

## Effect of Ethanol Extract from Clove Flower (*Syzygium aromaticum*) on the Growth of *Trichophyton rubrum* in vitro

Khusnul\*, Pepin Meilani Hildawati, Dewi Peti Virgianti

Medical Laboratory Technology Program, Sekolah Tinggi Ilmu Kesehatan Bakti Tunas Husada  
Jl. Cilolohan No. 36 Kel. Kahuripan Tawang, Kota, Kec, Tasikmalaya, Jawa Barat 46115, Indonesia

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### ABSTRACT

Indonesia is one of the countries with a tropical climate that has high temperature and humidity, a good atmosphere for fungal growth so that fungi can be found somewhere. Fungus *Trichophyton rubrum* is a fungal disease that attacks the nails, skin, hair. One of the preventions of this disease is by giving traditional medicines, namely clove flowers (*Syzygium aromaticum*) which contain chemical compounds saponins, tannins, flavonoids. Serves as an antioxidant that can prevent dermatosis. The purpose of this study was to determine the inhibition power of clove flowers (*Syzygium aromaticum*) on the growth of *Trichophyton rubrum* fungi. This research was carried out an experimental method with the Kirby Bauer method. Concentration dilution of clove ethanol extract (*Syzygium aromaticum*) from concentration 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%. The results of this study showed that the clove ethanol extract (*Syzygium aromaticum*) inhibit the growth of *Trichophyton rubrum* fungus from the concentration of 10% inhibition zone 14 mm, 20% inhibition zone 26 mm, 30% inhibition zone 36 mm, 40% inhibition zone 41 mm, 50% 45 mm inhibition zone, 60% 46 mm inhibition zone, 70% 48 mm inhibition zone, 80% 49 mm inhibition zone, 90% 51.0 mm inhibition zone, 100% inhibition zone of 56 mm.

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\*Corresponding author:

Khusnul

Medical Laboratory Technology Program, Sekolah Tinggi Ilmu Kesehatan Bakti Tunas Husada

Email: [khusnul@stikes-bth.ac.id](mailto:khusnul@stikes-bth.ac.id)

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## INTRODUCTION

The development of fungal infections in Indonesia as a tropical climate country is caused by high rainfall and humidity so that the growth of fungi is very good. Skin disease caused by several types of fungi is a problem in tropical countries such as Indonesia. Skin conditions that are prone to sweating and moisture, poor personal hygiene and a lack of knowledge about health are factors that allow the growth of skin disease fungi (Hezmela, 2006).

Fungi are one of the causes of infection in diseases, especially in tropical countries. Fungal skin disease is a skin disease that often appears in Indonesian society. The tropical climate with high humidity in Indonesia is very supportive of fungal growth. A large number of fungal infections is also supported by the fact that many Indonesians are still below the poverty line so that environmental hygiene, sanitation, and healthy lifestyles are less of a concern in the daily life of Indonesians.

One of the fungal infections is dermatophytosis caused by dermatophytes. Dermatophytes are a group of fungi capable of digesting keratin in the epidermis. *Trichophyton* One example of fungi that cause dermatophytosis other than *Microsporum* and *Epidermophyton*. *Trichophyton* has many species, which are widely known namely *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Trichophyton schoenleinii*, *Trichophyton tonsurans*, *Trichophyton verrucosum*, and *Trichophyton violaceum*. The fungus *Trichophyton rubrum* is the fungus that most often causes chronic dermatophytosis (Chandra, 2006).

The use of plants to treat fungal skin diseases has long been known by our ancestors, but when compared to antibacterial drugs, anti-fungal drugs are relatively few. Herbal plants are types of plants that have functions. Herbal plants are classified as spices and fruit plants that can be used to treat various diseases. The advances of modern medical discoveries have made traditional medicine look out of date. Many modern medicines are made from medicinal plants, it's just that the compounding is done in clinical laboratories so

that they seem modern. The discoveries of modern medicine also support the use of traditional medicines (Hariana, 2008).

Research on bioactive compounds essential oils from plants as solutions to overcome microbial infections have been experiencing increasing resistance (Cui *et al.*, 2019). essential oil widely developed as an alternative material natural from synthetic compounds which are available. (Simas *et al.*, 2017). This plant is considered as one of the source of bioactive compounds which can give an effect antimicrobials (Mesquita and Tavares, 2018).

Quantity and composition of essential oil varies from person to person Plants depend on genetics and plant physiology and current conditions planted, harvested, after harvest, and environmental conditions (Costa *et al.*, 2008). Cloves (*S. aromaticum*) is used in society as antibacterial, antioxidant, spices, and food seasoning (Rivas *et al.*, 2015).

Chemical compound content cloves can produce a variety of biological activity. Chemical compounds that contained in cloves are phenols, flavonoids, hydroxy benzoic acid, and hydrokinetics, containing main chemical compound eugenol (Cortes Rojas *et al.*, 2014). Chemical composition of clove essential oil, between 80-90% is eugenol, 15% of eugenol acetate and between 5-12% of beta caryophyllene (Alma *et al.*, 2007).

Several studies related to this study have not had any research with the object of the fungus *Trichophyton rubrum*, previous studies have mostly examined the inhibition of bacteria such as research by Andries *et al.* (2014) with the title Anti-Bacterial Effectiveness Test of Clove Flower Extract (*S.aromaticum*) Against *Streptococcus mutans* Bacteria In vitro, research from Paliling *et al.* (2016) Inhibitory Test of Clove Flower Extract Against *Porphyromonas gingivalis* Bacteria. Research related to this fungus is by Nurhayati (2017) with the title the effect of clove flower extract (*Syzygium aromaticum*) on the fungal inhibition zone (*Trichophyton rubrum*) using n-hexane as a solvent with diffusion method and extract dilution using 15% dimethyl sulfoxide.

In this regard, research on the effect of the ethanol extract of clove flower *Syzygium aromaticum* on the growth of the fungus *Trichophyton rubrum* has not been investigated and is interested in studying.

## MATERIALS AND METHODS

### Materials and Equipment

The materials used were a pure culture of *Trichophyton rubrum*, clove flower extract, and SDA media. The equipments used are maceration tool, autoclave, stirring rod, blender, petri dish, vial bottle, erlenmeyer, measuring cup, sparkling lamp, incubator, dry sterilizer, hot plate, cotton pad, umbrella paper, analytical balance, refrigerator, methylated spirits.

### Method

The research on the inhibition test of clove flower ethanol extract (*Syzygium aromaticum*) on *Trichophyton rubrum* fungus was experimental, with samples were taken from pure cultures that had been cultured on *Saboroud Dextrose Agar* media and given ethanol extract of clove flower (*Syzygium aromaticum*) then incubated at 37°C for 24 hours. The culture results were seen and the size of the inhibition zone formed was observed. The concentration used for this study was the concentration of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, with the diffusion method with samples using SDA media, the fungi *Trichophyton rubrum*, clove flower ethanol extract. For positive control using SDA media, 2% ketoconazole, *Trichophyton rubrum* fungi and negative control using SDA media, *Trichophyton rubrum* fungi and sterile aquadest.

### Procedure

- 1) Preparation of clove flower extract (*S. aromaticum*)
  - a. The powdered clove flower that has been pulverized is weighed 100 grams and then put into a beaker with 1000 ml 96% ethanol solvent. Soak for 3 times 24 hours, stirring occasionally, then filtered.
- b. The filtered filtrate is evaporated to evaporate the ethanol solvent that is still mixed so that a pure extract is obtained at a temperature of 70° C so that a concentrated extract is obtained.
- 2) Antifungal Testing of Clove Flower Extract Leaf Extract Against *Trichophyton rubrum*
  - a. Standard 0.5 Mc Farland 1%
    - 9.5 sulfuric acids (H<sub>2</sub>SO<sub>4</sub>) solution is put into a sterile tube and add 1% BaCl<sub>2</sub> as much as 0.5 ml. Then shake until you get 0.5 Mc Farland turbidity
  - b. Making Fungi Suspensions
    - The fungi culture was taken using a sterile loop needle and then put in a physiological NaCl solution, shake it until it reaches 0.5 Mc Farland turbidity. The suspension is homogenized just before inoculation on a petri dish to prevent deposition.
- 3) Making Variations of Clove Flower Ethanol Extract Solution
  - The ethanol extract of langsat seeds in this study was made in concentrations of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 10% by calculating w/v (g/10ml). That concentration made by weighing the extracts 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 grams, then dissolved each with sterile distilled water to a volume of 10 mL.
- 4) Activity Test of Clove Flower Leaf Extract
  - a. 100µL of fungi suspension was poured into Saboroud Dextrose Agar medium.
  - b. Add 20µ clove flower ethanol extract to each media that has been placed on discs with various concentrations of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%. And sterile distilled water was used as a negative control.
  - c. The media was incubated at room temperature (25-30° C) for 4 days. The results are seen in the inhibition zone around the disc.

### Data Analysis

The research design used a completely randomized design (CRD) with the type of treatment in the form of several concentrations of clove flower extract, in each treatment 3 repetitions were carried out so that a total of 30 experimental units were obtained. The antifungal activity was observed from the clear zone around the paper disc. The data obtained were then analyzed statistically using One-way Analysis of Variance with a validity level of 95%, the level of significance between treatments then analyzed using the Duncan test.

### RESULTS AND DISCUSSION

Based on research results with a sample of 30 tests, at a concentration of 10% to concentration 100% contained zone of inhibition (mm), where the zone is marked as a no the growth of fungi around the discs that each have different in diameter, where is the fungi *Trichophyton rubrum* (*T.rubrum*) at a concentration of 10% of 14 mm, and at a concentration of 100% 56 mm with positive control equal to 47 mm. One-way or one-

way analysis of variance data one way ANOVA shows the difference which was very evident between the treatments several concentrations of flower extract cloves to the inhibition zone diameter *T.rubrum*, where  $p = 0.000$  or  $p$ -value  $< 0.05$ . This matter shows that the concentration variation the ethanol extract of clove flower has an effect against the growth of *T.rubrum*, from these results, can be carried out a further test with using the Duncan test. The Duncan test results were mean the different diameter of resistivity of every concentration. Result of flower extract treatment cloves almost the entire concentration showed different effectiveness. The test results are presented in Table 1 and Figure 1.

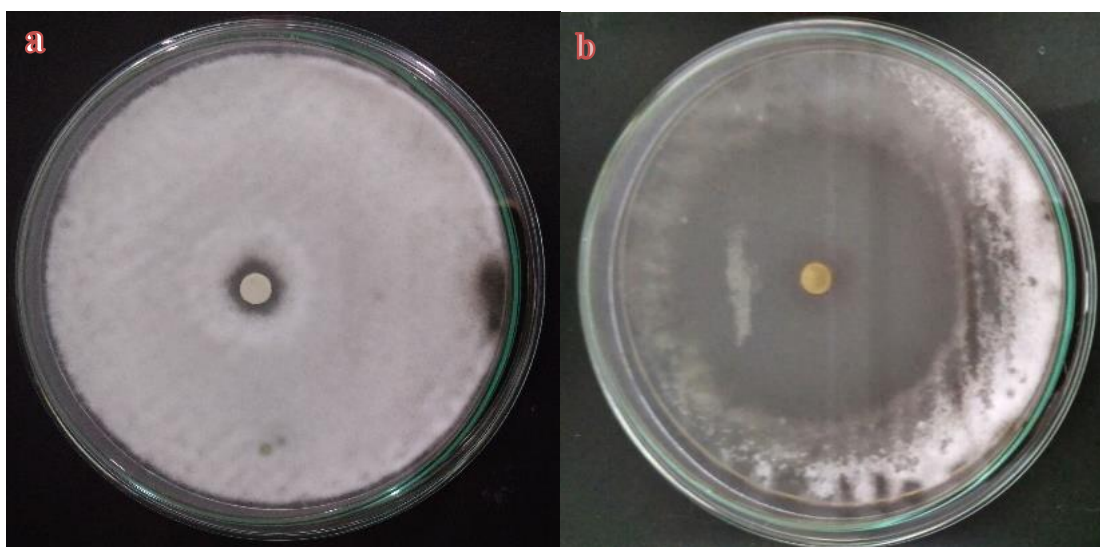
Based on the results of further tests on Table 1, shows that the ability of every concentration clove flower extract against *Trichophyton rubrum* produces growth distinct zone of inhibition, however several concentrations are its inhibitory ability is not significantly different namely between the concentrations 50% ; 60% with 70%, 60% ; 70% with 80%, and 80% equals 90%, at a concentration of 100% showed the maximum inhibition with an inhibition of 56 mm.

**Table 1.** The test results of ethanol extract (*S.aromaticum*) on the growth of *Trichophyton rubrum* fungus with various concentrations.

Treatment(s)	The Diameter of clear zone of Inhibitory (mm)	
10%	14 <sup>a</sup>	Weak
20%	26 <sup>b</sup>	Very Strong
30%	36 <sup>c</sup>	Very Strong
40%	41 <sup>d</sup>	Very Strong
50%	45 <sup>e</sup>	Very Strong
60%	46 <sup>e f</sup>	Very Strong
70%	48 <sup>e f g</sup>	Very Strong
80%	49 <sup>f g</sup>	Very Strong
90%	51 <sup>g</sup>	Very Strong
100%	56 <sup>h</sup>	Very Strong
Control (+)	47	Very Strong
Control (-)	0	

Note(s):

- Number followed by the same alphabet showed a similar based on Duncan test
- Very Strong response signified  $> 2$ cm, Strong 1.6-2cm, Medium 1-1.5cm, and Weak  $< 1$ cm.



**Figure 1.** Macroscopic picture of the *Trichophyton rubrum*  
(a) 10% extract concentration (b) 100% concentration

The zone of inhibition that is formed is due to the presence of compounds active capable of influencing fungal growth so that it forms the clear zone around the disc. Test the active compound can be done using screening phytochemical test where positive results can be seen in the presence change color of extract after addition of certain solvents. Result Phytochemical test screening on the ethanol extract *S. aromaticum* shows this flavonoid compounds, saponins, and tannins as presented in table 2. The results of phytochemical screening test of the ethanol extract of *S. aromaticum* were shown in Table 2 as follows.

Tannins change the function of the membrane in the process of transporting compounds so that they can cause fungal growth to be stunted and die. Saponins as a plant defense system from fungi by lowering the cell wall membrane. Flavonoids function to denaturation cell proteins which can inhibit the work of enzymes in cells so that the cell wall formation process is imperfect. In the content of clove

flower extract compounds which function as anti-fungi, which can inhibit fungal growth due to the content of eugenol compounds which are phenol components. These phenol components can damage cell microorganisms by causing protein coagulation and causing leakage of cell wall membranes and can cause inactivation of enzymes that are important in microorganism cell metabolism (Sundari *et al.*, 2001).

Other active compounds found in clove flowers are eugenol compounds. Eugenol is a commonly used compound in the pharmaceutical industry because it has many pharmacological activities as antiseptic, anti-inflammatory, antiviral, antimicrobial, antifungal, antispasmodic, stimulant, anesthetic local (Alisa *et al.*, 2015). Eugenol can inhibit bacteria gram-positive and also negative up to in bacteria that are resistant to antibiotics. Due to the nature of hydrophobic, the compounds will damage cell structure by entering into the existing lipopolysaccharide in cell membranes (Utami, *et al.* 2019)

**Table 2.** Phytochemical screening test results on ethanol extract of clove flower on *Trichophyton rubrum*

Phytochemical Test	Results	Note(s)
Tannin	Positive	There was color change to dark.
Saponin	Positive	Foam was formed.
Flavonoid	Positive	Yellow sedimentation was formed.

Several studies related to this research, namely by Andries *et al* (2014) with the title Anti-Bacterial Effectiveness Test of Clove Flower Extract (*S.aromaticum*) Against *Streptococcus mutans* In vitro, with 5 repetitions at concentrations of 40%, 60%, and 80 % had the inhibitory power at a concentration of 40% 20.41 mm, 60% 21.0 mm 80% 25.81 mm. Another study conducted by Paliling *et al* (2016) Inhibitory Test of Clove Flower Extract Against *Porphyromonas gingivalis* Bacteria with 96% ethanol solvent and the Kirby Bauer method showed the inhibitory power of clove flower extract against *Porphyromonas gingivalis* bacteria with an average of 13.0 mm. And another study by Nurhayati (2017), namely the effect of clove flower extract (*Syzygium aromaticum*) on the inhibition zone of fungi (*Trichophyton rubrum*) using n-hexane solvent with diffusion method and extract dilution using 15% dimethyl sulfoxide. Based on the results of the research on the effect of various concentrations of clove flower extract in 9 concentrations, the results were obtained with a concentration of 10% inhibition zone 11 mm, 20% inhibition zone 15 mm, 30% inhibition zone 21 mm, 40% inhibition zone 23 mm, 50% inhibition zone 26 mm, 60% inhibition zone 28 mm, 70% inhibition zone 30 mm, 80% inhibition zone 34 mm, 90% inhibition zone 40 mm.

Research conducted by Lova *et al* (2018) on clove flower essential oil to bacteria was able to produce the greatest antibacterial activity, namely 25.85 mm - 26.75 mm while flower stalk essential oil produced activity with an inhibition zone of 20.60 mm - 21, 20 mm and clove essential oil produced an inhibition zone of 18.04 mm - 18.58 mm. This proves that the essential oil from the clove flower has the best activity against *P.acnes* compared to the essential oil from the flower stalk and clove leaf. Thus, the essential oil from the flower stalk and clove leaf are not comparable to that from the clove flower when used as an antibacterial for *P. acnes*.

The difference from previous studies with research conducted by researchers lies in the type of solvent and diluent solution. The type of

solvent used by the previous research was n-hexane which was non-polar, while the solvent used by the researcher was polar ethanol, because ethanol is a more effective solvent for attracting active compounds in clove flowers. The results of research that have been carried out on clove flower extract using n-hexane solvent have inhibitory power at a concentration of 10%, namely 11 mm and a concentration of 100%, while the clove flower extract with ethanol solvent at a concentration of 10% is 14 mm and a concentration of 90% is 51 mm. So the results obtained from the ethanol extract were greater than the n-hexane extract. Another study on the fungus *Trichophyton rubrum* has been conducted by Khusnul *et al.* (2017) regarding the effectiveness test of the ethanol extract of galangal rhizome (*Alpinia Galanga* L) against the growth of the fungus *Trichophyton rubrum*. At a concentration of 10% to 20% there is no inhibition and a concentration of 30% inhibition zone 3 mm, 40% inhibition zone 6 mm, 50% inhibition zone 12 mm, 60% inhibition zone 12 mm, 70% inhibition zone 14 mm, 80% inhibition zone 14 mm, 90% inhibition zone 16 mm, 100% inhibition zone 18 mm. Galangal rhizome extract had a smaller inhibition on the growth of *Trichophyton rubrum* while clove flower extract had a larger inhibition zone on the growth of *Trichophyton rubrum*.

Cloves have activity broad antimicrobial because it can inhibit bacteria, fungi, protozoa, and viruses. MIC value against Gram bacteria positive and Gram-negative show good drag. Clove show killing power against some bacteria (Pathirana *et al*, 2019). The inhibition against Gram-positive bacteria greater than the resistance at Gram-positive bacteria (Saikumari *et al*, 2016), but cloves shows the MIC value against some Gram-negative bacteria very low or very strong (Moon *et al*, 2011; Pandey dan Singh, 2011)

The greater the concentration of clove flower extract produced, it was formed due to the presence of active compounds that we're able to affect the growth of fungi so that a clear zone was formed around the disc. The active compound test

can be carried out by screening phytochemical tests where positive results can be seen by the change in the color of the extract after the addition of certain solvents

## CONCLUSION

Based on research results can prove that the ethanol extract flower clove (*Syzygium aromaticum*) can effect on inhibition the growth of the fungus *Trichophyton rubrum*.

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## CONFLICT OF INTEREST

We have no conflict of interest related to this work.

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