Comparison of The Effectiveness Between Red Galangal Rhizome's (Alpinia Purpurata K.Schum) Extract And Ketoconazol 2% To Inhibit Growth Of Dermatophyte Fungi In Vitro

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Abstract: Red galangal rhizome (Alpinia purpurata k. Scum) has larger size and has more therapeutic properties. Red galangal has compounds that can inhibit fungal growth namely flavonoids and saponins which can interfere the function of the fungal cytoplasmic membrane, besides that they can cause leakage and aggregation of the outer cell membrane, which can inhibit the growth of dermatophytes. This study is a laboratory experimental study, using antifungal activity test with diffusion method using paper discs to see the comparison of the red galangal extract effectiveness and ketoconazole 2% on the growth of dermatophytic fungi Microsporum canis, Microsporum gypseum, and Trichophyton metagrophytes by measuring the inhibition zone around the fungal colonies. The concentrations of red galangal extract used were 10%, 20%, 30%, 40%, and 50% and used 2% ketoconazole as control. The data was analyzed using the One Way Anova test. Result of this study shown that all concentration with out 10% of red galangal have inhibit zones of Microsporum canis, Microsporum gypseum, and Trichophyton metagrophytes but not better that ketoconazol 2% activities.

Keywords: Red galangal extract (Alpinia purpurata k. Scum), Ketoconazole 2%, Dermatophytes

INTRODUCTION

Dermatophytes are a class of fungi that cause dermatophytosis. This group of fungi has keratin digesting properties. Dermatophytes belong to the fungi impeti class, which is divided into 3 genera, namely Microsporum, Trichophyton, and Epidermophyton. Dermatophytosis is also called "tinea" infection which is further grouped based on the location of the
infection, namely: *Tinea capitis, Tinea corporis, Tinea cruris, Tinea manus et pedis, Tinea unguium, Tinea barbae* and *Tinea imbrikata*.\(^1\)

including white rhizomes (white galangal) and red rhizomes (red galangal). Red galangal rhizome has a larger size and more therapeutic efficacy.\(^2\) Red Galangal (*Alpinia purpurata k. Schum*) has been studied in various studies and it has been proven that this plant has various biological effects such as anti-inflammatory, antioxidant, antifungal, antiviral, antibacterial, and anticancer activity.\(^3\) The content of galangal contents include 0.5-1.5% volatile oil containing 1.8-sinerol component 5.6%, eugenol, methylcinamic 2.6%. The biting heat and warmth comes from a mixture of volatile compounds namely diarilheptanoid (galangol), and there are also ginerols, acetoicirichol acetate, acetyleneulgen acetate, and karyophilenol-1. In addition to essential oils, there are also flavonoids derived from quercetin, kaemferida, 7-hydroxy-3,5-dimethoxy flavone, galangin (3,5,7, - trihydroxyplavone), alpinine, isoramnetin, kaemferol, kaemferol-7-methyleter, kaemferol-4 -metileter, quercetin, and quercetin-3-methyleter.\(^4\) Some flavonoids in galangal rhizome have been identified such as kaemperol, kaemferide, galangin and alpinin. The mechanism of action of flavonoids in inhibiting fungal growth is by causing disruption of fungal cell membrane permeability. Hydroxyl groups found in flavonoid compounds cause changes in organic components and nutrient transport which will eventually result in fungal cells becoming lysis.\(^5\) Based on research conducted by Haraguchi, antifungal activity of galangal by diterpene compounds is associated with changes in fungal lipid membrane permeability.\(^6\) Meanwhile, according to research conducted by Brian J Kopper regarding the effect of diterpene on fungi, it has been proven that it is efficacious as an antifungal by reducing fungal cell division and fungal mycelial growth.\(^7\) Based on research conducted by Silvina, galangal rhizome extract 10% more effective when compared with 2% ketoconazole in inhibiting the growth of Candida albicans in candidiasis vaginalis.\(^8\)

**METHODS**

This research is a type of laboratory experimental research using post test only control group design to see the comparison of the effectiveness test of red galangal rhizome extract (*Alpinia purpurata k.schum*) with a concentration of 10%, 20%, 30%, 40%, 50%, and ketoconazole 2% in inhibiting the growth of dermatophyte fungi. Red galangal extract in this study was obtained with a weight of 160 grams in the form of a thick extract.

This research was conducted at the Microbiology and Phytochemistry Laboratory of the Faculty of Pharmacy, University of North Sumatra. The sample of this study was dermatophyte fungi taken from the Microbiology Laboratory of the Faculty of Pharmacy, University of North Sumatra which consisted of *Microsporum canis, Microsporum gypseum*, and *Trichophyton metagrophytes*.

The extraction process is carried out using the maceration method. Antifungal testing was carried out by the diffusion method using disc paper soaked in red

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galangal extract that had been made in each dilution concentration with DMSO solution, i.e. concentrations of 10%, 20%, 30%, 40%, and 2% ketoconazole implanted into the fungus Microsporum canis, Microsporum gypseum, and Trichophyton metagrophytes on Sabouraud Dextrose Agar media.

Analysis of the inhibition zone diameter data was performed using the Shapiro Wilk test and then continued with the One Way Anova test.

RESULTS

Galangal has several known varieties, Phytochemical screening results of red galangal ethanol extract in table 1 show the presence of flavanoids, glycosides, saponins and triterpenoids.

The results of inhibition zones of red galangal extract with various concentrations in table 2 show that for T. Metagrophytes, inhibitory zones were seen at all concentrations ranging from 10% to 50%, while the shows of M. canis and M. gypseum inhibitory zones were seen starting at concentrations of 20%. Ketoconazole as a positive control showed inhibition zones in each repetition (table 3). Comparison of mean diameter of inhibition zone of red galangal rhizome extract based on concentration compared to ketoconazole showed a significant difference in all fungal species (table 4).

Table 1. Phytochemical screening results of red galangal ethanol extract

| No. | Screening | Results |
|-----|-----------|---------|
| 1.  | Alkaloids | -       |
| 2.  | Flavonoids| +       |
| 3.  | Glycosides| +       |
| 4.  | Saponin   | +       |
| 5.  | Tannin    | -       |
| 6.  | Triterpenoids | +     |

Notes: (+) = contains a class of compounds; (-) = does not contain compounds

Table 2. Results of Inhibition Zones of Red Galangal Extract with Various Concentrations on Fungal Growth Microsporum canis, Microsporum gypseum, and Trichophyton metagrophytes.

| No | Dermatophytes fungi | Inhibition Zone Diameter of Each Concentration Red Galangal Extract (mm) |
|----|---------------------|--------------------------------------------------|
|    |                     | 50%    | 40%    | 30%    | 20%    | 10%    |
| 1. | M. canis (I)        | 8.4    | 8.0    | 7.4    | 6.9    | -      |
|    | M. canis (II)       | 8.8    | 8.4    | 7.0    | 6.8    | -      |
|    | M. canis (III)      | 8.3    | 7.8    | 7.1    | 6.6    | -      |
|    | Mean:               | 8.5    | 8.06   | 7.16   | 6.76   | -      |
| 2. | M. gypseum (I)      | 9.5    | 8.3    | 7.4    | 7.0    | -      |
|    | M. gypseum (II)     | 9.0    | 8.2    | 7.6    | 6.8    | -      |
|    | M. gypseum (III)    | 9.4    | 8.4    | 7.8    | 6.9    | -      |
|    | Mean:               | 9.3    | 8.3    | 7.8    | 6.9    | -      |
| 3. | T. metagrophytes (I)| 9.9    | 8.8    | 8.4    | 7.6    | 6.9    |
|    | T. metagrophytes (II)| 10.0  | 8.9    | 8.6    | 8.0    | 6.8    |
|    | T. metagrophytes (III)| 9.7   | 9.0    | 7.9    | 7.4    | 7.0    |
|    | Mean:               | 9.86   | 8.9    | 8.3    | 7.66   | 6.9    |

Notes:
(1)= Repetition 1; (II) = Repetition 2; (III) = Repetition 3
Table 3. Results of Ketoconazole Tablets 2% Inhibition Zones Against Fungal Growth *Microsporum canis*, *Microsporum gypseum*, dan *Trichophyton metagrophytes*.

| No. | Dermatophytes fungi | Ketoconazole Tablet 2% Inhibition Zone Diameter (mm) | Mean |
|-----|---------------------|----------------------------------------------------|------|
|     |                     | (I)       | (II)      | (III)     |      |
| 1.  | *M. canis*          | 27.9      | 28.4      | 28.9      | 28.4 |
| 2.  | *M. gypseum*        | 29.3      | 30.0      | 29.7      | 29.67|
| 3.  | *T. metagrophytes*  | 31.6      | 30.9      | 31.2      | 31.23|

Notes:
(I) = Repetition 1; (II) = Repetition 2; (III) = Repetition 3

Table 4. Difference in Average Diameter of Inhibitory Zone of Red Galangal Rhizome Extract Based on Concentration and Ketoconazole 2% in Each Fungal.

| Fungal | Treatment | Inhibitory Zone Diameter (mm) | Mean     | SD        | Sig.    | p-value |
|--------|-----------|--------------------------------|----------|-----------|---------|---------|
| *M. canis* | Konsentrasi 50% | 8,5000 ± 0,26458 | 0,365a   | 0,000*    |         |         |
|         | Konsentrasi 40% | 8,0667 ± 0,30551 | 0,637a   | 0,000*    |         |         |
|         | Konsentrasi 30% | 7,1667 ± 0,20817 | 0,463a   | 0,000*    |         |         |
|         | Konsentrasi 20% | 6,7667 ± 0,15275 | 0,637a   | 0,000*    |         |         |
|         | Ketokonazol 2%  | 28,4000 ± 0,50000 | 1,000a   | 0,000*    |         |         |
|         | Blank (DMSO)    | 0                         | 0             | 0         | 0.000*  |         |
| *M. gypseum* | Konsentrasi 50% | 9,3000 ± 0,26458 | 0,365a   | 0,000*    |         |         |
|         | Konsentrasi 40% | 8,3000 ± 0,10000 | 1,000a   | 0,000*    |         |         |
|         | Konsentrasi 30% | 7,6000 ± 0,20000 | 1,000a   | 0,000*    |         |         |
|         | Konsentrasi 20% | 6,9000 ± 0,10000 | 1,000a   | 0,000*    |         |         |
|         | Ketokonazol 2%  | 29,6777 ± 0,40000 | 0,956a   | 0,000*    |         |         |
|         | Blank (DMSO)    | 0                         | 0             | 0         | 0.000*  |         |
| *T. metagrophytes* | Konsentrasi 50% | 9,8667 ± 0,15275 | 0,637a   | 0,000*    |         |         |
|         | Konsentrasi 40% | 8,9000 ± 0,10000 | 1,000a   | 0,000*    |         |         |
|         | Konsentrasi 30% | 8,3000 ± 0,36056 | 0,637a   | 0,000*    |         |         |
|         | Konsentrasi 20% | 7,6667 ± 0,30551 | 1,000a   | 0,000*    |         |         |
|         | Ketokonazol 2%  | 31,2333 ± 0,3511 | 0,843a   | 0,000*    |         |         |
|         | Blank (DMSO)    | 0                         | 0             | 0         | 0.000*  |         |

Notes:
a = Shapiro Wilk test: p>0.05 : normally distributed data; * = One way Anova test: p<0.05: significant differences

DISCUSSIONS
In this study the inhibition zone diameter of red galangal extract with a concentration of 10%, 20%, 30%, 40%, and 50% were tested on fungal *Microsporum canis*, *Microsporum gypseum*, and *Trichophyton metagrophytes*, indicates antifungal activity. Antifungal activity of red galangal extract is classified as moderate. This is in accordance with the opinion of Greenwood, which states that if the diameter of the inhibitory power is 5-10 mm, the inhibitory activity is classified as moderate.9
Based on the phytochemical screening results, red galangal extract contains flavonoids, glycosides, saponins, and triterpenoids. Antifungal contents found in red galangal extract include flavonoids and saponins. Flavonoids are anti-inflammatory, antimicrobial, analgesic and anti-oxidant compounds. Flavonoids are the largest group of phenol compounds, phenol compounds have the property of effectively inhibiting the growth of viruses, bacteria, and fungi. Flavonoids cause damage to the permeability of fungal cell walls. The anti-inflammatory mechanism occurs through its inhibitory effect on the arachidonic acid metabolism pathway, the formation of prostaglandins, the release of histamine in inflammation. Flavonoids contain phenol compounds. Phenol is a type of acidic alcohol so it is also called carbolic acid. Phenols have the ability to denaturate proteins and damage fungal cell walls.\textsuperscript{10}

Saponin compounds can disrupt the stability of cell membranes in fungi that cause damage to cell membranes and cause the release of various important components of the fungal cells, namely proteins, nucleic acids and nucleotides.\textsuperscript{11}

Based on the measurement results show that the fungus Trichophyton metagrophytes has the largest average diameter of the inhibitory zone. This is because the fungus Trichophyton metagrophytes has an anthropophilic living habitat that is transmission from human to human. Anthropophilic species cause relatively mild and chronic infections in humans, produce little conidia in culture, and can be eradicated. The Trichophyton metagrophytes colony has a granular cotton form showing
many groups of round micronidia shaped like grapes in terminal 20 branches. Tricophyton dermatophytes with fine-walled microscopic morphology have adapted to humans as hosts, and will cause a mild to non-inflammatory response.\textsuperscript{12}

Based on the measurement results show that the Microsporum gypseum fungal had the second largest inhibitory zone diameter after Trichophyton metagrophytes. This is because the Microsporum gypseum fungal has a goefilik living habitat that is transmission from soil to humans. As a species that evolved from soil habitats to human hosts, the Microsporum gypseum fungus loses the ability to produce asexual conidia and reproduce sexually to produce spores. Microsporum gypseum forms a light brown colony like powder and many four-to-six-celled thick-walled macronidia.\textsuperscript{13}

Based on the measurement results show that the Microsporum canis fungal has the smallest inhibitory zone diameter. This is because the Microsporum canis fungus has a zoophilic living habitat that is transmission from animals to humans. Microsporum canis form colonies with white surfaces such as cotton, thick-walled macronides, 8 to 15 cells, and often have curved and hooked ends. Zoophilic species infections usually produce severe inflammation.\textsuperscript{13}

In a study conducted by Silvina on the Comparative Test of the Effectiveness of Galangal Rhizome Extract (Alpinia Galanga) 10\% with Ketoconazole 2\% In Vitro Against the Growth of Candida Albicans in Vaginal Candidiasis. It was found that Candida albicans grows more on Sabouraud Dextrose Agar media containing 2\% ketoconazole compared to Sabouraud Dextrose agar media containing 10\% galangal rhizome extract so that it can be concluded that there is a difference in effectiveness between 2\% ketoconazole and 10\% galangal rhizome extract in vitro.\textsuperscript{8}

From this research it can be seen that the higher the concentration of red galangal extract, the higher the diameter of the inhibition zone formed. The same thing was also found in research on Comparison of the Effectiveness of Red Galangal (Alpinia purpurata k schum) and White Galangal (Alpinia galanga) on the growth of Candida Albicans Fungal in vitro. The size of the inhibition zone is influenced by the concentration of the extract given, increasing the concentration of the extract causes an increase in the content of the active ingredient that functions as an antifungal so that its ability to inhibit the growth of a fungus is also greater. Unequal increase and decrease in inhibition zone can be caused by the solubility of the active substance in the extract and the difference in the diffusion rate of the agar media.\textsuperscript{14}

**CONCLUSIONS**

Measurement of inhibition zone diameter of red galangal extract of various concentrations in Trichophyton metagrophytes fungi had the largest average diameter of inhibitory zone. Measurement of the diameter of the inhibition zone of red galangal extract of various concentrations in Microsporum canis fungi has an average diameter of the smallest inhibitory zone. Comparison of the effectiveness of red galangal rhizome extract and ketoconazole 2\% in dermatophyte fungi is that the antifungal activity of 2\% ketoconazole is more effective than the red galangal rhizome extract, although the antifungal activity of red galangal extract is classified as moderate. The higher the concentration of red galangal extract, the wider the inhibition zone dimension.
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