Effect of clove essential oil (*Syzygium aromaticum*) against the growth of dandruff scalps-causing fungal pathogen using Kirby-Bauer method in vitro

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Abstract

One of the strongest contributing factors in dandruff scalps is the presence of fungal pathogen or called as Malassezia. Several studies have found a connection between Malassezia and scalp health, one of which is caused by pathogenic fungal infections including *Pityrosporum ovale*, *Microsporum gypseum*, and *Candida albicans*. Clove flowers are known to contain eugenol and its derivative compounds which have antimicrobial, antifungal, antiseptic, and local anesthetic activity. This research aims to determine the effect of clove flower essential oil (*Syzygium aromaticum*) against some dandruff-causing fungi. The research method was to test the inhibitory power of clove flower essential oil using the diffusion method (Kirby Bauer technique). Samples of pathogenic fungi were cultured on SDA media (Saboroud Dextrose Agar) and given discs that had been given several concentrations of clove flower essential oil. The concentrations used were 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100%, positive control using 2% ketoconazole and negative control using virgin coconut oil (VCO). The results showed a significant effect between the ability of essential oils to inhibit the growth of some fungi that cause dandruff. The treatment of clove flower essential oil against *M. gypseum* at the concentration of 10% has an inhibitory ability of 33.05 mm and at the concentration of 100% amounted to 63.95 mm. Meanwhile, the inhibition percentage in *P. ovale* with a concentration of 10% and 100% of extract were 14.75 mm and 46.15 mm, respectively. The inhibition rate in *C. albicans* with the concentration of 10% extract and 100% of extract was 13.5 mm and 39.35 mm. The research shows that clove flower essential oil inhibits the growth of several pathogenic fungal in vitro.

Keywords: dandruff scalps, fungal pathogen, in vitro, Kirby-Bauer, *Syzygium aromaticum*

Introduction

Indonesia is a tropical country with a perfect location for the development of diseases caused by fungal pathogen. Most of fungi can live optimally in humid environmental conditions. In addition, these climatic conditions allow the scalp to become more humid, which has the potential for the growth of pathogenic fungi supported by air pollution, dust, and sunlight that can cause damage to the hair and scalp.
In humans, the scalp is unlike any other skin. It has a thick layer of skin, a high follicle density, and numerous sebaceous glands. The hair shaft has a pH of 3.67 and the scalp has a pH of 5.5. The existence of these glands, together with the scalp's dark and warm environment, renders it more prone to fungal infections like dandruff and seborrheic dermatitis, as well as parasitic infections like Pityriasis capitis. Despite the fact that scalp problems are not among the most common diseases that cause serious physical illness or morbidity, they have a significant social impact. The state of one's scalp and hair has a greater psychological impact on the general population. Even minor changes in hair such as gray hair and dandruff, affect a person's self-confidence. Scalp disorders including fungus and bacterial infestation causing problems such as Tinea capitis (Narshana and Ravikumar, 2017). Pityriasis capitis (dandruff) is a squamous scalp disorder that is practically physiological and is defined by the formation of small scales without indications of irritation. It is commonly thought to be a minor type of seborrheic dermatitis.

Herbal medications, in addition to synthetic drugs, can be employed as a drug that can be used for our bodies. Antifungal chemicals have been found in a variety of herbal plants in Indonesia, including clove flower or Syzygium aromaticum. However, most of people chose alternative medicine first because of lower risk of medication side effects, more affordable price, and easier application. Clove flowers are a type of spices in Indonesia which is commonly used as an alternative. Clove flowers contain a variety of beneficial chemicals. According to Prianto, et al. (2013), clove flowers contain eugenol, trans-caryophyllene, alpha-humulene, eugenol acetate, caryophyllene oxide, and trimetoxycacetophenone. The content of eugenol and its derivative compounds has pharmacological activity as an analgesic, anti-inflammatory, antimicrobial, antiviral, antifungal, anti-septic, anti-spasmodic, antiemetic, stimulant, and local anesthetic (Towaha, 2012).

The results of previous research conducted by Yuliana (2014) showed that clove flower essential oil had antifungal activity on wood with an inhibition zone of 29 mm. Research conducted by Febrianti and Riyanta (2012) also found that clove leaf essential oil could effectively inhibit the growth of Candida albicans with a volume concentration of 0.3 ml extract. Subsequent research conducted by Alfauziah and Budiman (2016) has also successfully found that clove essential oil with a concentration of 1% exhibiting antifungal activity with an inhibition zone of 1.12 cm. Emulsion with 0.5% CMC emulsifier was the best formula and showing inhibition activity against wood fungi of 2.9 cm.

Based on the previous relevant studies, no research has been conducted on the inhibitory activity of clove flower essential oil against some dandruff fungus (P. ovale, C. albicans, and M. gypseum). Many researchers conclude that clove flower essential oil can be an alternative herbal medicine against antifungals but there is no specific study on the inhibitory this so that it is important for the finding of new alternatives for the scalp treatment.

Materials and methods

Sample preparation

The materials used in this study are Sabouraud Dextrose Agar (oxid), Physiological NaCl, 1% BaCl2, 1% H2SO4, 2% ketoconazole, clove flower essential oil, stock culture of P. ovale, M. gypseum, C. albicans, and aquadest sterile. The equipment used were autoclave (Portable M300), light microscope (Olympus CX23), evaporator (IKA RV 10), dry sterilizer (Corona ZTP8A-7 (IR Ray), incubator (Memmert UNB 4000, analytical balance (Excellent Analytical), balance AB HZK-2104, oven (T100-200), stir bar, mattress thread, petri dish, beaker, Erlenmeyer, measuring cup, spirit flask, round loop, hot plate, micropipette, tip, spatula, test tube, gauze , umbrella paper. In this study, the inhibition activity of clove flower essential oil against the growth of some dandruff scalp-causing fungal pathogen (P. ovale, C.
albicans, and M. gypseum) was tested in vitro using the Kirby-Bauer method with samples taken from pure cultures that have been cultured on Saboraud Dextrose Agar (SDA). The sample was then given clove flower essential oil followed by incubation at a temperature of 25°-30°C for 4 days.

Preparation of Mc.Farland's standard

The Mc.Farland's standard 0.5 was done by pipetting 0.05 ml of 1% H2SO4 solution and adding 9.95 ml of 1% BaCl solution, the two solutions were mixed (Clinical And Laboratory Standard Institute, 2012).

Preparation of suspension of pathogenic fungi

Three collected tubes containing physiological NaCl were prepared, followed by the suspension of pure cultured strains (P. ovale, M. gypseum and C. albicans) was taken 1 oose of each and put into a tube containing physiological NaCl. It was then homogenized properly and the turbidity was calculated compared to the standard 0.5 Mc Farland's (Clinical and Laboratory Standards Institute, 2012).

Inhibition activity testing of clove flower essential oil against the three pathogenic fungi (P. ovale, M. gypseum, and C. albicans)

The fungal suspension was planted on the media using a scattering technique by pipetting 100 µl of the fungal suspension into a petri dish that already contained Saboroud Dextrose Agar and was then leveled with a stirring rod. The discs that have been pre-soaked with a concentration of clove flower essential oil with concentrations of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100% was then put into the agar media. The media were wrapped in plastic and with umbrella paper which was then incubated for 2 x 24 h for the growth of C. albicans and P. ovale and 3 x 24 hours for the growth of M. gypseum. The results were observed by measuring the inhibition zone formed around the disc using a caliper (Pratiwi et al., 2008).

Data analysis

The results of the diameter of the inhibition were analyzed using a parametric test with the One way ANOVA method (one-way analysis of variance) with a 95% confidence level or \( p = 0.05 \), if the test results showed significant results, it is followed by Duncan's test or DMRT.

Results and discussion

Based on the results of the study, it was shown that there was a clear zone between the paper discs, at a concentration of 10% to 100% there was an inhibition zone (mm). The treatment with the greatest inhibitory ability was the treatment on M. gypseum, with the average inhibition at 10% concentration of 33.05 mm, and at 100% concentration of 63.95 mm with positive control of 24.7 mm. On the inhibition zone then compared to the fungus C. albicans and the fungus P. ovale, this M. gypseum is larger.

Analysis of one-way variance data or one-way ANOVA showed a very significant difference between treatments of several concentrations of clove flower essential oil on the average diameter of the inhibition zones of several dandruff-causing fungi (M. gypseum, P. ovale, and C. albicans), where \( p = 0.000 \) or \( p\)-value < 0.05. This matter shows the significant results of the concentration variation of clove flower essential oil against the dandruff-causing fungal growth so that a further test was carried out using Duncan's test.
Duncan's test results show the mean different diameters of resistance of any concentration or type mold. Clove essential oil treatment results showed that almost all concentration showed different effectiveness, but some concentrations has a same effective inhibition. The test results are presented in Table 1, 2, 3 and Figure 1.

Table 1. The average diameter of the inhibition zone of clove flower essential oil on the growth of *Pityrosporum ovale* after Duncan's test.

| Treatment(s) | The average diameter of inhibition zone (mm) | Interpretive Category |
|--------------|---------------------------------------------|-----------------------|
| 10%          | 14,75±0,25                                 | Intermediate          |
| 20%          | 17,4b ± 1                                  | Intermediate          |
| 30%          | 21,05c ± 0,35                              | Susceptible           |
| 40%          | 26,9d ± 0,1                                | Susceptible           |
| 50%          | 28ke ± 0,6                                 | Susceptible           |
| 60%          | 29,1e ± 0,3                                | Susceptible           |
| 70%          | 31,05f ± 0,95                              | Susceptible           |
| 80%          | 35,35g ± 0,65                              | Susceptible           |
| 90%          | 40h ± 1                                    | Susceptible           |
| 100%         | 46,15i ± 1,05                              | Susceptible           |
| Control (+)  | 45,jl ± 0,1                                | Susceptible           |
| Control (-)  | 0                                          |                       |

Note(s):
- Number followed by the same alphabet was identical based on Duncan test
- Susceptible response signified ≥ 20 mm, Intermediate 15 -19mm, and resistant < 14 mm. (CLSI 2018)

Table 2. The average diameter of the inhibition zone of clove flower essential oil on the growth of *Microsporum gypseum* after Duncan's test.

| Treatment(s) | The average diameter of inhibition zone (mm) | Interpretive Category |
|--------------|---------------------------------------------|-----------------------|
| 10%          | 33,05a ± 0,55                              | Susceptible           |
| 20%          | 37,85b± 6,65                               | Susceptible           |
| 30%          | 39,45c ± 5,65                              | Susceptible           |
| 40%          | 45,93d ± 4,38                              | Susceptible           |
| 50%          | 49,05d ± 0,05                              | Susceptible           |
| 60%          | 50,95e± 0,75                               | Susceptible           |
| 70%          | 55,65f± 1,75                               | Susceptible           |
| 80%          | 56,85g± 2,55                               | Susceptible           |
| 90%          | 58,35h± 3,65                               | Susceptible           |
| 100%         | 63,95i± 4,15                               | Susceptible           |
| Control (+)  | 25,7a ± 0,1                                | Intermediate          |
| Control (-)  | 0                                          |                       |

Note(s):
- Number followed by the same alphabet was identical based on Duncan test
- Susceptible response signified ≥ 20 mm, Intermediate 15 -19mm, and resistant < 14 mm. (CLSI 2018)
Table 3. The average diameter of the inhibition zone of clove flower essential oil on the growth of *Candida albicans* after Duncan's test.

| Treatment(s) | The average diameter of inhibition zone (mm) | Interpretive Category |
|--------------|--------------------------------------------|-----------------------|
| 10%          | 13.5 ± 0.17                               | Susceptible           |
| 20%          | 19.23 ± 0.30                              | Susceptible           |
| 30%          | 25.03 ± 0.66                              | Susceptible           |
| 40%          | 27.2 ± 0.2                                | Susceptible           |
| 50%          | 33.26 ± 0.41                              | Susceptible           |
| 60%          | 35.66 ± 0.15                              | Susceptible           |
| 70%          | 36.4 ± 0.55                               | Susceptible           |
| 80%          | 36.56 ± 0.35                              | Susceptible           |
| 90%          | 37.23 ± 0.55                              | Susceptible           |
| 100%         | 39.53 ± 0.15                              | Susceptible           |
| Control (+)  | 34.23 ± 0.25                              | Susceptible           |
| Control (-)  | 0                                         |                       |

Note(s):
- Number followed by the same alphabet was identical based on Duncan test
- Susceptible response signified ≥ 20 mm, Intermediate 15 -19 mm, and resistant < 14 mm. (CLSI 2018)

Figure 1. The clear zone shows the inhibitory activity of clove essential oil against *Microsporum gypseum* on saboraud dextrose agar medium at 30°C for 4 days. (a. concentration 100%, b. positive control: ketoconazole 2%).

Table 4. Clove flower essential oil phytochemical screening test results

| Phytochemical Test   | Results |
|----------------------|---------|
| Saponin              | Negative|
| Phenol and Tannin    | Negative|
| Flavonoid            | Positive|
| Alkaloid             | Negative|
| Poli Fenolat         | Positive|
| Kuinon               | Positive|
| Steroid              | Positive|
| Menoterpen           | Positive|
Based on the results of Duncan’s test, it showed that the ability of each concentration of clove flower extract to the growth of *M. gypseum* resulted in a significantly different inhibition zone. The ability of the 10% concentration was significantly different and better than the control treatment of 2% ketoconazole, namely the inhibitory power at a concentration of 10% was 33.05 mm while the ability of 2% ketoconazole was 25.7 mm. Likewise, the ability of clove flower essential oil to have an inhibitory zone of *C. albicans*, at a concentration of 60% was significantly different and better than the control, with inhibitory abilities of 35.66 mm and 34.23 mm, respectively. The ability of clove flower essential oil to the *P. ovale* inhibition zone, the inhibitory ability between 100% concentration and 2% control was not significantly different, namely the inhibition diameters were 46.15 mm and 45.7 mm, respectively. The inhibition zone was showed as the clear zone around the disc. It implied that the active compounds able to inhibit fungal growth.

The active compounds contained in clove flower essential oil has antimicrobial activity because it contains eugenol and flavonoids (Table 4). Eugenol belongs to the polyphenolic group which has bacteriostatic or bactericidal activity depending on the concentration. Eugenol inhibits the biosynthesis of ergosterol - an important component in fungal cell membranes so that fungal cell membranes are damaged and their function decreases. Because eugenol is a lipophilic compound, eugenol can penetrate the lipid bi-layer membrane which is composed of fatty acid chains, thereby changing the fluidity and permeability (Alfauziah and Budiman, 2016). Flavonoids act by denaturing cell proteins, which can inhibit the work of enzymes in cells, resulting in a faulty cell wall construction process. Because of the inclusion of eugenol chemicals, which are phenol components, clove flower extract contains antifungal substances that can suppress fungal growth. These phenol components can harm cell microorganisms by producing protein coagulation and cell wall membrane permeability, as well as inactivating enzymes involved in microorganism cell metabolism (Sundari *et al*., 2001).

Eugenol compounds are other active chemicals discovered in clove flowers. Eugenol is a widely utilized substance in the pharmaceutical industry due to its wide range of pharmacological properties, including antiseptic, anti-inflammatory, antiviral, antibacterial, antifungal, antispasmodic, stimulant, and local anesthetic properties (Alisa *et al*., 2015). Eugenol has been shown to inhibit gram-positive and gram-negative bacteria, as well as those resistant to antibiotics. Because of their hydrophobic character, the chemicals will harm cell structure by interacting with the lipopolysaccharide already present in cell membranes. (Utami *et al*., 2019).

Several studies linked to this subject, like Andries *et al* (2014), reported that clove flower extract (*S. aromaticum*) has antibacterial activity against *Streptococcus mutans* in vitro, with 5 repetitions at concentrations of 40%, 60%, and 80%. It indicated the inhibitory zone at 40 percent 20.41 mm, 60 percent 21.0 mm, and 80 percent 25.81 mm concentrations, in that order. Nurhayati (2017) discovered that the clove flower extract (*Syzygium aromaticum*) inhibited the growth of bacteria. Paliling *et al.* (2016) found that the clove flower extract can prevent the growth of *Porphyromonas gingivalis* using 96 percent ethanol solvent and a diameter of 13.0 mm using the Kirby Bauer method.

Khusnul *et al.* (2017) did another research on the fungus *Trichophyton rubrum*, this time testing the effects of an ethanol extract of galangal rhizome (*Alpinia Galanga* L) against *Trichophyton rubrum* growth. There is no inhibition at concentrations of 10% to 20%, and there is inhibition at concentrations 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, and 100% inhibition zone 18 mm. *Trichophyton rubrum* growth is inhibited to a lesser extent by galangal rhizome extract, whereas *Trichophyton rubrum* growth is inhibited to a greater extent by clove flower extract.
Lova et al. (2018) found that clove flower essential oil had the greatest antibacterial activity, with an inhibition zone of 25.85 mm - 26.75 mm, followed by flower stalk essential oil with an inhibition zone of 20.60 mm - 21, 20 mm, and clove essential oil with an inhibition zone of 18.04 mm - 18.58 mm. When compared to the essential oils from the flower stem and clove leaf, the essential oil from the clove flower exhibits the best activity against P. acnes. When used as an anti-bacterial for P. acnes, the essential oil from the flower stem and clove leaf is not similar to that from the clove flower.

Clove can inhibit bacteria, fungus, protozoa, and viruses, giving them a broad spectrum of antibacterial activity. Gram-positive and Gram-negative bacteria have different MIC values. Clove flowers have been shown to destroy germs (Pathirana et al, 2019). Although the inhibition against Gram-positive bacteria is larger than the resistance against Gram-positive bacteria (Saikumari et al, 2016), cloves has a very low or very strong MIC value against some Gram-negative bacteria, depending on the bacterium types (Moon et al., 2011; Pandey and Singh, 2011).

Conclusion
Based on the results of this study, it can be concluded that the essential oil clove flower (S. aromaticum) can affect fungal growth that causes dandruff (Microsporum gypseum, Pityrosporum ovale, and Candida albicans).

Conflict of interest
The authors state no conflict of interest from this manuscript.

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Author contributions
All authors have reviewed the final version of the manuscript and approved it for publication. KK designed the study; PPA performed research and collected the data; KK analysed the data; KK, DPV wrote and reviewed the paper. KK is the main contributor of this manuscript.

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