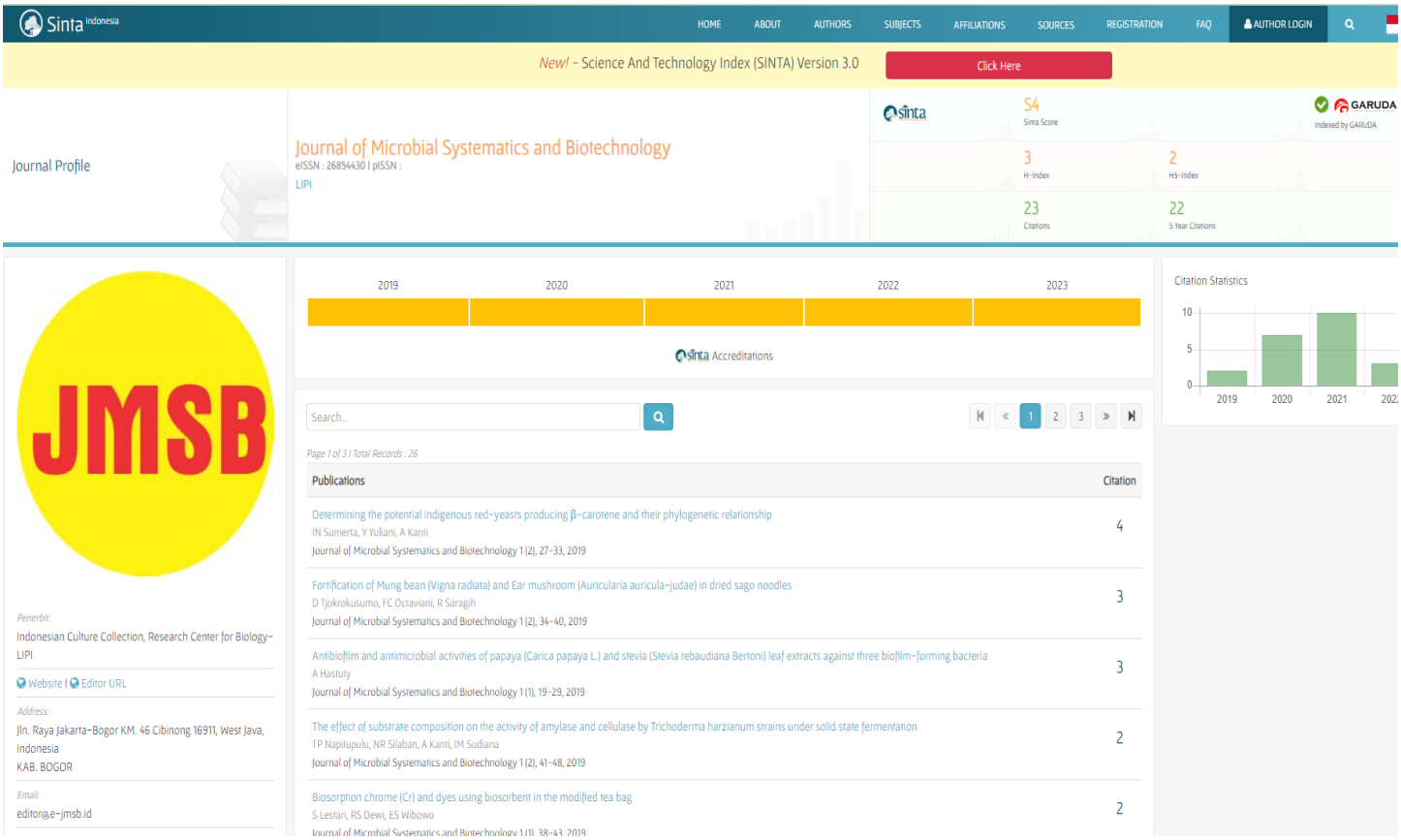

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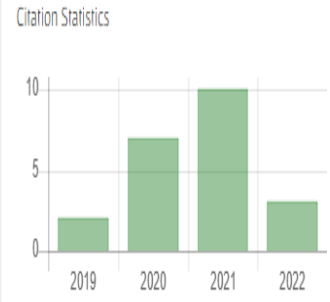

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Effect of ethanol extract from Karuk leaf (*Piper sarmentosum* Roxb.) on the growth of *Malassezia furfur* in vitro

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Khusnul, Kusmayanti A, Rahman LA, Ratnaningtyas NI. 2020. Effect of Ethanol Extract from Karuk Leaf (*Piper sarmentosum* Roxb.) on The Growth of *Malassezia furfur* In Vitro. Journal of Microbial Systematics and Biotechnology 2(2), 22-27

Abstract

In Indonesia, there are numerous therapeutic plants found. Some of the plants used in herbal medicine are Karuk leaf (*Piper sarmentosum* Roxb.) belong to the *Piperaceae* family. Karuk leaf has chemical contents such as saponins, polyphenols, flavonoids, and essential oils and many tests are carried out on several bacteria, but testing of fungi is rarely studied. This study aims to determine the ethanol extract activities from karuk leaf in inhibiting the growth of the *Malassezia furfur* fungus and to determine its minimum inhibition by using the Kirby-Bauer method. The study was conducted by an experimental method of the *M. furfur* fungus using the Kirby-Bauer method. The ethanol extract from karuk leaf was made in various concentrations and tested on 0.5 McFarland fungus by diffusion on Sabouraud Dextrose Agar (SDA). The results of this analysis showed that the ethanol extract of Karuk leaf could inhibit the *M. furfur* fungus at a concentration of 30% by 5.3 mm, 40% by 6.6 mm, 50% by 7.6 mm, 60% by 11.3 mm, 70% by 12.5 mm, 80% by 15.6 mm, 90% by 17.4 mm, and 100% by 19.5 mm. Based on the results of the study and the data analysis, it can be concluded that several concentrations of ethanol extract of Karuk leaf affect the growth of *M. furfur* in vitro.

Keywords: effect, Karuk leaf, Kirby-Bauer, *Malassezia furfur*, mycosis

Introduction

Indonesia is a tropical country with high humidity for the growth and development of fungi. However, the growth and development of fungi are not only influenced by the climate but also affected by a dirty environment, lack of public knowledge about healthy lifestyles. (Khusnul *et al.* 2019). These climatic conditions supports several pathogenic fungi to grow well and causes infection in humans, one of which causes superficial mycosis. Superficial mycosis is a fungal disease that may invade the surface of the stratum corneum as the skin layer, the hair, and the nails. Superficial mycosis is classified into two classes, caused by fungal, which is not dermatophytes (pityriasis versicolor) and caused by dermatophytes (Sutanto 2009). According Mansjoer *et al.* (2000), one type of mycosis is superficial mycosis which usually affects the skin, especially the dead and contains keratin such as nails and skin. Superficial mycoses are divided into two namely, dermatophytosis and non-dermatophytosis. Dermatophytosis is a disease of the tissue that contains horny substances, such as the stratum

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corneum of the epidermis, hair and nails caused by dermatophyte fungi, also called tinea, ringworm.

Malassezia belongs to a class of basidiomycetous yeasts whose survival depends on the lipid content of the skin and mucosa of humans and other warm-blooded animals. (Theelen *et al.* 2018). Fungal growth in human skin was found to be lower than that of bacteria. However, *Malassezia* fungi are the most common skin eukaryotes representing 50-80 per cent of the total skin mycobiomes reported using culture-independent methods. *Malassezia* species occupied all body sample sites with the exception of foot (Findley *et al.* 2013)

Several researchers have conducted research on the activity test of some plant extracts against *Malassezia furfur*, but research on the activity test of Karuk leaves (*Piper sarmentosum* Roxb.) against *M. furfur* has not been widely studied. In the previous report by Virgianti (2009), an inhibition test of the ethanol extract from Karuk leaf on *Streptoccocus pyogenes* bacteria is obtained inhibition zone results of 18.95 mm at 100% concentration. These results show that the Karuk leaf extract can inhibit the growth of *S. pyogenes* bacteria with a density of 1 McFarland standard. Some studies have also been carried out on the *M. furfur* fungus. Ethanol extract from Karuk leaf has the ability to inhibit *Candida albicans* with an inhibition zone approximately 31 mm (Shinta 2002). On the other hand, Khusnul *et al.* (2019) reported Karuk leaf ethanol extract has the ability to inhibit the growth of *C. albicans* and *Microsporum gypseum* in vitro.

Therefore, the application of Karuk leaf as an anti-fungus agent in daily life can be developed. For this reason, to consider the possibility of the efficacy of the Karuk leaf as an anti-fungus agent, We performed an experiment to test the effect of the Karuk leaf extract in terms of growth inhibition of *M. furfur* as a cause of mycosis.

Materials and methods

This study was experimental research by using the Kirby-Bauer method. The study was conducted at Microbiology Laboratory of Health Analyst of BTH Tasikmalaya. Some of the tools used in the research, including incubator, beaker glass, Petri dish, ose needle, disc paper, analytical balance, blender, erlenmeyer, measuring glass, stir bars, pipettes, test tubes, clinipettes, hot plates, cotton swabs, tube racks, and bunsen. Some materials that are used for testing, including distilled water, 30%-100% ethanol extract karuk leaves, 1% $BaCl_2$, disk antibiotic, 96% ethanol, Sabouraud dextrose agar (SDA), 1% H_2SO_4, Mueller-Hinton agar (MHA), pure strains of *M. furfur* fungus, karuk leaves, $FeCl_3$, NaCl, and HCl.

Samples collection

Karuk leaves were collected from Cineam, Tasikmalaya, East Java, Indonesia. Then the plant species was identified in the Plant Taxonomy Laboratory of the Faculty of Biology, Jenderal Soedirman University.

Simplicia preparation

Karuk leaves were washed with water until clean, then aerated and not direct sunlight. Then the leaves are dried in direct sunlight. After drying, the leaves were grinded to produce a fine powder. The powder is filtered using a coarse sieve. (Ditjen POM 1985)

Extraction of Karuk leaf

A 100 g of simplicia powder was weighed and put in Erlenmeyer flask. Absolute ethanol 96% was added to the simplicia in a ratio of 1:10 (simplicia : ethanol). The mixture was soaked for 3 days and stirring occasionally. The ethanol extract of the Karuk leaves was

filter using Whatman filter paper No. 41. The filtrate was evaporated using a rotatory evaporator under temperature < 65^0C to get a concentrate of the extract. Then extract was diluted using distilled water to reach a desired final concentration, 100% (without any dilution), 90%, 80%, 70%, 60%, 50%, 40% and 30% (Depkes RI 2000).

Anti-fungal test of ethanol extract of Karuk leaves against *M. furfur* growth.

SDA was poured at the temperature of 45°C, which is still liquid as much as 15-20 ml (thickness of ± 5-10 mm) into a sterile petri dish, flatten and allow to solidify. *M. furfur* fungus suspension with a standard of 0.5 McFarland was spread with sterile stirring rods onto SDA media. The plates should be left to solidify for 5 minutes. The disc paper was placed on a drip plate and then drop it with extracts from Karuk leaf with various concentrations using a micropipette. Then, the disc paper that has been dripped with Karuk leaf extract was placed on the top of agar aseptically. The plates were incubated at 37ºC for 24-48 hours. The positive control (SDA media + fungus suspension + ketoconazole), and negative controls (SDA media + fungus suspense + distilated water) were also prepared as a reference. Observed the existence of a barrier area in the form of a clear zone around the paper disc. (Khusnul *et al.* 2017)

Experimental design and data analysis

Completely randomized design (CRD) was used in this experiment, with treatment in the form of different types of concentration extract (30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100%) and control (+). Each treatment was carried out in triplicates so that a total of 27 experimental units were obtained. The clear zone around the paper disc were observed in order to determine anti-fungal activity. Data obtained were then analyzed statistically by means of one-way analysis of variance with a 95% level of validity, the significant level between treatment then analyzed using Duncan test (Steel 1991).

Results

There were different results shown from the test of inhibitory activity of the ethanol extract of Karuk leaves against *M. furfur in vitro* with various extract concentration on Mueller-Hinton agar (MHA) media (Table 1) and (Figure 1).

Table 1. The diameter of clear zone of inhibitory the ethanol extract of Karuk leaf upon *Malassezia furfur* at further testing

Treatment(s)	The Diameter of clear zone of Inhibitory (mm)	Interpretive Category
30%	$5.3^a \pm 0.18$	Resistant
40%	$6.6^b \pm 0.26$	Resistant
50%	$7.6^c \pm 0.20$	Resistant
60%	$11.3^d \pm 0.15$	Resistant
70%	$12.5^e \pm 0.20$	Resistant
80%	$15.6^f \pm 0.25$	Intermediate
90%	$17.4^g \pm 0.20$	Intermediate
100%	$19.5^h \pm 0.43$	Susceptible
Control (+)	$59.2^i \pm 0.20$	Susceptible
Control (-)	0	

Note(s):

- Number followed by the same alphabet was identical based on Duncan test
- Susceptible response signified ≥ 20 mm, Intermediate 15 -19mm, and resistant < 14 mm. (CLSI 2018)

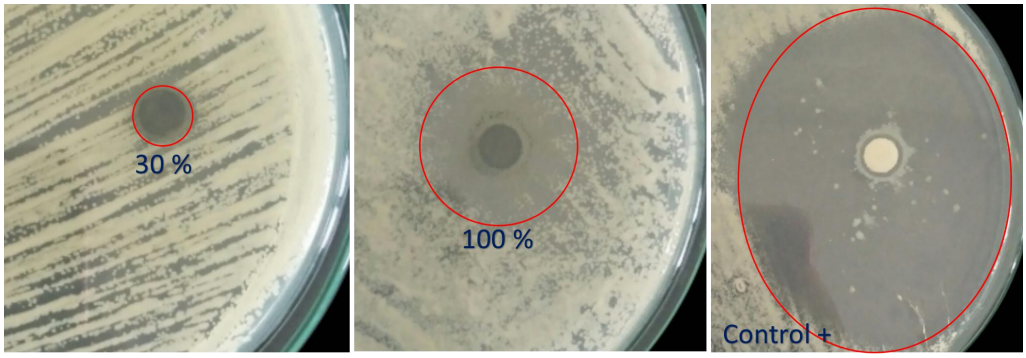

Figure 1. The clear zone indicated inhibition activities from the ethanol extract of Karuk leaf against *Malassezia furfur*

The findings of the phytochemical screening test for ethanol extract of Karuk leaves have been shown in Table 2.

Table 2. Phytochemical screening test results

Phytochemical Test	Results	Note(s)
Saponin	Positive	The foam was formed.
Phenol and Tannin	Positive	There was a color change to dark.
Flavonoid	Positive	Yellow sedimentation was formed.
Alkaloid	Positive	White sedimentation was formed.

Discussion

Based on the results from this study, there show that ethanol extracts from Karuk leaf inhibited the growth of *M. furfur* fungus. It could be detected by inhibition zones or clear areas produced on the agar media. This clear zone caused by tested fungi was not growing well or inhibited due to the presence of anti-fungal substances from the Karuk leaf extract. Active substances diffused and spread in agar media. The results of the further tests given in Table 1 showed different inhibition mean diameters. Almost all of concentration level tested showed different effectiveness. Meanwhile, at a concentration level of 100%, the clear zone reached 19.5 mm, so that classified as susceptible (CLSI 2018). However, its inhibitory zone was not much better than that of which with 2% ketoconazole, of which clear zone reached 59.2 mm.

Each concentration of ethanol extract from Karuk leaf has a difference in the inhibition zone diameter. The higher concentration of ethanol extract from Karuk leaf, the more concentrated of the solution and the higher amount of anti-fungal substances could be extracted. When the anti-fungal substance in the ethanol extract from Karuk leaf was increasing, the growth of *M. furfur* fungus was inhibited probably by body structure and its metabolic system disruption. This study also carried out phytochemical tests to see the presence of chemical compounds in the extracts from Karuk leaf. Some substrates were identified, including flavonoids, saponins, tannins, and essential oils. From the analysis process, positive results of flavonoids are indicated by the orange color on the test tube. Flavonoid may play an important role in anti-fungal activity. There well known that flavonoids can form complex compounds against extra cellular proteins that interfere with the integrity of the membrane and cell wall, disrupting cell metabolism by inhibiting nutrient transport (Nurhafani 2012). Whereas in tannin, positive results are obtained which showed the formation of a deep blue color, which means the presence of tannin compounds could

inhibit the formation of the enzyme C-14 demethylase, which plays a role in the synthesis of ergosterol and inhibited chitin synthesis in cell walls (Siswandono 2000). Saponin solution also contributes as an anti-fungal solution by lowering the surface tension of the sterol membrane in the fungal cell to improve its permeability. It can then damage the permeability of cell walls and eventually cause cell death (Noer *et al*. 2006).

The testing method used in this study was the Kirby-Bauer method or also called the disk method, considering this method was the most widely used method to determine the sensitivity of germs and was based on WHO standards. Besides, this method was not too complicated. Because it only required the type of hatchery media, namely SDA media. Based on its function, the SDA media was clarified as a testing medium, which was a medium for testing certain compounds with the help of antimicrobials. While the test fungus used in this study was the *M. furfur* fungus, because this fungus was one of the fungi that caused mycosis, found in many tropical countries (Sutanto 2009). Based on the research results described above, it shows that several concentrations of ethanol extract of Karuk leaves affect the growth of *M. furfur* in vitro. This is influenced by the presence of several active compounds of flavonoids, saponins, alkaloids, phenol, and tannins in the Karuk leaf plant which is an active compound as an anti-fungal.

Conflict of interest

The authors state no conflict of interest from this manuscript.

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Author contributions

All authors have reviewed the final version of the manuscript and approved it for publication. KK designed the study; AK, LAR performed research and collected the data; KK analysed the data; KK, NIR wrote and reviewed the paper. KK is the main contributor of this manuscript.

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