Global Scientific JOURNALS

GSJ: Volume 8, Issue 1, January 2020, Online: ISSN 2320-9186
www.globalscientificjournal.com

ANTIFUNGAL EFFECTIVITY OF INDONESIAN BAY LEAF (*Syzygium polyanthum*) AND KAFFIR LIME LEAF (*Citrus hystrix*) ETHANOL EXTRACTS ON THE GROWTH OF *Trichophyton mentagrophytes* FUNGI

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ABSTRACT

This study was aimed to determine the effectivity of Indonesian bay leaf and kaffir lime leaf, each with 12.5% and 25% concentrations on *Trichophyton mentagrophytes* with a positive control of Miconazole. *T. mentagrophytes* is a type of molds, which is included in dermatophyte group. The disease that it most often causes is dermatophytosis or ringworm. This study was conducted using experimental method and post-test only design, in which the samples were taken with purposive sampling technique. Effectivity assessment of bay leaf and kaffir lime leaf extracts on *T. mentagrophytes* was conducted using disc diffusion technique, by measuring inhibition zone diameter of fungi on disc papers moistened with bay leaf and kaffir lime leaf extracts and Miconazole as positive control. The results found in this study was analyzed using One-Way ANOVA, followed by Post Hoc. The results showed that there were significant differences between all treatments given with 95% confidence interval. In 12.5% bay leaf group, the effectivity on *T. mentagrophytes* was higher than 25% kaffir lime leaf extract and both were above Miconazole. The inhibition zone of 12.5% bay leaf extract was 12.5 mm, with similar antifungal effect as 25% kaffir lime leaf extract with 10 mm and *Miconazole* showed 8.5% inhibition zone on *T. mentagrophytes* growth.

Keywords: Antifungal, bay leaf extract, kaffir lime leaf extract, Tricophyton mentagrophytes.

1. INTRODUCTION

Indonesia is known as a nation with the largest biodiversity, second to Brazil. The condition has a great potential for natural antifungal materials, including bay leaf (*Syzygium polyanthum*) and kaffir lime leaf (*Citrus hystrix*). Bay leaf is widely found in the mountain forest above 1,800 masl. Bay leaf is known to contain vitamin A, vitamin C, and vitamin E which act as antioxidant. The essential oil content in bay leaf include citral, eugenol, tannin, simple phenol and flavonoid. Other chemical contents include saponin, triterpenoid, polyphenol, sesquiterpenoid and lactone. Bay leaf extract had antifungal and antibacterial effects. (Fitriani, Hamdiyanti, & Engriyani, 2012)

Bay leaf is a plant often used in the community as alternative medicine. The existence of bay leaf is common in the community and easily available andis expected to aid in education and introduction of bay leaf to the community as an herbal alternative for health. (Harismah & Chusniatun, 2016)

Kaffir lime is a fruit widely known in the community as a food source and known to contain active substances believed to be an herbal medicine with very high antioxidant activity, thus can be beneficial in daily needs, whether for medicinal, industrial, or home-use purposes. The use of the fruit and leaves of kaffir lime has been known by the community since a long time ago as an herbal medicine. The leaf and fruit parts are usually used for fatigue and to increase fitness and as food seasoning. (Halawa, Mendrofa, & Lubis, 2019)

One of the natural ingredients that can be used as antifungal is kaffir lime (*Citrus hystrix*). The leaves contain steroid triterpenoid, tannin (1.8%), and essential oil (1-1.5%). The skin contains saponin, tannin (1%) and essential oil containing citric (2-2.5%).(Halawa et al., 2019)

Serious infection caused by *Trichophyton mentagrophytes* has shown increased resistance to antifungal agents. Dermatophytosis is a disease caused by the colonization

of dermatophytes which attacks keratinized tissues, including the stratum corneum of the skin, hair, and nail in humans and animals. High resistance level of *T. mentagrophytes* on antifungal agent such as amphotericin-B and fluconazole requires an alternative in the form of active substances from plants, which is highly effective on *T. mentagrophytes*. According to the above problem, further *in vitro* studies should be conducted and developed regarding the antifungal activity of *S. polyanthum* (Wight) Walp. leaf ethanol extract on the growth of *T. mentagrophytes* causing candidiasis. (Devy dan Evrianti, 2016)

Bay leaf (*Syzygium polyanthum*) is an herb found in Indonesia and widely used as food seasoning and traditional medicine. Empirically, bay leaf has been known to have effect on diarrhea, hyperuricemia, diabetes, decreasing cholesterol level and blood pressure. These effects have been proven preclinically using animal models. (Verawati, Nofiandi, & Petmawati, 2017)

Various activities of herbal medicine are caused by secondary metabolites contained in it, including phenolate, alkaloid, saponin, steroid, terpenoid, tannin, etc. Phenolate is a secondary metabolite group with wide distribution in plants. Phenolate has several antioxidants, antibacterial, anti-inflammatory, and anticancer activities. Phenolate content in plants acts as natural antioxidant which can ward off various oxidants and free radicals harmful to health. (Verawati et al., 2017)

Kaffir lime (*Citrus hystrix*)is known to be beneficial in Indonesia and Asian countries and widely used as natural fragrance in several food and beverage ingredients.

Other than food seasoning, kaffir lime is also known to be used as traditional medicine.(Febrina, 2019)

Citronellal is a chemical compound found in kaffir lime, which comprises of 81.49%. Other than citronellal, linalool, citronellyl-acetate, citronellol, and geraniol are also identified in kaffir lime leaf. (Febrina, 2019)

The result of essential oil extraction from kaffir lime skin using steam distillation with controlled temperature conducted in under 3 hours produced essential oil yield of 13.4%. The main substances identified from kaffir lime skin oil were sabinene, β-pinene, limonene, α-pinene, camphene, myrcene, terpinen-4-ol, α-terpineol, linalool, terpinolene and citronellal. Kaffir lime leaf originates from Thailand and China and

produces citronellal as its main component, consisting of 80.04% using steam distillation method in under 4 hours.(Febrina, 2019)

2. METHODS

This is an experimental study conducted in the Biochemical Laboratory of Prima Indonesia University, Medan from 3 – 24 October 2019.

The tools used include 7 petri dishes, reaction tube, filter paper, measurement glass knife, autoclave, disk papers, cotton swabs, tweezers, sterile cottons, caliper, dropping pipette, Erlenmeyer flask, analytical balance, reaction tube rack.

The materials used were consisted of bay leaf (*S. polyanthum*), kaffir lime leaf (*C. hystrix*), *T. mentagrophytes*, 70% ethanol, 96% technical ethanol, Miconazole, alcohol, double distilled water, physiological NaCl, spiritus, Sabouraud dextrose agar (SDA) media, plastic wrap, aluminum foil.

A. Preparation
   a. The tools and materials were prepared then sterilized in the oven at 40-70^{o}C for± 2 hours.
   b. In the making of bay leaf (*S. polyanthum*) and kaffir lime leaf (*C. hystrix*) water, the ingredients were first rinsed in the water until cleaned.
   c. Afterwards, the bay leaf and kaffir lime leaf were cut in small pieces and blended.

B. The making of concentrations

Bay leaf and kaffir lime leaf extracts, in which ethanol was evaporated, were divided into two concentrations (25% and 12.5%) by dissolving them in 96% technical ethanol due to their essential oil content.

   a. For 25% concentration: 2.5 gram extract + 10 ml 96% technical ethanol
   b. For 12.5% concentration: 2.5 gram extract + 10 ml 96% technical ethanol.

C. The making of bay leaf (*S. polyanthum*) and kaffir lime leaf (*C. hystrix*) extracts

The extracts were made by macerating bay leaf and kaffir lime leaf water was rinsed in running water and then drained and dried. Afterwards, the ingredients were smoothened with a blender to form powder. The resulting ingredients were taken for 1 kg as samples, then dissolved or immersed in 10 liters absolute ethanol solvent (96%) per each ingredient. The solution wasstirred daily for an hour and the maceration process were repeated 3 times.

D. The making of SDA media (Sabouraud Dextrose Agar)

Sixty-five grams of the mediawas suspended to a liter distilled water into Erlenmeyer flask and stirred using hot plate stirrer for a minute or until dissolved. Then, the media were sterilized in autoclave for 15 minutes at 121^{o}C, then let still until the temperature reached 40-45^{o}C to be poured to petri dishes and chilled till frozen.

E. The making of *T. mentagrophytes* suspension

The fungi used in this study was obtained from Pharmacy Laboratory of USU. One loop of *T. mentagrophytes*were taken from the colony, then inserted to a tube containing physiological NaCl, then stirred until all fungal colony was dissolved in NaCl.

F. Diffusion test of *T. mentagrophytes*

a. Cotton swab containing *T. mentagrophytes* suspension was smeared evenly on all hardened SDA media surfaces.
b. One sterile blank disk was taken, then dipped into 50% concentration and placed on top of the media previously smeared with *T. mentagrophytes*. The same treatment was performed on all concentrations and separated to avoid the unification of inhibition zones.
c. The treatment was repeated 3 times for data collection.
d. Afterwards, all isolates were incubated for 36-48 hours at 37^{o}C in incubator.
e. After 36-48 hours, the inhibition zone was measured using caliper.

3. RESULTS AND DISCUSSION

Data collection was performed on April 2019 in the laboratory. The samples used were bay leaf (*S. polyanthum*) and kaffir lime leaf (*C. hystrix*) on the growth of *T.*

mentagrophytes. According to the obtained and analyzed data, the concluding results were as follows:

The inhibition zone of bay leaf extract and kaffir lime leaf extract on the growth of *Trichophyton mentagrophytes*

The inhibition zone produced by bay leaf ethanol extract showed inhibition zone (clear zone) surrounding the concentrated disk paper. After 36-48 hours, the inhibition zone was measured using caliper. The measurement was repeated three times with Miconazole as control (+) and distilled water as control (-).

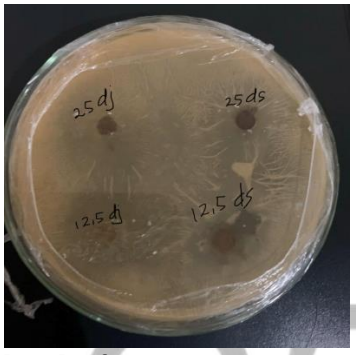

Figure 3.1 Inhibition zone of bay leaf extract (*Syzygium polyanthum*) on the growth of *Trichophyton mentagrophytes*.

Figure 1 showed that the inhibition zone of *T. mentagrophytes*was higher in accordance to increasing concentration of bay leaf extract (*Syzygium polyanthum*). The extract concentrations used in this study were 12.5% and 25%. The test was repeated three times with Miconazole as control (+) and distilled water as control (-).

Figure 3.2 Inhibition zone of control (+) Miconazole and control (-) distilled water on *Tricophyton mentagrophytes*.

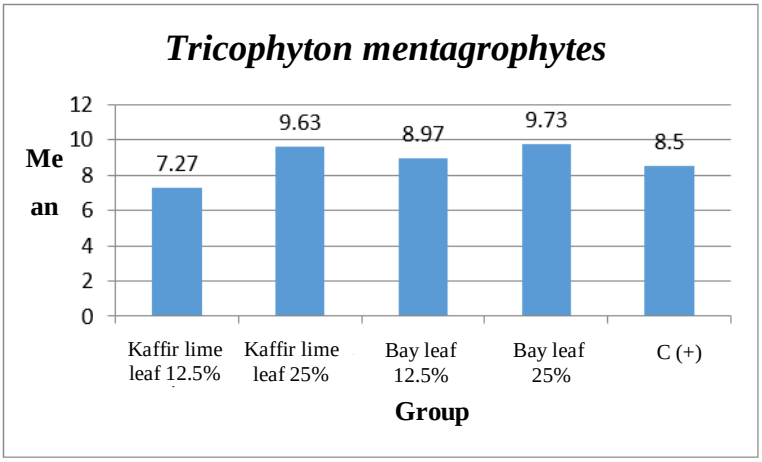

Figure 3.3 The graph of inhibition zone of bay leaf extract (*Syzygium polyanthum*) on the growth of *Tricophyton mentagrophytes*.

Table 3.1 The results of One-Way ANOVA

Group	Mean ± SD	Median (min – max)	p
Kaffir lime leaf 12.5%	7.27 ± 0.15	7.3 (7.1 – 7.4)	0.637*
Kaffir lime leaf 25%	9.63 ± 1.52	9.9 (8 – 11)	0.705*
Bay leaf 12.5%	8.97 ± 3.07	7.4 (7 – 12.5)	0.125*
Bay leaf 25%	9.73 ± 1.07	10.3 (8.5 – 10.4)	0.089*
C (+)	8.50 ± 0.00	8.5 (8.5 – 8.5)	–

* Normal ($p > 0.05$)

Table 3.2 The inhibition zone of bay leaf extract (*Syzygium polyanthum*) and kaffir lime leaf extract (*Citrus hystrix*) on the growth of *Tricophyton mentagrophytes*.

Group	Mean ± SD	p	Homogeneity
Kaffir lime leaf 12.5%	7.27 ± 0.15	0.379	0.004
Kaffir lime leaf 25%	9.63 ± 1.52		
Bay leaf 12.5%	8.97 ± 3.07		
Bay leaf 25%	9.73 ± 1.07		
C (+)	8.50 ± 0.00		

* significant ($p < 0.05$); ** homogenous ($p > 0.05$)

Table 3.2 showed the inhibition zone data produced by bay leaf extract (*S. polyanthum*) with 12.5% and 25% concentrations, whereas the first 12.5% concentration test revealed 7.0 mm, second test revealed 12.5 mm, and third test revealed 7.4 mm. Meanwhile, the first test of 25% concentration of bay leaf revealed inhibition zone of 10.4 mm, second test revealed 10.3 mm, and third test revealed 8.5 mm.

According to the study, the comparison of bay leaf extract and kaffir lime leaf extract on the growth of *T. mentagrophytes* showed significant difference with p value < 0.05, shown by the result of One-Way ANOVA with p value = 0.000, which means that

p <α, where α< 0.05, thus Ha was accepted and Ho rejected, which concludes that the result was significant. The result of One-Way ANOVA of 0.04 was significant.

4. DISCUSSION

Based on the obtained data in this study, there was an effect of bay leaf extract (*Syzygium polyanthum*) and kaffir lime leaf extract (*Citrus hystrix*) on the growth of *Trichophyton mentagrophytes*. The effectivity was shown by inhibition zones formed around disk papers. These inhibition zones were used to assess the effectivity produced by each extract on *T. mentagrophytes*.

Phenolate level and antioxidant activity from plant extract are affected by several factors, including extraction method. This study used several extraction methods on bay leaf, thus the correct method of extraction that can be implemented to bay leaf can be known.

The resulting inhibition zone of kaffir lime ethanol extract on *T. mentagrophytes* was under 10 mm, which showed that ethanol extract of kaffir lime was less effective on *T. mentagrophytes*. The highest inhibitory power was found in the second repetition of 25% concentration, which consisted of 11 mm.

The result of essential oil extraction from kaffir lime skin using steam distillation with controlled temperature conducted under 3 hours can produce yield of 13.4%. The main substances identified from kaffir lime skin oil were sabinene, β-pinene, limonene, α-pinene, camphene, myrcene, terpinen-4-ol, α-terpineol, linalool, terpinolene and citronellal. Kaffir lime leaf originates from Thailand and China with citronellal as its main component, which comprises of 80.04% obtained by steam distillation technique in under 4 hours.

The best concentration of bay leaf ethanol extract for inhibiting *T. mentagrophytes* was 12.5%, which can be seen in the second repetition with zone diameter of 12.5 mm. For control (+), Miconazole was used, and the inhibition zone found was 8.5 mm and in control (-) that used distilled water did not show any inhibition zone on *T. mentagrophytes.*

Figure 1 showed that the inhibition zone diameter of *T. mentagrophytes* increased along with concentration increase of kaffir lime leaf extract.

5. CONCLUSION

Based on the obtained data, there were effects of bay leaf extract and kaffir lime leaf extract on the growth of *T. mentagrophytes*. The effectivity was shown by inhibition zone produced around disk papers. The average inhibition zone produced by kaffir lime leaf extract on *T. mentagrophytes* was under 10 mm, which showed that kaffir lime leaf was less effective on *T. mentagrophytes*. The best inhibitory power of bay leaf ethanol extract on *T. mentagrophytes* was on 12.5% concentration, which can be seen from the second repetition with zone diameter of 12.5 mm. The analysis results of One-Way ANOVA followed by Post-Hoc showed p value = 0.000 which showed p $<\alpha$, with $\alpha< 0.05$, thus Ha was accepted, and Ho rejected. This result concludes that the difference was significant, whereas One-Way ANOVA result of 0.04 was significant.

This study suggests that further studies should be conducted whilst including factors affecting the effectivity of bay leaf extract and kaffir lime leaf extract on the growth of *T. mentagrophytes*.

6. ACKNOWLEDGEMENT

The author, along with other members of the team, acknowledges dr. Sri Lestari Ramadhani M.K.M as an advisor, and dr. Linda Chiuman, M.K.M., AIFO-K as the Dean of Faculty of Medicine, who facilitated this study.

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