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Formulation and Antifungal Activities of *Syzygium Aromaticum* Essential Oil Contained in Natural Bath Soap

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ABSTRACT

Syzygium aromaticum essential oil (clove oil) has a promising potential as an antifungal. However, the potential has never been applied in bath soaps. This study aimed to produce an antifungal natural soap using clove oil as the additive. Natural bath soap containing clove oil was made through a cold saponification reaction of vegetable oils, sodium hydroxide, and various contents of clove oil (1, 2, and 3% w/w) as an antifungal agent. The soap quality was tested based on Indonesian National Standards (INS), including water content, total fat, insoluble matters in ethanol, free alkali, and unsaponified fatty matter. With a Kirby-Bauer diffusion method, the researchers carried out an antifungal activity test of clove oil soap on *Candida albicans* and *Trichophyton mentagrophytes*. All samples in the quality test of clove oil natural bath soap have met the standard. The antifungal activities of all tested clove oil soap performed growth inhibition of *Candida albicans* yeast and *Trichophyton mentagrophytes* mould in all treatments. However, there is no relationship between the clove oil concentration and antifungal activities in the range of clove oil content in this study. The formulation of clove oil in natural bath soap executed excellent antifungal activities against both yeast and mould. Due to the potential of clove oil as an antifungal agent, clove oil in bath soap is efficient to be used as natural cosmetics and medicinal soap in the future.

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INTRODUCTION

The COVID-19 pandemic has spread throughout the world since the end of 2019. There is no vaccine or drug yet that has been proven effective to beat this virus infection. Our skin is the primary defence against virus infections because the fastest transmission is caused by contacts of contaminated hands with mouth, nose or eyes. Skincare products contain moisturizers and reduce irritation can maintain skin health (Davarnia and Khaki, 2020). Although washing hands with soap is useful for preventing COVID-19, it should not be done too frequently because it will cause dermatitis (Beiu *et al.*, 2020). Therefore, natural soap is crucial for a skincare product to avoid excessive use of chemicals.

Nowadays, people have begun to understand the significance of natural food, medicine or cosmetic products. Green and sustainable extraction methods of natural products involve greener processes by reducing chemicals as basic ingredients. In the cosmetic or medicinal industry, chemicals are replaced by safer ingredients with various biological activities (Chanchal and Swarnlata, 2008). Some potential natural sources of antifungal, antibacterial and antioxidant in food industries are essential oils of *Biden pilosa* Linn (Deba *et al.*, 2008), *Allium sativum, Azadiracta indica, Ocimum sanctum* (Bansod and Rai, 2008), *Thymus vulgaris, Satureja hortensis and Syzygium aromaticum* (Omidbeygi *et al.*, 2007).

Natural soap is the results of the saponification reaction of fatty acids from vegetable oil with a base. By adding the natural ingredients with certain biological activities, natural bath soap can be used as a medicinal soap (Ruckmani *et al.*, 2014; Wmank and Perera, 2016). Since medicinal soap interacts directly with the skin, it must at least be able to inhibit the growth of microbes that create their habitats on the skin. Skin infections are usually caused by fungi such as *Trichophyton mentagrophytes* (Woodfolk, 2005) and *Candida albicans* (Lachke *et al.*, 2003). Therefore, to optimize the natural soap into a medicinal soap, it must at least functions as an antimicrobial and antifungal agent. The selection of raw ingredients determines the physical and chemical properties of soap. Relatively insoluble fats can make the soap texture a bit hard and foamy.

In comparison, easily dissolved coconut oil in soap can help produce foam easily (Barel *et al.*, 2001). Olive oil, an extract from the *Olea Europea* fruit, has long been used as a primary ingredient in body care and beauty care products (Boskou, 2006). Olive-based soap has higher moisture than ordinary soap's (Beetseh and Godwin, 2015). Castor oil can also be used as a raw material for solid or liquid bath soap. The main content of castor oil is ricinoleate which serves a function as anti-inflammatories (Vieira *et al.*, 2001). The right combination of several types of vegetable oils can provide physical and chemical properties according to the expected quality and function of the soap.

Clove (*S. aromaticum*) is a native spice plant in Maluku Islands and has been cultivated and traded for generations in the form of smallholder plantations. The main content of clove oil is eugenol (83%) and caryophyllene (9%) (Xing *et al.*, 2010). This chemical content is nearly the same as the essential oil's from *S. caryophyllatum* (Bhuiyan, 2012). The biological activity of clove oil has been investigated on several microorganisms and parasites, including pathogenic bacteria, herpes simplex, and hepatitis C virus. In addition to its antimicrobial, antioxidant, antifungal, and antiviral activities, clove oil is excellent with its anti-inflammatory, cytotoxicity, mosquito repellent and anaesthetic properties (Chaieb *et al.*, 2007). Antifungals are antibiotics that can inhibit the growth of fungi (fungistatic) or even kill the fungi (fungicidal). Antifungal drugs, also known as antimycotic drugs, are used to treat two types of fungal infections: superficial fungal infections on the skin or mucous membranes and systemic fungal infections on the lungs or central nervous system. *Mucocutaneous mycosis*, a fungal infection of the mucosa and moist skin layers, is usually caused by candida. *C. albicans* is an opportunistic commensal organism found on our skin. *Sygyzium aromaticum* is a potential antifungal agent that can inhibit the growth of *C. albicans* (Yanisse *et al.*, 2020). Therefore, natural bath soap with clove oil as an antifungal agent needs to produced and tested against *C. albicans* fungi. The exact dose of an antifungal agent is crucial in antifungal technology because *Candida albicans* is more fungistatic than fungicidal (Berman and Sudbery, 2002). Other studies have reported the use of *T. mentagrophytes* to test the pathogenesis of dermatophyte infections on human skin (Duek *et al.*, 2004). Besides, the essential oils of clove (*S. aromaticum*) also perform an antifungal activity against pathogenic fungi in plants and animals such as *Fusarium moniliforme* NCIM 1100, *Fusarium oxysporum* MTCC 284, *Aspergillus sp.*, *Mucor sp.*, *Trichophyton rubrum* and *Microsporum gypseum* (Rana *et al.*, 2011). *In vitro*, fungitoxicity has indicated that *S. aromaticum* essential oil acquires a fungicidal activity against phytopathogenic fungi (Beg and Ahmad, 2002). In this study, natural bath soap was made using *Sygyzium aromaticum* as an antifungal agent. Our investigation focused on the effect of clove oil as an antifungal agent on natural bath soap.

MATERIALS AND METHODS

Reagents and Chemicals

Palm oil, VCO, castor oil, olive oil and clove oil were purchased from a local market in Yogyakarta, Indonesia. Chemicals, *C. albicans* ATCC 10231, *T. mentagrophytes*, Nutrient Broth, chloramphenicol, Potato Dextrose Agar (PDA), and paper discs were obtained from Oxoid.

Soap formulation

Olive oil (121 g), virgin coconut oil, VCO (106 g), palm oil (106 g), and castor oil (15 g) were weighed and mixed in a plastic container. NaOH (50 g) in 140 mL distilled water was added into the oil mixture. The mixture was stirred slowly until thickened to form the soap. Clove oil (1%) was added to the thickening soap while it was stirred. The homogeneous thickened bath soap was poured into the mould and left for four weeks. The procedure was then repeated using clove oil 2% and 3%. The quality test on solid soap based on the INS had been carried out in previous study (Handayani *et al.*, 2018).

Antifungal tests

The test was carried out by modifying the methods of Cavalieri *et al.* (2005) and Balouiri *et al.* (2016) using the Kirby-Bauer diffusion method on the yeast of *C. albicans* and mould *T. mentagrophytes*. *C. albicans* isolate was grown on Nutrient Broth (NB) medium for 24 hours at 37 °C. Meanwhile, *T. mentagrophytes* was grown on *Potato Dextrose Agar* (PDA) slant media for seven days before making the spore suspension. The yeast suspension was inoculated on the nutrient agar (NA) plate with the spread plate method. The samples with a variety of treatments and controls were placed on the NA plates. The plates were then incubated for 24 hours at 37 °C, and the diameter of the clear zone was measured using the vernier calipers at the 12^{th}, 24^{th} and 30^{th} hours. The diameter of the inhibition of fungal growth was analyzed statistically using SPSS version 21.

RESULTS AND DISCUSSION

The natural bath soap was made through a cold saponification reaction at room temperature (Warra *et al.*, 2011). Natural bath soap is classified as herbal cosmetics using various natural additives from plant parts such as leaves, flowers, and the like through unique formulation methods (Shivanand *et al.*, 2010). Besides, natural bath soap can be used as a medicinal soap if the added ingredients perform biological activities such as antibacterial, antioxidants, and antifungal. Therefore, clove oil is an excellent ingredient used as a natural antifungal agent in soap products. In this study, clove oil was added to the natural soap and tested for the quality and antifungal activity against *C. albicans* and *T. mentagrophytes*. Since the maximum concentration of additives that can be added to soap is 3%, the added clove oil used in this study was 1%, 2%, and 3%.

The quality test on the solid bath soap presented in Table 1 shows that the quality of all soap meets the INS. In previous studies, it was reported that a clove oil concentration of 0.03% made a high cytotoxic effect. This effect is caused by high eugenol content (Prashar *et al.*, 2006). In this study, the addition of 1% clove oil on natural bath soap results in a high-quality soap that met the standards. These results are not completely different from the soap quality in previous studies using 2 and 3% of clove oil (Handayani *et al.*, 2018). Therefore, to avoid the high cytotoxic effects that may appear in clove oil soap, the use of clove oil with the lowest concentration (1%) is highly recommended.

The growth inhibition of *C. albicans* ATCC 10231 yeasts and *T. mentagrophytes* moulds was observed in all treatments. Antifungal compounds were diffused into the agar media, and no growth was observed around the paper disc as indicated by the formation of inhibitory zones. The wider the diameter of the inhibition, the greater the inhibitory power of the antifungal compound. The growth inhibition of *C . albicans* and chloramphenicol as the positive controls is shown in Figure 1. The positive control (chloramphenicol 10 ppm) inhibited the growth of fungi. Both fungi continued to grow upon contact with distilled water used as a negative control.

The diameter of the growth inhibition zone of *C. albicans* yeast was measured during 30 hours of incubation. The data of the inhibition zone, which were observed at 18^{th}, 24^{th} and 30^{th} hours are summarized in Table 2. The inhibition zone disappeared after 42 hours of incubation. It shows that the inhibition zone decreases with increased incubation time. The higher concentration of soap treatment did not always indicate an increase in the average diameter of the inhibition zone. This observation contradicted the previously reported studies which stated that the higher the clove oil concentration, the higher the inhibition diameter of the same fungus (Xing *et al.*, 2010). As expected, the positive control (chloramphenicol 10 ppm) was able to inhibit the growth of *C. a lbicans* but not always as large as that in the treatment. In contrast, the negative control (distilled water) was consistently unable to inhibit the growth of the fungi.

The ANOVA analysis performed significant differences in the inhibition zone of *C. albicans* ($p<0.05$) with the soap containing clove oil of 1%, 2% and 3%, and chloramphenicol as positive controls at the 12, 24 and 30 hours of incubation. Also, significant differences occurred with the concentrations of soap in the *C. albicans* inhibition zone ($p<0.05$) with the concentration of soap 2.5%, 5%, 7.5%, and 10% at the 12, 24 and 30 hours of incubation. There was a significant interaction ($p<0.05$) between the type of soap and the concentration of clove oil, which affected the inhibition zone of *C. albicans* at 12, 24 and 30 hours of incubations.

The Inhibition zone at 12^{th} hour was higher than that at 24^{th} and 30^{th} hours of incubation in all treatments. The treatment of 1%-clove oil soap with a concentration of 7.5% in 12 hours incubation time showed the highest inhibition zone. It was reported that the antifungal properties of clove oil were lower than the synthetic antimycotic antibiotics used in the *in vitro* treatment of candidiasis (Nzeako *et al.*,

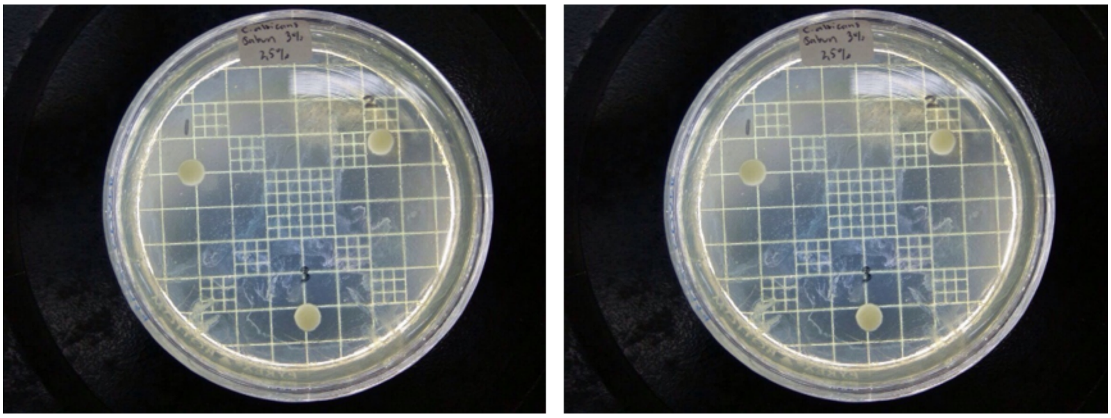

Figure 1: Sample photos of growth inhibition on *Candida albicans* by clove oil soap

Table 1: The Quality test of natural bath soap with clove oil as an additive

Clove Oil (%)	Water content (%)	Total fat (%)	Insoluble matter in ethanol (%)	Free alkali (%)	Unsaponified fatty matter (%)	Reference
1	1.180	88.860	1.730	0.010	0.006	
2	1.370	90.480	1.260	0.006	0.032	(Handayani *et al.*, 2018)
3	1.520	92.050	0.800	0.005	0.003	(Handayani *et al.*, 2018)
INS	max 15.0	min 65.0	max 5.0	max 0.1	max 0.5	

Table 2: Diameters of inhibition zones on clove oil soap against *C. albicans* (mm)

Clove oil in natural bath soap (%)	The concentration of soap (%)	The diameter of the inhibition zone observed after (hours)		
		12	24	30
1	2.5	19.80	8.03	8.30
	5.0	19.57	9.77	15.86
	7.5	21.94	13.47	11.09
	10	21.09	12.89	12.66
2	2.5	10.30	8.41	7.54
	5.0	13.65	10.82	9.53
	7.5	20.22	13.97	9.60
	10	17.60	11.93	10.63
3	2.5	21.01	16.26	8.14
	5.0	20.09	12.39	8.58
	7.5	20.27	19.48	9.07
	10	12.27	11.41	9.54
Chloramphenicol	10	17.70	16.37	15.33
Aquadest		0	0	0

Table 3: Diameters of inhibition zones on clove oil soap against *T. mentagrophytes* (mm)

Clove oil in natural bath soap (%)	The concentration of soap (%)	The diameter of the inhibition zone observed after (hours)		
		12	24	30
1	2.5	7.88	7.76	7.03
	5.0	7.43	7.23	6.86
	7.5	9.11	8.99	8.43
	10	9.66	9.51	8.70
2	2.5	7.70	7.64	7.36
	5.0	8.25	8.14	8.09
	7.5	8.56	8.47	8.04
	10	9.74	9.68	9.04
3	2.5	7.48	7.30	6.87
	5.0	8.47	8.28	7.81
	7.5	8.88	8.70	8.19
	10	8.69	8.57	8.24
Chloramphenicol	10	8.23	7.99	7.01
Aquadest		0	0	0

2008). However, previous research has also shown that clove oil indicated a broad spectrum of antimicrobial activities with a maximum zone diameter of 35 mm for C. albicans (Ayoola *et al.*, 2008). The minimum inhibition concentration of clove oil against *C. albicans* was 2.5% for direct application in dilution assay (Chee and Lee, 2007). Other studies have shown that antifungal activities of clove oil and eugenol were relatively high against the relevant fungi clinically, including the strains resistant to fluconazole (Pinto *et al.*, 2009). They supported the results of this study in which the addition of *S. aromaticum* essential oil to the bath soap was able to inhibit the growth of *C. albicans* fungi. Thus, the synthesized clove oil bath soap is potential to be a natural medicinal soap.

Moreover, the diameter of the inhibition zone of *T. mentagrophytes* mould was measured during the 30-hour incubation since the inhibition zone disappeared after 42 hours of incubation. The data in Table 3 are the results of the observations collected at the 18^{th}, 24^{th}, and 30^{th} hour of incubation. The results showed a similar pattern, as observed in *C. albicans.* The inhibition zone decreased along with the increased incubation time. However, the higher concentration of soap treatment did not show any increase in the average diameter of the inhibition zone. As expected, the positive control (chloramphenicol 10 ppm) showed the inhibition of the growth of *T. mentagrophytes*, but the effect was not always as large as the treatment. The negative control (distilled water) was undoubtedly unable to inhibit fungal growth.

The analysis data suggested a significant difference in the *T. mentagrophytes* inhibition zone (P <0.05) among the soaps with clove oil content of 1%, 2%, 3% and chloramphenicol in all cases of incubation, i.e. the 18^{th}, 24^{th} and 30^{th} hours. The results of the ANOVA analysis also indicated the significant differences on the *T. mentagrophytes* inhibition zone (P<0.05) among the soap concentrations of 2.5%, 5%, 7.5%, and 10% at the 18^{th}, 24^{th}, and 30^{th} hours of incubations. Moreover, the ANOVA analysis results showed a significant interaction (P<0.05) between the soap type and the concentration which affected the *T. mentagrophytes* inhibition zone at the 18^{th}, 24^{th}, and 30^{th} hours of incubations.

Inhibition zone at the 12^{th} hour was higher than that of the 24^{th} and 30^{th}- hour incubations in all cases of the treatments. The treatment using 2% of clove oil soap with a concentration of 10% and 12^{th} hour of incubation showed the highest inhibition zone. Clove oil also inhibited the growth of *T. mentagrophytes* at the minimum concentration inhibition of 1% (v/v) (Chee and Lee, 2007). This difference in the effectiveness of inhibition can be easily understood from the point of view that the content of clove oil in the natural bath soap in this study is only 2% which is lower than the previously reported results.

In general, natural bath soap containing clove oil has shown more effective inhibition on the growth of *C. albicans* than *T. mentagrophytes*. The compound which is responsible for the inhibition activity of fungal growth in clove oil is eugenol (Park *et al.*, 2007). This conclusion is supported by the fact that the antibacterial properties of clove oil are almost the same as eugenol's and higher than caryophyllene (Moon *et al.*, 2011). The cytotoxicity data from previous studies indicated that essential oils or their components must be used in a highly diluted form, especially when they have direct contact with the skin (Prashar *et al.*, 2006). In this study, different concentrations of clove oil in the natural bath soap provided no significant difference in its antifungal activities. Therefore, it is advisable to add clove oil in bath soaps at the lowest concentration to prevent unwanted effects since it is applied directly to the skin.

CONCLUSION

The soap quality test shows that all samples have met the Indonesian National Standard. The addition of clove oil to bath soap performed significant inhibition on the growth of *C. albicans* and *T. mentagrophyte* bacteria. Soap containing clove oil can be a promising alternative as natural antifungal soap in the future. Based on the diversity of the clove oil's function, further research on the use of clove oil as an antifungal agent for other skincare and cosmetic products such as lotions or moisturizers is necessary to conduct soon.

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Conflict of Interest

The authors declare that there is no conflict of interest for this study.

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