Antifungal Combination of Miconazole and Sulfur for the Treatment of Dermatophytosis

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Abstract. Dermatophytosis is a fungal infection of keratinized tissue (skin, hair, and nails) caused by three genera of dermatophytes: *Epidermophyton*, *Microsporum*, and *Trichophyton*. Dermatophytosis is a zoonotic disease that easily spreads to other animals as well as to humans. Long treatment will give great loss not only to livestock but also to humans. Miconazole and sulfur are antifungals that have often been used to treat fungal-caused dermatophytosis. The purpose of this study was to obtain the composition of sulfur and miconazole combination which was effective in killing dermatophytes fungus. The fungus used was *Trychophyton mentagrophytes*. The antifungal doses used were single miconazole with a graded dose of 10mg/g, sulfur with a graded dose of 30 mg/g, and a combination of miconazole and sulfur. The test method used was the diffusion test to see the width of the inhibition zone and the dilution test (pour plate) to calculate the growth of the fungus. The concentration of fungus used for the test was 10^6 cfu/ml. Solutions of antifungal and fungus active ingredients were tested using Sabouraud Dextrose Agar media. The results of the in vitro test showed that the width of the inhibition zone on miconazole 10 mg/g was 35.2 mm (diffusion test) with 0 fungal growth (dilution test). Sulfur concentration 30 mg/g the inhibition zone was 4,83 mm (diffusion test) with 34 cfu/ml fungus growth (dilution test). In the combined of sulfur 7,5 mg/g and miconazole 5 mg/g, the width of the inhibition zone was 30.3 mm with the growth of fungus colonies is 0. Based on the results of the diffusion and dilution tests, the best combination composition between sulfur and miconazole was obtained at a concentration of 7,5 mg/g sulfur and 5 mg/g miconazole.

Keywords: Dermatophytosis · miconazole · sulfur · combination

1 Introduction

Superficial skin disease caused by three genera of dermatophytes, namely *Epidermophyton*, *Microsporum*, and *Trichophyton*, is called dermatophytosis or ringworm [1]. Dermatophytosis is a zoonotic disease that easily spreads from humans to animals and contaminates the environment [2]. The target that is attacked by dermatophytes is the keratin layer on the skin, nails and hair of animals and humans. Clinical signs that

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appear are the presence of reddish circular sores with clear boundaries and slightly crusted. Treatment of dermatophytosis is usually carried out using antifungals from the polyenes, azoles, allylamines, echinocandins and the other class (griseofulvin dan flucytosin) based on active ingredients and it work [3, 4]. Each of which has a different active substance and target of action against fungi [5]. Antifungals in their work will target several important parts of the fungal cell system that built up fungi and their metabolism, namely cell walls, ergosterol pathways (components in cell membranes), nucleic acids and fungal protein biosynthesis. Commercial antifungal drugs can find on the market in topical drugs (ointments, creams) and oral drugs (tablets, capsules).

In addition to the above antifungal, sulfur can also be used as an antifungal. Sulfur is known to function as a keratolytic, in the market you can often find commercial sulfur preparations with a concentration of 6% in ointments, powders and shampoos. The target of sulfur work against fungi is on the cell wall structure by lysing it so that the fungi is destroyed, sulfur can also disrupt fungi homeostasis through fungal sulfur metabolism, resulting in fungal cell death [6, 7].

This study aims to obtain the best dose for the combination of sulfur and miconazole. The role of sulfur as a keratolytic agent is expected to synergize with miconazole, sulfur is expected to be able to lyse the keratin layer of the patient's skin which usually occurs thickening (crust/scaly) due to dermatophyte fungal infection, so that miconazole can easily reach fungal cells and start its target work against dermatophyte fungi.

2 Materials and Methods

2.1 Trichophyton. Mentagrophytes

The fungus *T. mentagrophytes* used in this study was isolate from BBLITVET Culture Collection with isolate number BCC F0127. The fungus was cultured in Sabouraud Dextrose Agar (SDA) media. The fungal suspension was made in sterile aquadest, the suspension was allowed to settle for 5 to 10 min to remove heavier particles, and conidia were counted using a haemocytometer. The concentration of the fungi was made at 10^6 cfu/ml which was ready to be used for agar diffusion test. This conidial suspension was then diluted, made in 10 ml of each lot adjusted to the final concentration of 1×10^3–3×10^3 cfu/ml which was ready to be used for agar dilution test [8].

2.2 Active Substance

For difusion test, miconazole was prepared as a salves with concentrations of 1,25 mg/g, 2,5 mg/g, 5 mg/g, 10 mg/g. Sulfur was prepared in a salves with a concentration of 3,75 mg/g, 7,5 mg/g, 15 mg/g, 30 mg/g. The combination of sulfur and miconazole made a salves with a concentration of Sulfur 30 mg/g + Miconazole 1,25 mg/g, Sulfur 15 mg/g + Miconazole 2,5 mg/g, Sulfur 7,5 mg/g + Miconazole 5 mg/g, Sulfur 3,75 mg/g + Miconazole 10 mg/g. For dilution test, each concentration of the active substance is prepared in 10 ml for use in the test.

2.3 Activity of Miconazole, Sulfur and Their Combination by Agar Diffusion and Dilution Methods

Miconazole and sulfur antifungal tests were carried out using the agar diffusion and dilution methods. The agar diffusion test was carried out by means of the fungus being cultured on the surface of SDA media in sterile petri dishes, then making wells with a diameter of 0.5 cm. The active substance to be tested is inserted into the hole. Incubation was carried out at 37 °C for ± 14 days. As a Positive control, the well filled with a salves of 20 mg/g miconazole, for negative control, the well was not filled with active substance. Observations were made by measuring the radius of the clear zone formed [9]. At this stage, the width of the inhibition zone was obtained which indicated the effectiveness of miconazole, sulfur or a combination of both against T. *mentagrophytes*.

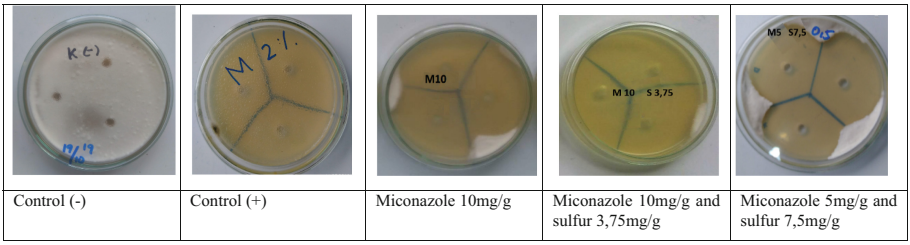
The dilution test was carried out by pouring 1 ml of the test fungus 10^3 cfu/ml into a sterile petri dish together with 1 ml of the active substance to be tested. SDA media which is still liquid (temperature 45 °C) is poured into a petri dish which already contains a solution of the active substance, the SDA solution and the active substance are homogenized and then incubated at 37 °C for ± 14 days. As a positive control, 1 ml miconazole 10 mg/ml and 1 ml fungal solution was poured into sterile petri dish, for negative control, 1 ml of the test fungal solution was poured into a sterile petri dish without adding the active substance solution, then SDA media was added and incubated at 37 °C. The results were checked by looking at the growing population of fungal colonies compared to the control. The dilution of the extract that did not show colony growth was the value of the Minimum Inhibitory Concentration (MIC) [9]. The test was carried out with 3 repetitions. The resulting data will be tested with a one-way ANOVA test.

3 Results

The diffusion test from various concentrations of miconazole, sulfur and their combination, the results are shown in Fig. 1. The widest radius is produced by the active ingredient miconazole 10 mg/g and the combination of sulfur 3,75 mg/g miconazole 10 mg/g, while the smallest radius of inhibition zone is produced by sulfur 3,75 mg/g. The growth of fungus colonies from the diffusion test results is shown in Fig. 2, from the 12 treatments carried out, the use of miconazole 10 mg/g and the combination of miconazole 10 mg/g sulfur 3.75 mg/g showed an inhibition zone width of 35.20 mm, in the combination of miconazole 5 mg/g. g sulfur 7.5 mg/g indicates the width of the inhibition zone is 30,30 mm. In the dilution test depicted in Fig. 3, it can be seen that a solution of the active substance miconazole with various dilution levels and a combination solution of sulfur and miconazole at various ratios can inhibit the growth of the test fungal to 0. Based on the results of the diffusion and dilution test, composition of the best combination of sulfur and miconazole was obtained at a sulfur 7.5 mg/g and miconazole 5 mg/g (Tables 1 and 2).

Table 1. Diffusion test of miconazole, sulfur and their combination against *T. mentagrophytes.*

No	Treatment	Inhibition zone diameter (mm)
1	Control + (Miconazole 20 mg/g)	37.33
2	Control -	0
3	Miconazole 1.25 mg/g	24.17
4	Miconazole 2.5 mg/g	25.83
5	Miconazole 5 mg/g	27.17
6	Miconazole 10 mg/g	35.2
7	Miconazole 3.75 mg/g	1.33
8	Sulphur 7.5 mg/g	1.82
9	Sulfur 15 mg/g	3.13
10	Sulfur 30 mg/g	4.83
11	Sulfur 30mg/g + Miconazole 1.25mg/g	23.87
12	Sulfur 15mg/g + Miconazole 2.5 mg/g	25.37
13	Sulfur 7.5 mg/g + Miconazole 5mg/g	30.3
14	Sulfur 3.75 mg/g + Miconazole 10 mg/g	35.2



Control (-) | Control (+) | Miconazole 10mg/g | Miconazole 10mg/g and sulfur 3,75mg/g | Miconazole 5mg/g and sulfur 7,5mg/g

Fig. 1. Inhibition zone witdh of miconazole, sulfur and their combination against *T. mentagrophytes*

Table 2. Dilution test of miconazole, sulfur and their combination against *T. mentagrophytes*

No	Treatment	Colony Forming Unit
1	Control + (Miconazole 20 mg/ml)	0
2	Control -	843
3	Miconazole 1,25 mg/ml	0
4	Miconazole 2,5 mg/ml	0
5	Miconazole 5 mg/ml	0

(*continued*)

Table 2. (*continued*)

No	Treatment	Colony Forming Unit
6	Miconazole 10 mg/ml	0
7	Sulfur 3,75 mg/ml	454
8	Sulfur 7,5 mg/ml	228
9	Sulfur 15 mg/ml	67
10	Sulfur 30 mg/ml	34
11	Sulfur 3 mg/ml + Miconazole 1.25 mg/ml	0
12	Sulfur 15 mg/ml + Miconazole 2.5 mg/ml	0
13	Sulfur 7,5 mg/ml + Miconazole 50mg/ml	0
14	Sulfur 3,75 mg/ml + Miconazole 10 mg/ml	0

4 Discussion

Antifungals of the azole group (miconazole, ketoconazole, fluconazole, etc.) work by inhibiting the synthesis of ergosterol contained in the cell membrane layer of dermatophytes. Miconazole works by inhibiting ergosterol biosynthesis in C-14 demethylation, namely by inhibiting cytochrome P450-dependent 14α-lanosterol demethylase (Cyp51) which is encoded by the ERG11 gene by converting lanosterol into ergosterol [10]. Inhibition of C-14 demethylation can lead to depletion of ergosterol and accumulation of lanosterol and other intermediates in dermatophyte cells, and ultimately disrupt the integrity of their plasma membranes [5]. Inhibited ergosterol synthesis will cause accumulation of toxic sterols in fungal cell membranes (lanosterol or eburicol) which replace methylated sterols and cause release of ergosterol from fungal membranes, resulting in unstable cell membranes, impaired growth and cell death [11].

Fungi require several important elements to support their metabolic processes, including sulfur. Sulfur plays a role in the metabolism of the proteogenic amino acids cysteine (Cys) and methionine (Met), defense of oxidative stress [glutathione (GSH) and ergothioneine (EGT)], methylation of [S-adenosylmethionine (SAM)], biosynthesis of the toxin epipolythiodioxopiperazine (ETP) (e.g., gliotoxin) and iron metabolism (Fe–S groups) [12, 13]. Fungi can obtain sulfur from inorganic and organic sources, the availability of this sulfur is important for fungal pathogenesis and must be sourced from external sources for its survival [12, 14]. There are differences in sulfur assimilation pathways between humans and fungi themselves, enzymes and intermediates produced by fungi represent possible drug targets for the treatment of fungal infections. In addition, interactions occur between many components of this sulfur metabolism pathway and other systems in fungal pathogens [15], this may provide opportunities for the development of targeted antifungal drugs with sulfur active ingredients.

In this study, sulfur showed an increase in its effectiveness after being combined with miconazole, this is evidence that the action of sulfur synergizes with the action of miconazole in killing *T. mentagrophytes*. Sulfur as a keratolytic agent and also kills bacteria, fungi and other parasites. Sulfur has a keratolytic effect on human and animal

skin, which works to soften and thin the epidermis. The skin of patients with dermatophytosis will develop a crust, with the keratolytic function of sulfur, it is hoped that it will make it easier for the antifungal active substance to reach the target of dermatophyte fungus. Sulfur acts as an antifungi in addition to damaging the cell walls of fungi, it also disrupts the balance of fungal sulfur metabolism in the amino acid chain cysteine, methionine which is important for the biosynthesis of glutathiones antioxidants (GSH), S-adenosylmethionine (SAM), methyl groups in cell transmethylation reactions and S. -adenosylhomocysteine (SAH) [6]. When sulfur is combined with miconazole, it will synergize to kill the test fungi by physically and biochemically damaging the fungal cell structure by disrupting the balance of cell metabolism and ergosterol biosynthesis. Based on the results of this study, sulfur has a dual role, namely as a keratolytic on the skin of the host (patient) and as an antifungal active substance that can affect the life of fungus.

5 Conclusion

The best combination composition between miconazole and sulfur was obtained at a concentration of 5 mg/g miconazole and 7.5 mg/g sulfur. Even though the combination of miconazole 10 mg/g and sulfur 3.75 mg/g showed a wider width of the inhibition zone, the role of sulfur was not very visible (this was evidenced by the width of the inhibition zone in a petri dish with a single active ingredient of miconazole 10 mg/g).

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