

## Fungicidal Effects of Chloroform Extract of Red Galangal (*Alpinia purpurata* (Vieill.) K. Sch) on the Growth of *Trichophyton rubrum*

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**Abstract:** *Trichophyton rubrum* is the most common causative agent of anthropophilic dermatophytosis worldwide. Treatment of dermatophytosis can use natural ingredients. In Indonesia, several medicinal plants have been used, one of which is red galangal (*Alpinia purpurata* (Vieill.) K. Sch). This study aims to determine the effectiveness of red galangal rhizome chloroform extract on the growth of *Trichophyton rubrum*. This study is a True Experiment study with Posttest Only With Control Group Design consisting of 8 treatments namely positive control, extract control, 0 mg/mL (negative control), 20 mg/mL, 30 mg/mL, 40 mg/mL, 50 mg/mL, and 60 mg/mL extract of red galangal rhizome chloroform with 3 repetitions. The antifungal activity test was carried out using the determination of Minimum Inhibitory Concentration (MIC), Minimum Fungicidal Concentration (MFC), and Bioautography Test. The results showed that the red galangal rhizome chloroform extract in the Agar Overlay Bioautography Test contained an inhibition zone on the growth of *Trichophyton rubrum*. The determination of MIC and MFC were 20 mg/mL and 40 mg/mL, respectively. The study concludes that the red galangal rhizome chloroform extract affects the growth of the fungus *Trichophyton rubrum*. Research can be continued by knowing more specifics about the bioactive compounds from the red galangal rhizome, which have antifungal activity against *Trichophyton rubrum*.

**Keywords:** Red galangal rhizome; *Trichophyton rubrum*; chloroform; bioautography test; minimum inhibitory concentration.

## INTRODUCTION

Dermatomycosis is most often caused by dermatophytosis<sup>1</sup>. Dermatophytosis is one of the causative agents of fungal infections of the superficial skin and the most common cause of skin diseases globally. Dermatophytosis is keratolytic and destroys keratinized tissue that accounts for most superficial skin infections, hair, and nails. Anthropophilic dermatophytosis *Trichophyton rubrum* is the most common causative agent worldwide and is the cause of tinea pedis and tinea unguium<sup>1,2</sup>. The results of Karmila et al.'s research on elderly patients at Sanglah Hospital, Bali, Indonesia, reported that 40% of patients were infected with *Trichophyton rubrum* from culture examinations<sup>3</sup>. Another study of fish-slicing women in the Fisherman's Settlement in Bengkulu City, Indonesia, found that nine people (37.5%) out of 24 respondents were infected with *Trichophyton rubrum*<sup>4</sup>.

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According to data from basic health research in 2018, 24.6% of all ages in Indonesia have used family medicinal plants<sup>5</sup>. Medicinal plants are known to have various benefits because of their active compounds. Red galangal (*Alpinia purpurata* (Vieill.) K. Sch) is a medicinal plant that people in Indonesia widely use. Research on the content of red galangal has been done a lot. The results of phytochemical screening of red galangal rhizome ethanol extract found flavonoid compounds, saponins, carbohydrates, proteins, glycosides, terpenoids, resins, and tannins<sup>6</sup>. Another study by Kusriani and Zahra stated that the n-hexane extract of the red galangal rhizome contains katekat tannins, quinones, steroids/triterpenoids. In contrast, red galangal rhizomes' ethyl acetate and ethanol extracts contain flavonoids, tannins, quinones, and steroids/triterpenoids<sup>7</sup>. The results of chloroform extract and aqueous solvents of red galangal rhizome obtained higher alkaloids and tannins<sup>8</sup>.

There has not been much research on the effect of red galangal rhizome on *Trichophyton rubrum*. A research result showed that the juice of the red galangal rhizome effectively inhibited the growth of *Trichophyton rubrum*. At the same time, in the form of essential oils and methanol extract, it effectively inhibited the growth of *Malassezia furfur*, *Aspergillus flavus*, *Candida albicans*, *Aspergillus niger*, *Candida vulgaris*, and *Candida tropicalis*<sup>4,9,10,11</sup>. It is necessary to conduct research using other non-polar solvents such as chloroform. This study aimed to identify the active compounds contained in the red galangal rhizome chloroform extract and determine the effect of the red galangal rhizome chloroform extract on the growth *Trichophyton rubrum* by determining the MIC, MFC, and bioautography methods for overlaying.

## MATERIALS AND METHODS

The type of research used is an actual experiment. The research design used in this study was Posttest only with a control group design. This study compared the growth inhibition of *Trichophyton rubrum* against red galangal rhizome chloroform extract with concentrations of 20 mg/mL, 30 mg/mL, 40 mg/mL, 50 mg/ml, and 60 mg/mL from the results of the dilution test and bioautography test for overlaying. With a positive control group and a negative control group.

The galangal rhizome used in the study came from the Banjarbaru area of Indonesia. The galangal rhizome was processed into simplicia. The manufacture of red galangal rhizome extract using the maceration method with non-polar solvent chloroform produces a thick blackish brown extract with a yield of 7.04%. The extract was taken partly to be tested for its phytochemical content qualitatively. Subsequently, it was tested by Thin Layer Chromatography (TLC) by spotting the extract in TLC and eluted with hexane, ethyl acetate, and ethanol in a ratio (5:3:2)<sup>12</sup>. The TLC plate with the eluted stain was then tested for overlay bioautography by placing the TLC plate into a sterile petri dish with a diameter of 15 cm, pouring *Trichophyton rubrum* suspension in Sabouraud's dextrose agar (SDA) with a ratio of 107cfu/mL: 9 mL at 50°C until a layer was formed, incubated at room temperature for seven days.

Determination of MIC by dilution method, namely by planting *Trichophyton rubrum* in TSB with a ratio of 107cfu/mL then mixed with various concentrations of chloroform extract, incubated at room temperature for seven days. The results were read by measuring the absorbance value of each treatment on a photometer using a wavelength of 420 nm. Meanwhile, the determination of MFC by the dilution method was done by

pipetting as much as 20 l of each control and concentration treatment in the tube for determining the MIC spread over SDA. incubated at room temperature for seven days. This research was conducted at the Microbiology Laboratory and Applied Chemistry Laboratory, Health Analyst Department, Health Polytechnic Banjarmasin, Indonesia. Laboratory of the Faculty of Mathematics and Natural Sciences (FMIPA) and the Laboratory of Biochemistry and Biomolecular at Lambung Mangkurat University, Banjarmasin Indonesia.

## RESULTS AND DISCUSSION

Qualitative Phytochemical Test results, Thin Layer Chromatography (TLC) test results, and Bioautography test results for Overlay can be seen in Table 1,2,3.

Table 1. Qualitative Phytochemical Test Results

Parameters	Results
Saponin	+
Alkaloid	-
Steroid	+
Fenolik	+
Flavonoid	+
Triterpenoid	+

Table 2. Results of Rf TLC Extract of Red Galangal Rhizome Chloroform

Repetition	Rf-value	Dot Colour
1	0,91	Yellow
2	0,90	Yellow
3	0,91	Yellow

\*Retardation factor (Rf)= Distance traveled by extract/Distance traveled by solvent.

Table 3. Bioautography Test Results Agar Overlay Chloroform Extract of Red Galangal Rhizome

Repetition	Growth Inhibition Zone
1	Yes
2	Yes
3	Yes

Bioautography results In order for the Overlay to show an inhibition zone around the stain formed from the TLC results, where the stain looks clear, which indicates the absence of fungal growth, with a size of about 5 mm. Determination of MIC using a photometer is done by comparing the absorbance values before and after incubation. A decrease in the absorbance value indicated the inhibition of fungal growth after incubation. On the other hand, an increase in absorbance value after incubation indicates that fungal growth is still occurring<sup>13</sup>.

Table 4. Average Results of Minimum Inhibitory Concentration (MIC) Red Galangal Rhizome Extract against *Trichophyton rubrum*

Concentration (mg/mL)	Average Absorbance		Results
	Before Incubation	After Incubation	
20	0,811	0,789	Decreased
30	0,815	0,688	Decreased
40	0,831	0,382	Decreased
50	0,834	0,434	Decreased
60	0,862	0,467	Decreased
Positive Control	0,821	0,811	Decreased
Negative Control	0	0	N/A
Extract Control	0,811	0,820	Increased

Based on the results of absorbance measurements using a photometer with a wavelength of 420 nm, the absorbance value of 20 mg/mL was the lowest concentration that decreased the absorbance value before and after incubation. This indicates the inhibition of fungal growth. So that the concentration of 20 mg/mL was determined as the Minimum Inhibitory Concentration (MIC) of the red galangal rhizome chloroform extract on the growth of *Trichophyton rubrum*.

Table 5. Average results of Minimum Fungicidal Concentration (MFC) Red Galangal Rhizome Extract against *Trichophyton rubrum*

Concentration (mg/mL)	Average Colonies Number
0 (kontrol negatif)	∞
20	701
30	201
40	0
50	0
60	0
Kontrol positif	0
Kontrol ekstrak	0

Determination of the MFC value of red galangal rhizome chloroform extract on the growth of *Trichophyton rubrum* at a concentration of 40 mg/mL was expressed as MFC because it was the lowest concentration no fungal colony growth was found.

The results of the phytochemical test showed that there were chemical compounds in the chloroform extract of the red galangal rhizome (table 1) except for the alkaloid compound, in contrast to the study of Chan and Wong (2015), which showed that the chloroform extract and aqueous solvents of the red galangal rhizome were found to be high in alkaloids and tannins. The results of TLC with a mixed eluent of hexane, ethyl acetate, and ethanol (5:3:2) obtained one chromatogram with an average R<sub>f</sub> of 0.91 (table 2). TLC results were used for overlay bioautography as a screening test for antifungal activity.

The results in table 5 show a decrease in fungal growth and an increase in concentration. However, in the positive control and control extract, it was seen that there

was a decrease in the absorbance value of the positive control and an increase in the control extract after incubation. The increase in absorbance in the control extract was made possible by extracting particles that were not completely dissolved, apart from an error in the measurement on the photometer; dirty cuvette walls, air bubbles, and insoluble particles<sup>14</sup>.

The MIC yield of chloroform extract of red galangal rhizome against *Trichophyton rubrum* was 20 mg/mL. Previous studies used Ethyl Acetate as a solvent with a MIC value of 1.56 mg/mL<sup>15</sup>. These results show differences because the solvents used are different; ethyl acetate is a semi-polar solvent while chloroform is a non-polar solvent. The results of the determination of MFC (Table 5) chloroform extract of red galangal rhizome on the growth of *Trichophyton rubrum* at a concentration of 40 mg/mL were expressed as MFC because growth inhibition occurred at that concentration.

The study's limitations were contamination with *Aspergillus niger*, *Aspergillus flavus*, and *Penicillium* fungi. Contamination control has been carried out using sterile tools and materials, and the work process is carried out in the Biosafety Cabinet. Although aseptic techniques have been used to prevent contamination, according to Hafsani, microorganism contamination can occur through the operator's hands, gloves, or laboratory coats<sup>16</sup>.

Red galangal extract has been proven in vitro to have potential as a fungicide, especially for *Trichophyton rubrum*. Research can be continued by knowing more specifics about the bioactive compounds from the red galangal rhizome, which have antifungal activity against *Trichophyton rubrum*. Developing this natural ingredient of red galangal requires in vivo research and clinical trials, so that it becomes an alternative treatment for fungal infections.

## CONCLUSION

Secondary metabolites of red galangal rhizome chloroform extract are flavonoids, phenolics, steroids, saponins, and triterpenoids. These secondary metabolites have zones of inhibition on the growth of *Trichophyton rubrum* based on the Agar Overlay Bioautography Test. The results of inhibition of red galangal rhizome chloroform extract on the growth of *Trichophyton rubrum* were Minimum Inhibitory Concentration (MIC) 20 mg/mL and Minimum Fungicidal Concentration (MFC) 40 mg/mL. The development of this natural ingredient of red galangal requires in vivo research and clinical trials, so that it becomes an alternative treatment for fungal infections.

## ACKNOWLEDGMENT

The author would like to thank those who have helped carry out this research, especially for Poltekkes Kemenkes Banjarmasin, Poltekkes Research Unit, and Laboratory in Medical Laboratory Technologist Campus, who have facilitated this research.

## CONFLICT OF INTEREST

The authors declare no conflict of interest and have not received any funds for this study.

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