Effect of Karuk Leaves (*Piper Sarmentosum* Roxb) and White Galangal Rhizome (*Alpinia Galanga* L) Ethanol Extract on the Growth of *Microsporum Gypseum* and *Candida Albicans* in Vitro

Khusnul¹*, R Suhartati¹, DP Virgiianti¹, M Fathurohman¹, ATK Pratita¹

¹ School of Technology Laboratory Medic, Sekolah Tinggi Ilmu Kesehatan Bakti Tunas Husada Tasikmalaya, Indonesia

*khusrul@stikes-bth.ac.id

**Abstrak.** *Piper sarmentosum* Roxb are wild plants that are widely used as an itch and cough medicine. *P. sarmentosum* and *Alpinia galanga* contain flavonoid, saponin, and alkaloid which has a function as an antifungal. This study aims to determine the effect of extract ethanol of Karuk leaves and white galangal rhizome in influencing the growth of *Microsporum gypseum* in vitro. The study was carried out with an experimental method of fungi using the Kirby Bauer. The results showed that the ethanol extract of Karuk leaves (*Piper sarmentosum*) and white galangal rhizome (*Alpinia galanga*) had an effective inhibitory effect on the growth of fungi *M. gypseum* and *C. albicans*. The best inhibitory zone of Karuk leaf extract is at a concentration of 100% with a diameter of 34.2 mm inhibitory zone which is categorized as very strong (*M. gypseum*), diameter of 16.3 mm inhibitory zone which is categorized as strong (*C. albicans*) and while the inhibitory zone of galangal rhizome extract is the best, namely at a concentration of 100% with a diameter of 12.3 mm (*C. albicans*) and 32.06 mm (*M. gypseum*) inhibition with a very strong inhibitory response.

1. Introduction
In Indonesia, health issue has become the most serious problem. There have been a number of health issues Indonesian people have to conquer, which are commonly classified as infectious and non-infectious diseases and caused by a pathogenic microorganism, to name viruses and fungi. Therefore, it is necessary that we maintain our health to keep staying away from infectious diseases. One of the common health issues the Indonesian people are often suffering from is dealing with dandruff, which in fact, is instigated by species from Fungi classification named as *Dermatophytosis*, *Candidiasis*, and *Pityriasis Capitis*. In fact, dandruff refers to a process of automatic peeling of excessive dead skin cells on the head. In nature, the mentioned process is deemed normal, as long as there is just a little amount of the peeling [1]. The three aforementioned fungi are normal flora which is alive on hair. However, their growths are significantly dependent on such a number of factors as temperature, humidity, excessive sebum, and immunity degradation [2]. *Dermatophytosis* is known as an infectious fungus that assaults keratin tissues due to fungal colonization from Dermatophyte species [3]. *Candidiasis* constitutes a fungal disease that is categorized as acute or sub-acute caused by *Candida albicans* species which could grow on some areas such as mouth, vagina, skin, nail, bronchus, and lung. In addition, the species might beset those, men and women, in all ages [4]. *Pityriasis capitis*
(dandruff) is defined as a squamous disorder of head skin which is physiologically initiated by the existence of soft squamate without any inflammatory signs, which is frequently considered as a mild symptom of seborrheic dermatitis [5].

In addition to synthetic medicine, an herbal one is also good for human’s medical treatment. It has been identified that there are various medicinal plants in Indonesia which contain an anti-fungal compound, some of which are Karuk leaves (Piper sarmentosum RoxB) and white galangal rhizome (Alpinia galanga L). There are three species from Fungi class that might result in dandruff, to mention Microsporum gypseum (M. gypseum), Candida albicans (C. albicans), and Pityrosporum ovale (P. ovale) [6]. Many scientists have made a number of studies about the three, but none of whom put their concerns on how ethanol extract of Karuk leaves and white galangal rhizome affect M. gypseum. One of the studies about Karuk leaves was conducted by Shinta [7], focusing on examining the ethanol extract of Karuk leaves upon C. Albicans species. The result showed that under 1250-mg/ml degree of concentration of ethanol extract, the diameter of the inhibition zone signified 31 mm. Unfortunately, there has not been any activation test of Karuk leaves toward M. gypseum. In addition, there was also a study from Gholib [8] that attempted to see the effect of the ethanol extract of Karuk leaves upon Trichophyton mentagrophytes. The study, further, showed that under 50% degree of concentration of ethanol extract, the average range of the inhibition zone signified 17.33 mm. Meanwhile, there are also some studies that focus on the effect of ethanol extract of white galangal rhizome upon the fungal growth. A study from Sutrisno [9] pinpointed that the ethanol extract of white galangal rhizome could obstruct the fungal growth which resulted in dandruff, to be specific Pityrosporum ovale, under 60% degree of concentration. Similar with it, Khusnul [10] exhibited that the extract of white galangal rhizome could hamper the growth of Trichophyton rubrum under 30% degree of concentration, with 3.00-mm inhibition zone.

2. Experimental

The experimental method was used to support this current study. Meanwhile, all the data were collected with respect to the results of laboratory analysis, including ethanol extraction of Karuk leaves (Piper sarmentosum) and white galangal rhizome (Alpinia galanga), which was testified upon the fungal growth of Microsporum gypseum and Candida albicans. An anti-fungal method was directed through a diffusion method by means of Kirby-Bauer one.

2.1. Instruments and materials

There were some instruments that were made in use for the research, including autoclave, Petri dish, beaker glass, blender, Bunsen, funnel, Erlenmeyer, Ose needle, analytical balance, disc paper, measuring glass, chemical glass, test tube, tube rack, and incubator. In addition, some materials were also made of use for the research including antibiotic, aqua, Barium Chloride (BaCl2) 1%, disk antibiotic, ethanol extract 10% - 100%, ethanol 96%, Sulfuric acid (H2SO4) 1%, medium Sabouraud Dextrose Agar, Muller Hinton Agar Natrium Chloride (NaCl), and isolate Microsporum gypseum and Candida albicans.

2.2. Procedures

2.2.1. Material Preparation

The samples of Karuk leaves (Piper sarmentosum) and white galangal rhizome (Alpinia galanga) were gathered from Cineam, Tasikmalaya, East Java. Further, a determination of suitability of the species had been formerly piloted in Laboratory of Plant Taxonomy in Faculty of Biology of University of Jenderal Soedirman.

2.2.2. Extraction of Karuk Leaves and White Galangal Rhizome

a. Ethanol Extract Creation from Karuk Leaves and White Galangal Rhizome by means of Maceration Method

The principle of Maceration: the sieving process of active substance was by soaking the simplicial powder in the extracting fluid which had been set on the proper temperature and was protected from light.
a. The softened simplicial powder was weighed as much as 100 grams before being poured into Erlenmeyer.
b. As much as 1000-mL ethanol fluid with 96% concentration (under 1:10 ratio) was added and soaked for about 3x24 hours with only a few stirrings.
c. The filtrate of the ethanol extract of Karuk leaves and white galangal rhizome was sieved by using Whatman sifter paper no. 41 so that the final result would be the filtrate and pulp.
d. The filtrate of the ethanol extract of Karuk leaves and white galangal rhizome was evaporated by means of rotatory evaporator under temperature < 65 Celsius degree so as to produce a thick substance of ethanol extract.
e. The extract was diluted by using the aqua, to reach a 100% concentration (without any dilution), 90%, 80%, 70%, 60%, and 50%

2.2.3. Anti-fungal Test from the Ethanol Extract of Karuk Leaves and White Galangal Rhizome upon Microsporum gypseum and Candida albicans

a. Standard of 0.5 Mc Farland
   1. As much as 9.95-mL Sulfuric Acid (H₂SO₄) 1% was poured into a sterile tube.
   2. 0.05-mL Barium Chloride (BaCl₂) 1% was added.
   3. Both substances were homogenized, to reach 0.5 Mc Farland of turbidity.
   4. The solution turbidity was set as the standard turbidity of fungal suspension.

b. Suspension Formation of Microsporum gypseum and Candida albicans
   1. Each of fungi was grown up and taken out by means of sterile Ose before being put into Sodium Chloride with 0.85% of concentration. The compound was shaken and stirred to reach the turbidity standard, as significant as 0.5 Mc Farland.
   2. After shaking and stirring, each suspension was inoculated on a slab to get a perfectly solid formation. The suspension was shaken and stirred for a couple of minutes before inoculation on each Petri dish to restrain any sedimentation in the suspension.

c. Treatment
   1. The suspension of M.gypseum and Candida albicans was taken out as much as 100µ and spread aseptically over MHA media by means of the sterile stirring rod.
   2. The blank paper disk was dyed to an extract of cardamom with several variations of concentration starting from 10% to 100%. In addition, positive and negative controls were set; the formerly contained ketoconazole 2%, while the latter sterile aqua.
   3. The dyed blank paper disk was taken out and affixed on the surface of MHA media in the Petri dish.
   4. The blank paper disk was incubated under the standard temperature (between 20 to 30 Celsius degree) for two days. Over the paper, the formed inhibition zone was observed and measured.

3. Data Analysis
   The observed parameter in the research referred to the diameter of the inhibition zone which was, in this case, in form of the vibrant zone around the paper. Data of the test result would be analyzed statistically by means of One-way Analysis of Variance with 95% level of validity or as significant as α= 0,05, and be continued to Duncan test.

4. Result and Discussion

There were different results shown from the test of inhibitory power of the ethanol extract of Karuk leaves (Piper sarmentosum) and white galangal rhizome (Alpinia galanga L) upon Microsporum gypseum and Candida albicans in vitro with various concentration on Muller Hinton Agar (MHA) media, whether on the basis of inhibitory power or of the fungal growth. The inhibitory power was analyzed by means of one-way analysis of variance and continued to Duncan test. The detailed result is elaborated in the following Table 1.
The results of a phytochemical screening test of the ethanol extract of Karuk leaves (*Piper sarmentosum*) and white galangal rhizome (*Alpinia galanga L.*) were shown in Table 2 as follows.

### 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. |

The results of the further testing provided in Table 1 displayed different diameter averages of inhibitory power, both from the extracts of Karuk leaves and white galangal rhizome. Referring to the treatment with the extract of Karuk leaves, almost all the concentration levels showed different effectiveness, but there were some concentration levels signified the same inhibitory power, to name on 50% level signifying 31.1 mm, 60% level 31.4 mm, 70% level 31.5 mm, and 80% level 31.7 mm. Meanwhile, on a 100% level of concentration, it was revealed the most excellent power compared to the others. However, its inhibitory power was not much better than that of which with 2% ketoconazole of which inhibitory power reached 40 mm.

Alluding to the treatment with the extract of white galangal rhizome, it was shown that the diameter averages of each level of concentration resulted in real difference, except one with 10% level of concentration (with 5.2 mm) which was equal to one with 20% level of concentration (with 6.2 mm). In addition, one with 20% level of concentration was also similar to another with a 30% level of concentration (with 7.2 mm). It pointed out that each level of concentration had different ability in inhibiting the growth of *M. gypseum*. 100% level of concentration (with 32 mm) was shown as the most effective concentration for inhibition among others, but less effective if it was compared to 2% ketoconazole. Counterproductively, 100% concentration of white galangal rhizome had greater inhibitory power compared to the extract of Karuk leaves in the same level of concentration.

Another difference was evident on a 10% level of concentration. The extract from Karuk leaves could inhibit as significant as 22.3 mm, while the other from white galangal rhizome was only able to inhibit
the growth of *M. gypseum* as significant as 5.3 mm. Besides, there were different results of diameters in inhibitory power. In addition, there were also different responses based on the diameters of the inhibitory power. In the extract from Karuk leaves, there were shown some differences, which were all responses at all levels of concentration were categorized very strong as all the levels could effectively inhibit the growth of *M. gypseum* more than 20 mm.

The responses of the inhibitory power from the extract of white galangal rhizome was smaller than that of Karuk leaves since not all levels of concentration has a very strong ability; only some of those were deemed very strong, such as 70%, 80%, 90% and 100%, which were all equal to the response from ketoconazole. The formation of the inhibitory zone in each concentration level of ethanol extract was generated by the contribution of active substances or secondary metabolite solutions which were allowed to inhibit the fungal growth. Based on the phytochemical test from the extracts of Karuk leaves and white galangal rhizome resulted in anti-fungal solution in forms of flavonoid, saponin, tannin, phenol dan alkaloid.

The results (table 1) of the treatment of Karuk leaf extract almost all concentrations showed different effectiveness, but some concentrations had the same inhibitory effectiveness, namely at a concentration of 10% (6.2 mm), with 20% (6.3 mm) while the concentration of 100% (16.3 mm) has the best ability of other concentrations, but no better inhibitory ability when compared with 2% ketoconazole control with a diameter of 40 mm inhibition.

The treatment results of galangal rhizome extract, ie the mean diameter of the inhibitory power of each concentration had a very significant difference, except the concentration of 10%, 20%, 30%, and 40% were not able to inhibit the growth of *C. albicans*. Concentrations other than others have different inhibitory abilities. Concentration of 100% (12.3 mm) is the treatment that has the best ability of the other concentrations, when compared with control of ketoconazole 2% the inhibitory ability is smaller, and at 100% concentration of galangal rhizome extract the inhibitory power is smaller when compared with the inhibitory effect of Karuk leaf extract at the same concentration.

The anti-fungal solution had several mechanisms for fungal inhibition by neutralizing any enzyme which was intercorrelated with fungal invasion and colonization, obstructing fungal cell membrane, inhibiting fungal enzyme system to prevent the growth of hyphae, and influencing synthesis process of nucleate acid and protein [11].

Flavonoid is defined as a solution that contributes to preventing the formation of the cell membrane or cell wall of fungi so that the formation process of the cell wall remains unformed. This occurs due to the fact that denaturalization of cell protein could prevent the enzyme function within the cell.

Meanwhile, tannin refers to any solution that generates an imbalance in fungal cytoplasm and membrane by changing membrane permeability and transform membrane function during a transportation process of essential solutions so as to trigger a metabolic imbalance which inhibits and cuts down the fungal growth [10]. Saponin solution, in addition, contributes as anti-fungal by lowering down the surface tension of sterol membrane within the fungal cell so that the permeability will increase.

5. Conclusion

Based on the results the ethanol extract of Karuk leaves (*Piper sarmentosum*) and white galangal rhizome (*Alpinia galanga*) had an effective inhibitory effect on the growth of fungi *Microsporum gypseum* and *Candida albicans*. The best inhibitory zone of Karuk leaf extract is at a concentration of 100% with a diameter of 34.2 mm inhibitory zone which is categorized as very strong (*Microsporum gypseum*), diameter of 16.3 mm inhibitory zone which is categorized as strong (*Candida albicans*) and while the inhibitory zone of galangal rhizome extract is the best, namely at a concentration of 100% with a diameter of 12.3 mm (*Candida albicans*) and 32.06 mm (*Microsporum gypseum*) inhibition.
with a very strong inhibitory response, the same as the inhibitory response of 2% ketoconazole as a positive control.

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