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RESEARCH ARTICLE

The Antifungal Effect of Kesum Leaf (*Polygonum minus* Huds) in Ethanol Extract on *Microsporum gypseum*

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ABSTRACT

Introduction: *Dermatophytosis or tinea is a disease found in the keratinized tissues caused by dermatophytes. The prolonged use of antifungal drugs has adverse effects on humans. Kesum leaves (Polygonum minus Huds) contains the secondary metabolite properties which can act as anti-fungi.* **Objective:** *This study aimed to determine the antifungal activity of ethanol extract of kesum leaves in inhibiting the Microsporum gypseum.*

Methods: *Phytochemical analysis was performed using a test tube method and thin layer chromatography. The antifungal activity test used a disc diffusion method with the extract concentrations of 5%, 10%, 20%, 40%, and 80%. Kesum leaves macerated using ethanol 96% solvent. Itraconazole 8µl/disk and DMSO4 were respectively used as positive and negative control.*

Results: *Phytochemical analysis on the ethanol extract of kesum leaves showed the secondary metabolite groups of flavonoids, alkaloids, terpenoids, phenolics, and saponins. Ethanol extract of kesum leaves did not form an inhibitory zone on the growth of Microsporum gypseum. Meanwhile in the positive control, in the form of itraconazole 8 µl/disk, the inhibition zone was formed around the disc with a mean value of 37.5 mm.*

Conclusion: *Ethanol extract of kesum leaves (Polygonum minus Huds) has no antifungal activity against the growth of Microsporum gypseum.*

Keywords: *Antifungal, ethanol extract of kesum leaves (Polygonum minus Huds), Microsporum gypseum*

ABSTRAK

Latar Belakang: Dermatofitosis atau tinea merupakan penyakit yang ditemukan pada jaringan yang mengandung zat tanduk, disebabkan oleh golongan jamur dermatofita. Penggunaan obat antijamur pada manusia secara berkepanjangan memiliki efek samping yang merugikan. Daun kesum (*Polygonum minus* Huds) mengandung kandungan metabolit sekunder yang dapat berperan sebagai antifungi.

Tujuan: Penelitian ini bertujuan untuk mengetahui aktivitas antifungi ekstrak etanol daun kesum dalam menghambat pertumbuhan *Microsporum gypseum.*

Metode: Analisa fitokimia dilakukan dengan metode tes tabung dan kromatografi lapis tipis. Uji aktivitas antifungi menggunakan metode difusi cakram dengan konsentrasi ekstrak 5%, 10%, 20%, 40%, dan 80%. Daun kesum dimaserasi menggunakan pelarut etanol 96%. Itrakonazol 8µl/disk dan DMSO4 masing-masing digunakan sebagai kontrol positif dan negatif.

Hasil: Analisa fitokimia ekstrak etanol daun kesum menunjukkan kelompok metabolit sekunder flavonoid, alkaloid, terpenoid, fenolik, dan saponin. Ekstrak etanol daun kesum tidak membentuk zona hambat terhadap pertumbuhan *Microsporum gypseum.* Sementara pada kontrol positif berupa itrakonazol 8µl/disk terbentuk zona hambat di sekitar cakram dengan nilai rerata sebesar 37.5 mm.

Kesimpulan: Ekstrak etanol daun kesum (*Polygonum minus* Huds) tidak memiliki aktivitas antifungi terhadap pertumbuhan *Microsporum gypseum.*

Kata Kunci: Antifungi, ekstrak etanol daun kesum (*Polygonum minus* Huds), *Microsporum gypseum*

INTRODUCTION

The incidence of fungal diseases in human (dermatophytosis) remain high, yet the existing antifungal medication relatively limited compared to antibacterial medication. Prolonged use of antifungal medication has adverse effects, for example hepatotoxic effect for ketoconazole usage (Widaty & Budimulja, 2016). Therefore, antifungal medication of natural ingredient kesum leaf (Persicaria minor, syn. *Polygonum minus* Huds.) which may inhibit the growth of *Microsporum gypseum*, is needed. Kesum (*Polygonum minus* Huds.) grows wildly in highland and humid areas such as on the bank of a lake or river (Murdopo, 2014). Kesum (*Polygonum minus* Huds.) commonly found

in Southeast Asia, especially in Malaysia, Vietnam, Thailand, and Indonesia. In Indonesia, particularly West Kalimantan, kesum (*Polygonum minus* Huds) is widely used as the main material of padas porridge. The typical aroma and taste of kesum (*Polygonum minus* Huds.) makes padas porridge commonly known as the special cuisine of West Kalimantan. Some studies also show that kesum has antifungal effect (Alves et al., 2001; Phatik et al., 2014).

Dermatophytosis, also called ringworm or tinea, caused by dermatophytes and found in tissue containing keratin substance, such as stratum corneum in epidermis, hair, and nail. Dermatophytes invade all layers of stratum corneum, activate host immune response, thus lead to symptoms (Widaty & Budimulja, 2016). The most common found forms of dermatophytosis in Japan are tinea pedis, followed with tinea unguium (Sei, 2012). Meanwhile, in India, the most commonly found forms are tinea kapitis and tinea kruris (Hanumanthappa et al., 2012). The prevalence of dermatophytosis in Sweden is also relatively high. A retrospective analysis conducted at Karolinska University Hospital, Sweden for 4 years (2005-2009) finds the prevalence of dermatophytosis of 14.1% (Drakensjö & Chryssanthou, 2011). There are 153 cases (3.7%) of dermatophytosis in Indonesia, especially in Manado, in 2013 out of the 4099 (100%) cases reported. The distribution of dermatophytosis cases by age may be classified into the biggest group of 45-64 years old of 32.7% because of declining body endurance (Sondakh et al., 2016). According to the research conducted by (Sarika et al., 2014), dermatophytosis occurs to women more than men. Besides, (Sarika et al., 2014) also find dermatophytosis infecting fungi, one of which is Microsporium sp with incidents of 11.33%. From 2011-2015, in Crete, dermatophytosis inducing fungus, Microsporium gypseum, has distribution value of 1.8% (Maraki & Mavromanolaki, 2016). Kesum (*Polygonum minus* Huds.) has essential high fat contents such as geraniol, geranial, and β-caryophyllene (Baharum et al., 2010; Yaacob, 1990).

Kesum (*Polygonum minus* Huds.) also contains secondary metabolite content in the form of flavonoids such as flavone and quercetin which may potentially contribute to antifungal activity (Johnny et al., 2011; Yaacob, 1990). The study reported by Johnny et al. shows that the acetone extract from *Polygonum minus* Huds has antifungal activity on *Colletotrichum capsici* (Johnny et al., 2011). In addition, some studies also show that the extract of some spesies of genus *Polygonum* such as *Polygonum ferrugineum*, and *Polygonum hydropiper* has antifungal activity on fungi that attack plants (López et al., 2011; Phatik et al., 2014). Furthermore, the study reported by De Almeida et al. shows that *Polygonum punctatum* also has antifungal properties on yeast and dermatophytes (Alves et al., 2001). Similarly, the study conducted by Derita and Zacchino also shows that *Polygonum persicaria* has antifungal activity on yeast, *Microsporum gypseum*, *Trichophyton rubrum*, and *Trichophyton mentagrophytes* (M. Derita & Zacchino, 2011).

Based on the description above, further research is needed to prove whether the ethanol extract of Kesum/ Persicaria minor Leaf (*Polygonum minus* Huds.) can serve as antifungal on *Microsporum gypseum*.

METHODS

This pure experimental research employed a posttest only control group design. The research was conducted in stages, including simplicia's preparation, phytochemical screening, and antifungal activity test of kesum (*Polygonum minus* Huds.) leaves in ethanol extract on *Microsporum gypseum*. There were five treatment groups in the research, according to ethanol extract of kesum leaves concentration respectively 5%, 10%, 20%, 40%, and 80%, positive control and negative control were also applied. The positive control use itrakonazol 8µl/disk, an azole group with wide antifungal spectrum. While the negative control use DMSO4 since it could dissolve compounds existing in the extract well and had no antifungal effect (Badan Pengawas Obat dan Makanan (BPOM), 2010). The number of repetitions in the experimental research was formulated using the following Federer formula:

$(n-1)\ (t-1) > 15$
$(n-1)\ (7-1) > 15$
$(n-1)\ (6) > 15$
$6(n-1) > 15$
$6n-6 > 15$
$6n > 15+6$
$6n > 21$
$n > 3.5 = 4$

Explanation: n=number of repetition, t=number of groups (7), the number of repetitions needed in the research was 4 times using the Completely Randomized Design (RAL) experimental method.

Tools Sterilization and Materials

The tools were sterilized before they were used by having them wrapped with heat-resistant plastic and inserted into autoclave at 121°C at 15 psi (per square inch) for 20 minutes. Non-heat-resistant tools were sterilized using alcohol 70%. Inoculating loops

were sterilized by having them exposed to flame. The growth media was sterilized using autoclave at 121°C for 15 minutes.

Inoculation of the Tested Fungus

Microsporum gypseum was taken using one sterile inoculation loop, inoculated on media agar ASD scratching it, and incubated in incubator at 37° C for 18-24 hours.

Extraction of Kesum Leaf with Ethanol 96%

Washed kesum leaf was dried by leaving it drying naturally. The dry leaf was chopped. The simplicia was extracted using ethanol 96% as solvent for 72 hours and stirred periodically once every 12 hours. The extraction result was evaporated using rotary evaporator. In this research, the ethanol was chosen as the solvent since it was suitable for obtaining phenolic extract, especially flavonoid (Pietta et al., 2002). In addition, ethanol was also effective and safe to extract the secondary metabolite compounds existing in kesum leaf (*Polygonum minus* Huds) (Gberikon et al., 2015; Wang et al., 2010).

Phytochemical Analysis

Alkaloid Test

One gram of simplicia powder was mixed in 10 mL ethanol for 15 minutes, filtered, and its filtrate was evaporated until it was dry. The residue was dissolved into 2 mL ethanol and used as test solution KLT (Thin Layer Chromatography). The silica gel F254 plate was employed in the stationary phase, while DCM 100% used in the mobile phase. The solution used as detector was Dragendroff's reagent.

Saponin Test

Two grams of simplicia powder was inserted into test tube, added with 10 mL distilled water, mixed for 30 seconds, and observed for any changes. Saponin was indicated if foam was formed, and remain for at least 30 seconds.

Phenol Test

Two grams of simplicia powder placed in Erlenmeyer flask, 10 mL HCl 2M added, heated for 30 minutes, and filtered. The filtrate obtained was inserted into separatory funnel. The residue was re-filtered using the same method and solution. The filtrate was mixed with 20 mL ether, and left for splitting two solutions. The ether solution was separated and evaporated, leaving only 5 mL to be used as KLT test solution. In KLT test, the stationary phase used silica gel F254 plate and the mobile phase used DCM 100% with FeCl3 as the reagent solution.

Flavonoid Test

Ten milliliters of ethanol added to 2 g powder, heated on water bath for 15 minutes, and filtered. The filtrate obtained was used as KLT test solution. The stationary phase used silica gel F254 plate and the mobile phase used DCM 100%. Cerium sulfate solution was used to detect flavonoid content.

Terpenoid Test

Ten milliliters of ethanol added to 2 g powder, heated on water bath for 15 minutes, and filtered. The filtrate obtained was used as KLT test solution. The stationary phase used silica gel 60 F254 plate and the mobile phase used DCM 100%. Liebermann-Burchard solution was used to detect terpenoid content.

Antifungal Activity Test

The test on the inhibition capacity of ethanol extract of persicaria minor leaf on the growth of *Microsporum gypseum* was conducted using diffusion method on 5 mm diameter paper disk. The initial phase was sterile cotton swab immersed in its inoculum suspension for 15 minutes, then rotated several times and pressed into tube wall above the liquid to remove excessive inoculum of the cotton swab. *Microsporum gypseum* was then inoculated on the surface of media ASD by swabbing the entire surface of media. The procedure was repeated twice, rotated at about 60 degrees to ensure inoculum reddening. The next phase was placing paper disks given with sample of ethanol extract of kesum leaf at concentrations 5%, 10%, 20%, 40%, 80%, positive control itrakonazol 8 µg/disk, and negative control of DMSO4 on the surface of ASD plate inoculated with fungi using sterile tweezers each with 4 repetitions (replication). Further, the medium was incubated at 37° C for 72 hours. The inhibition zone formed was then measured.

RESULTS

The phytochemical analysis test with sample of kesum leaf (*Polygonum minus* Huds.) results in positive result in the test on secondary metabolite flavonoid with cerium sulphate as the reagent. Secondary metabolite test using Dragendroff reagent shows positive result alkaloid. Test using Liebermann Burchard reagent shows positive result, that the sample contains terpenoid compound. Secondary metabolite compound phenolic was also positively tested and existed in the sample using reagent FeCl3. Test on secondary metabolite compound saponin using tube test method in the form of shaking

Table 1. Result of phytochemical Analysis

No.	Secondary Metabolite	Reagent	Result
1.	Flavonoid	Cerium sulfate	+
2.	Alkaloid	Dragendroff	+
3.	Terpenoid	Liebermann Burchard	+
4.	Fenolic	$FeCl_3$	+
5.	Saponin	Mixed	+

Explanation: +: Positive, containing compounds; -: Negative, not containing compounds

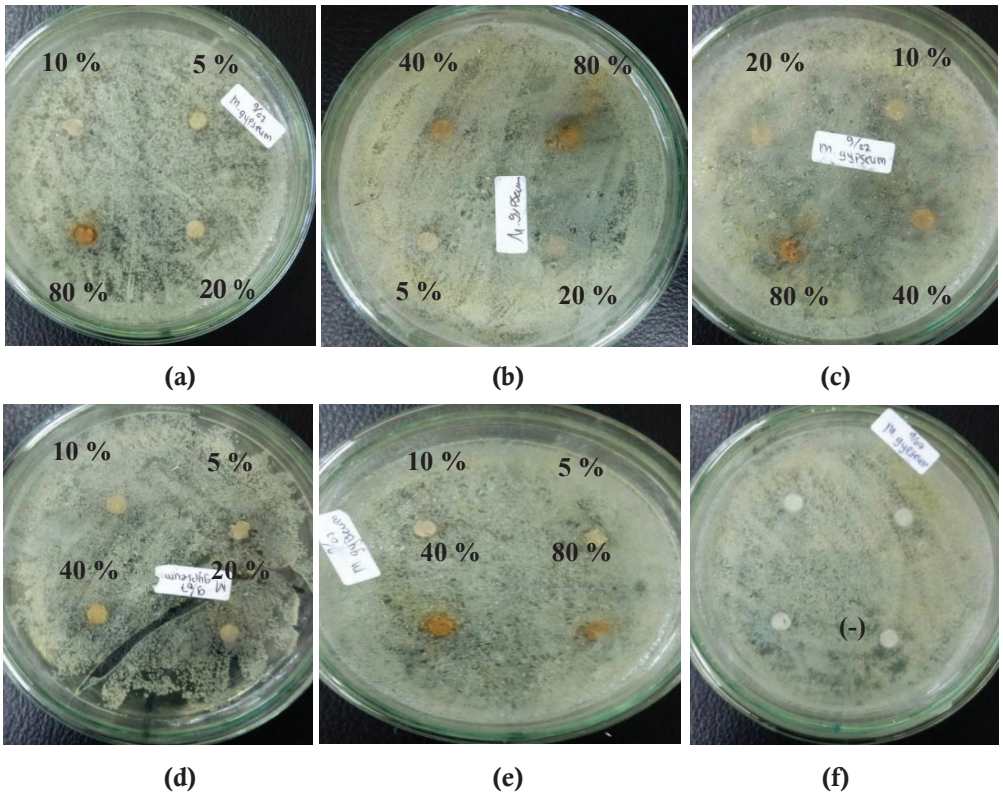

Figure 1. Antifungal activity test on the extract of kesum leaves (*Polygonum minus* Huds) (a) Concentration of 5%, 10%, 20%, and 40%. (b) Concentration of 5%, 20%, 40%, and 80%. (c) Concentration of 10%, 20%, 20%, and 80%. (d) Concentration of 5%, 10%, 20%, and 40%. (e) Concentration of 5%, 10%, 40%, and 80%, (f) Negative Control

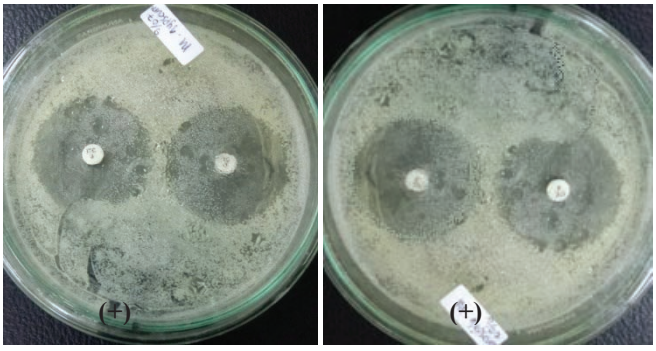

Figure 2. Positive control's Antifungal activity test on *Microsporum gypseum*

sample results in positive result (Table 1).

Microsporum gypseum with paper disk with respective test concentration and positive control and negative control was incubated for 3 days at 37° C. The result on the antifungal activity of ethanol extract of kesum leaf (*Polygonum minus* Huds) at concentrations

Table 2. Results of antifungal activity test on the ethanol extract of kesum leaves (*Polygonum minus* Huds.)

No.	Concentration (%)	Inhibiting Zone Diameter (mm)				Mean (mm)
		Repetition				
		I	II	III	IV	
1.	5	0	0	0	0	0
2.	10	0	0	0	0	0
3.	20	0	0	0	0	0
4.	40	0	0	0	0	0
5.	80	0	0	0	0	0
6.	Negative Control	0	0	0	0	0
7.	Positive Control	39	38	36	37	37.5

5%, 10%, 20%, 40%, and 80% on fungus *Microsporum gypseum* does not show any formation of inhibition zone (figure 1, table 2). Negative control DMSO4 also does not show any inhibition zone (Figure 1), while with positive control itrakonazol 8µl/disk an inhibition zone was formed around the disk with mean value of 37.5 mm (Figure 2 and Table 2).

DISCUSSION

The result of this research shows that the antifungal activity test with some concentrations 5%, 10%, 20%, 40%, and 80% of ethanol extract of kesum leaf (*Polygonum minus* Huds) does not successfully form inhibition zone around the disk. The same result also found in the use of DMSO4, not showing any inhibition zone. On the other hand, the use of itrakonazol 8µl/disk successfully forms inhibition zone around the disk with mean value of 37.5 mm (Table 2), which is classified as a very strong inhibition capacity (Kandoli et al., 2016). Itrakonazol as antifungal for *Microsporum gypseum* works by inhibiting ergosterol synthesis from lanosterol by impairing 14α-demethylase, forming inhibition zone on the fungi growing media (Lv et al., 2016; Nigam, 2015).

This result is different from that of the previous researches conducted by (Dewi, 2018; Melinda, 2018; Rosalim, 2018), showing that administration of extract of kesum leaf (*Polygonum minus* Huds.) to fungi of genera Trychophyton and Epidermophyton, which are also of dermatophytes, evidently presents positive result. The result of research conducted by (Dewi, 2018) shows that the test on the antifungal activity of ethanol extract of kesum leaf (*Polygonum minus* Huds.) on *Trichophyton rubrum* shows positive result at effective concentration 20% with mean diameter of inhibition zone 14 mm, which is classified as of a strong inhibition capacity. The research conducted by (Melinda, 2018) using ethanol extract of persicaria minor leaf (*Polygonum minus* Huds.) on Trichopyhton mentagrophtes shows positive result at effective concentration 80% with mean diameter of inhibition zone 20.625 mm, which is classified as of a very strong inhibition capacity. The difference between the results of this and previous researches is expectedly caused by some factors, such as fungal structural factor and the factor of secondary metabolite compound contained in kesum (*Polygonum minus* Huds) extract.

The wall structure of fungus genus Microsporum used in this research is different from the genera Trichophyton and Epidermophyton in the previous researches, although they are of the same group, dermatophytes. The wall structure Macroconidial of *Microsporum gypseum* is multiple septa with thick wall and rough or smooth surface (Lakshmipathy & Kannabiran, 2010). Meanwhile, the wall structure macroconidial of genus Trichophyton is thin smooth surface and of various shapes (Lakshmipathy & Kannabiran, 2010). Macroconidia in Epidermophyton grows in grup with thin wall and smooth wall surface (Lakshmipathy & Kannabiran, 2010). The thin macroconidial wall of Trichophyton and Epidermophyton expectedly causes the secondary metabolite compounds in the ethanol extract of kesum leaf (*Polygonum minus* Huds.) to easily penetrate cell wall and get into cell. Meanwhile, dermatophytes of genus Microsporum has thick macroconidial wall, making it difficult for the secondary metabolite compounds in the ethanol extract of kesum leaf (*Polygonum minus* Huds.) to penetrate into cell (Lakshmipathy & Kannabiran, 2010; Nigam, 2015). This supportive statement by Rosalim (2018), who tests the antifungal activity of ethanol extract of kesum leaf (*Polygonum minus* Huds.) on genus Microsporum canis. The test shows negative result for each concentration of the test (Rosalim, 2018).

The phytochemical analysis in this research shows that ethanol extract of kesum leaf (*Polygonum minus* Huds.) contains secondary metabolite compounds in the form of flavonoid, alkaloid, terpenoid, phenolic, and saponin compounds. However, the ethanol extract

of kesum leaf (*Polygonum minus* Huds.) containing five secondary metabolite compounds at concentrations 5%, 10%, 20%, 40%, and 80% is unable to inhibit the growth of *Microsporum gypseum*. The reason is that each secondary metabolite compound contained in plant is expected to have different type and group and, therefore, have different activity despite their classification into one group (Ahmad et al., 2016; Kurniawan, 2015).

The research conducted by (Hasan et al., 2009) and (Derita et al., 2009) reconfirm this presumption. *Polygonum* hydropiper, the same family with kesum (*Polygonum minus* Huds), namely Polygonaceae, is reported that the whole plant contains secondary metabolite compound flavonoid, namely flavone and glycoside flavonoid. (Hasan et al., 2009) test on the antifungal activity of *Polygonum hydropiper* root on one of dermatophytes, namely *Trichophyton rubrum*, shows positive result. The other antifungal activity research conducted by (Derita et al., 2009) on *Trichophyton rubrum* and *Microsporum gypseum* with the whole parts of the plant, including root, leaf, stem, and fruit of *Polygonum* acuminatum Kunth. Terpenoid compound in the form of isolated polygodial as the main compound responsible for positive antifungal activity, together with iso-polygodial, drimenol and confertifolin. The study conducted by (Derita & Zacchino, 2011) also reports other plant of genus Polygonum, that is *Polygonum* persicaria, to have antifungal activity on yeast, *Microsporum gypseum*, *Trichophyton rubrum*, and *Trichophyton mentagrophytes*.

The extract preparation method factor is also expected to influence the result of antifungal activity test (Sagar & Vidyasagar, 2013). Sagar and Vidyasagar (2013) test antifungal activity using methanol, alcohol, acetone, ether petroleum, chloroform and ethyl acetate to extract some plants in India. The test with these different solvents is conducted on five fungi of dermatophytes, one of which is *Microsporum gypseum*. The study also tests different antifungal activities, namely fungicidal and fungistatic activities, of each extract and concentration. The other research conducted by Ilagan (2015) states that decoction shows stronger inhibition capacity than organic extract. This shows that type of solvent and extraction method contribute to extracting secondary metabolite compound that will be active in its antifungal role (Ilagan et al., 2015).

CONCLUSION

The ethanol extract of Kesum leaf (*Polygonum minus* Huds) does not have antifungal effect on *Microsporum gypseum*.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest in this research.

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