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THE EFFECTIVENESS OF THE EXTRACT OF BAY LEAVES (*SYZYGIUM POLYANTHUM*) AND KAFFIR LIME LEAVES (*CITRUS HYSTRIX*) IN THE GROWTH OF *PITYROSPORUM OVALE* FUNGI

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Abstract:
Background: *Bay leaf contains vitamin A, vitamin C, and vitamin E which function as antioxidant. Its extract has the effect on anti-fungi and anti-bacteria. Kaffir lime is known as the source of food and assumed as active compound which can be used as herbal medicine with the highest antioxidant content. The objective of the research was to find out the effectiveness of the extract of bay leaves (Syzygium Polyanthum) and kaffir lime leaves (Citrus Hystrix) in inhibiting the growth of Pityrosporum ovale fungi.* ***Method:*** *The research used experimental laboratory research with sensitivity test method (Disc Diffusion Method). It was conducted in April, 2019. The samples were bay leaves and kaffir lime leaves obtained from the yards of the houses at Diski, Sunggal Sub-district, Deli Serdang.* ***Result:*** *It was found that the extract of bay leaves and kaffir leaves had the effectiveness of anti-fungi in inhibiting the growth of Pityrosporum ovale fungi which was indicated by the establishment of inhibitory zone or limpid zone surrounding disc paper. The diameter of inhibitory zone was measured by using vernier calipers to find out the power of anti-fungi. The concentration consisted of 50%, 75%, and 95%.* ***Conclusion:*** *It was concluded that the extract of bay leaves had the effect on inhibiting the growth of pityrosporum ovale microbe (fungi) with the inhibitory power of 16.20 mm in which miconazole that was used at its positive control had higher effect in inhibiting the growth of anti-fungus from Pityrosporum ovale with the inhibitory power of 27.1 mm. It is recommended that testing of guinea pig be done by using the extract of bay leaves and kaffir lime leaves with different cases.*

Keywords: Extract of Bay Leaves (Syzygium Polyanthum), Kaffir Leaves (Citrus Hystri), Growth of Pityrosporum Ovale Fungi

INTRODUCTION

Indonesia ranks the second in biodiversity after Brazil. This condition is very potential for natural anti-fungi, especially in bay leaves (*Syzgium polyanthum*) and kaffir lime leaves (*Citrus hystrix*). Bay leaves are ingredient leaves which are found in wild vegetation in highland with the altitude of 1,800 mdpl (meters above the sea level). They contain vitamin A, vitamin C, and vitamin E which function as antioxidant. Essential oil content in bay leaves contains citral, eugenol, tannin, simple phenol, and flavonoid compound. The other chemical compounds were saponin, triterpenoid, sesquiterpenoid, and lactones. Extract of bay leaves has the effect of anti-fungi and anti-bacteria.

Bay leaf plant is one of the plants which is often used for alternative therapy. Its existence is well-known by people since it is easily found. Therefore, it is expected that bay leaf plants can easily be used for education and introduction for people as one of alternative ingredients for herbal medicine.

Kaffir lime is fruit known by people as the source of food and assumed as containing active compound which is believed to be able to be used as herbal medicine which has the highest antioxidant so that it can be used in daily activities and in medical, industrial, and household activities. The use of the fruit and the leaves of kaffir lime has been known by people since long time ago as herbal medicine. Its fruit and leaves are usually used to cope with fatigue, to increase body fitness, and to be used as flavoring.

One of the natural ingredients which can be used as anti-fungi is kaffir lime (*Citrus hystrix*) in its leaves which contain steroid triterpenoid, tannin (1.8%), essential oil (1-1.5%). It also contains saponin, tannin (1%), and essential oil which contains citrate (2%-2.5%) on skin.

Pityrosporum ovale, yeast or a single cell fungus is the member of *Malassezia sp* genus and belongs to *Cryptococcaceae* family. It can cause superficial mycosis which attacks stratum corneum in epidermis layers. It is microorganism which is assumed as the main cause of dandruff. This type of fungus is really normal flora on scalp when hair has excessive sebaceous gland which makes it grow flourish.

FORMULATION OP THE PROBLEMS

Based on the background of the study, this research could be formulated as follows: "how about the test on the effectiveness of the anti-fungi of ethanol extract of bay leaves and kaffir lime leaves on the growth of *pityrosporum ovale* fungi.

OBJECTIVE OF THE RESEARCH

The objective of the research was to find out the effectiveness of the extract of bay leaves and kaffir lime leaves on the growth of *pityurosporum ovale* fungi.

RESEARCH METHOD

Operational Definition

Extract of Bay Leaves and Kaffir Leaves

Variable	Effectiveness test of bay leaves and kaffir lime leaves on the growth of *pityrosporum ovale* fungi
Operational Definition	Effectiveness test of bay leaves and kaffir lime leaves, using diffusion method
Method of Measurement	Dilution for getting concentration of 50%,75%, and 95%
Measurement Device	Calibrated beaker
Measurement Result	Concentration
Measurement Scale	Ordinal

Inhibitory Zone of *Pityrosporum ovale*

Variable	Inhibitory zone of *pityrosporum ovale*
Definition	Limpid zone or area surrounding blank disc which is not grown by fungi
Method of Measurement	By calculating the diameter of limpid zone or free from fungi surrounding disc paper in the media which have been grown by *Pityrosporum ovale* bacteria with the concentration of 50%,75%, 95%
Measurement Device	Calibrated beaker
Measurement Result	Diameter of inhibiting zone
Measurement Scale	Ratio

Research Design

This research used experimental laboratory with sensitivity test method (Disc Diffusion Method).

Research Time

The research was conducted in April, 2019.

Research Samples

The research samples were bay leaves and kaffir lime leaves obtained from the house yards at Diski, Sunggal Sub-district, Deli Serdang Regency.

Equipment and Ingredients

The equipment and ingredients used in this research were as follows:

Equipment:

7 (seven) Petri dishes, test tube, filter paper, knife, calibrated beaker, autoclave, incubator, disc paper, cotton swab, pin set, sterile cotton, vernier calipers, drip pipette, Erlenmeyer flask, analytic scales, reaction tube rack,

Ingredients:

Bay leaves *(szygium polyanthum),* kaffir lime leaves *(citrus hystrix),* fungi *(pityrosporum ovale)* ethanol 70%, technical ethanol 96%, miconazole, alcohol, aquadest, NaCl physiologies, spirituous content, sabouraud dextrose agar (SDA) media, plastic wrap, aluminum foil.

Method of Gathering Data

Preparation Stage

- Equipment and ingredients were firstly prepared and sterilized in an oven at the temperature of 40^0C -70^0C in ± 2 hours;
- In this stage of making the extract of bay leaves and kaffir lime leaves, they were cleaned up with clean water until they were clean completely;
- They were then sliced into small parts and blended in a blender.

Making Concentration

The extract of bay leaves and kaffir leaves which ethanol had been steamed was divided into 3 (three) percentages of concentration (50%), 75%, and 95%) by dissolving it by 96% of technical ethanol since there was essential oil content in it.

- For the concentration of 50% : 5 grams of extract + 10 ml of 96% technical ethanol;
- For the concentration of 75% : 7.5 grams of extract + 10 ml of 96% technical ethanol;
- For the concentration of 95% : 9.5 grams of extract + 10 ml of 96% technical ethanol.

Making the Extract of Bay Leaves and Kaffir Lime Leaves

Making the extract was done by using maceration method. Bay leaves and kaffir lime leaves were cleaned up with running water so that they became cleaned completely. They were then drained and dried up. After that, they were refined by using a blender until they became powder which would finally become *simplisia* powder. One kilo of the samples of *simplisia* powder was taken out. It was then dissolved or saturated by using 10 liters of absolute ethanol solvent (96% of ethanol) in each of the *simplisia* powder. The last thing which had to be done was stirring it up in one hour. This process of maceration was done 3 (three) times.

Preparing Ingredients

Bay leaves and kaffir lime leaves were washed cleanly with running water. They were then drained until they were dry. After that, they were put into a drying cabinet in 3 (three) days; and they were then refined by using a blender and sifted until they became fine powder.

Preparing Equipment

The first thing to do was that the equipment was sterilized and then to prepare 7 Petri dishes, test tube, and calibrated beakers by using autoclave at the temperature of 121^0C within 15 minutes while the equipment made of metal was sterilized with incandescent fire in one minute.

Making Sabouraud Dextrose Agar (SDA) Media

65 grams of medium were suspended in one liter of aquades into Erlenmeyer flask and stirred up with hot plate stirrer until they were dissolved. They were then sterilized with autoclave within 15 minutes at the temperature of 121^0C and wait until the temperature was 40^0 to 45^0C, directly poured into Petri dish, and made them cold until they were frozen.

Making *Pityrosporum Ovale* Suspension

The fungi used in this research were obtained from the Pharmacy Laboratory of the University of Sumatera Utara. The *Pityrosporum Ovale* was made by taking one microbe from the breeding place to make it tilted. It was then put into a tube containing physiological NaC1 and stirred up until the whole fungus colony was dissolved in the NaC1.

Diffusion Test of *Pityrosporum Ovale* Fungi

- SDA medium which had been hardened was rubbed equally on the medium surface by using cotton swab containing *Pityrosporum Ovale* fungus suspension;
- One sterilized blank disc was soaked in the concentration of 50% and put it on the medium surface which had been rubbed by *Pityrosporum Ovale* fungi. Treatment was done in the whole concentration with spacing in order to prevent the convergence of inhibitory zone.
- The experiment was repeated 3 (three) times to get the data;
- The whole isolate testing was incubated within 36 to 48 hours at the temperature of 37^0C in incubator.
- After 36 to 48 hours, the inhibitory zone was measured by using vernier calipers.

Table 1 Classification of Inhibitory Response to the Growth of Fungi (Puthera, at. al, 2007)

Diameter of limpid zone	Inhibitory Response to Growth
>2cm	Very Strong
1,6-2 cm	Strong
1-1,5 cm	Moderate
<1 cm	Weak

RESEARCH RESULT

Description of Research Location

This research was conducted in the Microbiology Laboratory of the Faculty of Medicine, Prima Indonesia University, Jalan Belanga No. 1 Ayahanda, Medan, North Sumatera Province.

Phytochemical Screening

The result of phytochemical screening of bay leaves and kaffir lime leaves was presented in the following Table:

Table 1: Result of Phytochemical Screening of Kaffir Lime Leaves *(Citrus Hystrix)*

No	Secondary Metabolite	Reactors	Result
1	Alkaloid	Dragendroff Bouchardat Meyer	+ + +
2	Flavonoid	Mg Powder + Amyl alcohol +HCl p	+
3	Glycoside	Molish + H_2 SO_4	+
4	Saponin	Hot water/shaken	+
5	Tannin	$FeCl_3$	+
6	Triterpenoid/Steroid	Lieberman-Bourchat	+

Table 2: Result of Phytochemical Screening of Bay Leaves *(Syzygium Polyanthum)*

No	Secondary Metabolite	Reactors	Result
1	Alkaloid	Dragendroff Bouchardat Meyer	- - -
2	Flavonoid	Mg Powder + Amyl alcohol +HCl p	-
3	Glycoside	Molish + H_2 SO_4	+
4	Saponin	Hot Wwater/shaken	+
5	Tannin	$FeCl_3$	-
6	Triterpenoid/Steroid	Lieberman-Bourchat	+

Based on the result of phytochemical screening as it was seen in Table 1, it was found that the extract of kaffir lime leaves had chemical content such as alkaloid, flavonoid, glycoside, saponin, tannin, and triterpenoid/steroid .Meanwhile, in Table 2 it was found that the extract of bay leaves had chemical content such as glycoside, saponin, and triterpenoid/steroid.

The Difference in Diameter of Inhibitory Zone in the Extract of Bay Leaves and Kaffir Lime Leaves from the Growth of *Pityrosporum Ovale* Fungi

The anti-fungi effectiveness of the extract of bay leaves and kaffir lime leaves for inhibiting the growth of *Pityrosporum ovale* fungi, using disc diffusion was indicated by the existence of inhibitory zone or limpid zone surrounding disc paper. Inhibitory zone was measured by using vernier calipers. The result of the research,

using the concentration of 50%, 75%, and 95% showed that the inhibitory zone could be seen in the following Picture and Table:

Extract of Bay Leaves

Picture 1: Diameter of inhibitory zone of the extract of bay leaves on the growth of *pityrosporum ovale* fungi

Table 3: Diameter of Inhibitory Zone of *Pityrosporum ovale* Fungi

Concentration	Diameter of Inhibitory Zone (mm)			Mean
	the 1st Petri	the 2nd Petri	the 3rd Petri	
50%	12.55	12.1	10.9	11.85
75%	15.9	14.4	12.8	14.37
95%	17.95	23.1	14.2	18.42
Mean	15.47	16.53	12.63	

From the Table above, it was found that the extract of bay leaves in the concentration from 50% until 95% was undergoing the increase in the diameter of inhibitory zone. The highest diameter of inhibitory zone was in the concentration of 95% (18.42 mm) while the lowest diameter of inhibitory zone was in the concentration of 50% (11.85 mm). When the result of diameter of inhibitory zone and the mean diameter of inhibitory zone were presented in the graph, the result was as follows:

Based on the curve above (Picture 4.3), it could be seen that in the concentration of 50%, the mean diameter of inhibitory zone was 11.85 mm, and it increased in the concentration of 75% at the mean diameter of inhibitory zone of 14.37 mm, and it increased again in the diameter of inhibiting zone in the concentration of 95% (18.42 mm). The diameter of inhibitory zone in positive control was 27.1 mm.

The data were then tested their normality and homogeneity before One Way ANOVA test was used. Normality test was used to find out whether the distributed data were normal or not. Normality test used Shapiro Wilk test with SPSS software 22.0 for windows with the result as follows:

Table 3.1 Result of Normality Test (α = 0.05)

Concentration	P-value	Conclusion
50%	0.510	Normal
75%	0.964	Normal
95%	0.827	Normal

The result of normality test above indicated that the data of inhibitory zone which had been distributed was normal because p-value > α (0.05). The next thing to do was doing homogeneity test to find out the variance of data whether it was homogenous or not. Homogeneity test used Lavene test with SPSS 22.00 software for windows with the result as follows:

Table 3.2 Result of Homogeneity Test (α = 0.05)

Test of Homogeneity of Variances			
Inhibitory Zone			
Levene Statistics	df1	df2	P-value
2.569	2	6	.156

The result of homogeneity test above showed that the variance of homogenous data was at p-value > α (0.156 > 0.05) so that One Way ANOVA test could be done due to the assumption that normality and homogeneity had been fulfilled. One Way ANOVA test was used to find out whether there was the influence of

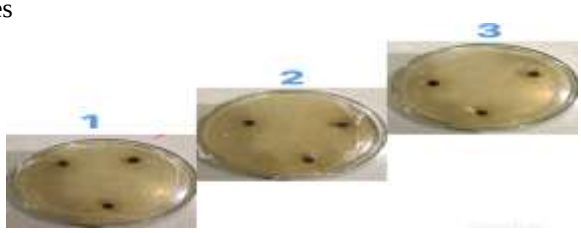
the extract of bay leaves on the growth of *Pityrosporum ovale* fungi. The result of One Way ANOVA test, using SPSS 22.0 software for windows showed in the following Table:

Table 3.3 Result of One Way ANOVA Test (α = 0,05)

ANOVA					
Inhibitory Zone					
	Sum of Squares	df	Mean Square	F	P-value
Between Groups	65.857	2	32.929	4.277	.070
Within Groups	46.193	6	7.699		
Total	112.051	8			

The result *One Way ANOVA* test above indicated that there was no influence of the extract of bay leaves on the growth of *Pityrosporum ovale* fungi. This was indicated by p-value > α (0.070 > 0.05) so that the next Post Hoc test was not continued, and it was concluded that there was no mean difference among the treatments.

Extract of Kaffir Lime Leaves



Picture 2 : Diameter of Inhibitory Zone of the Extract of Kaffir Lime Leaves on the Growth of *Pityrosporum ovale* Fungi

Table 4: Diameter of Inhibitory Zone of *Pityrosporum ovale* Fungi

Concentration	Diameter of Inhibitory Zone (mm)			Mean
	the 1st Petri	the 2nd Petri	the 3rd Petri	
50%	13.9	13.5	15.2	14.20
75%	15.3	14.8	16.1	15.40
95%	16	15.2	17.4	16.20
Mean	15.07	14.50	16.23	

From the Table above, it was found that the extract of kaffir lime leaves in the concentration from 50 until 95% was undergoing the increase in the diameter of inhibitory zone. The highest diameter of inhibitory zone was in the concentration of 95% (16.20 mm) while the lowest diameter of inhibitory zone was in the concentration of 50% (14.20 mm). When the result of diameter of inhibitory zone and the mean diameter of inhibitory zone were presented in the graph, the result was as follows:

Based on the curve above (Picture 4.2), it could be seen that in the concentration of 50%, the mean diameter of inhibitory zone was 14.20 mm, and it increased in the concentration of 75% at the mean diameter of inhibitory zone of 15.40 mm, and it increased again in the diameter of inhibiting zone in the concentration of 95% (16.20 mm). The diameter of inhibitory zone in positive control was 27.10 mm.

The data were then tested their normality and homogeneity before One Way ANOVA test was used. Normality test was used to find out whether the distributed data were normal or not. Normality test used Shapiro Wilk test with SPSS software 22.0 for windows with the result as follows:

Table 4.1 Result of Normality Test (α = 0.05)

Concentration	P-value	Conclusion
50%	0.433	Normal
75%	0.747	Normal
95%	0.702	Normal

The result of normality test above indicated that the data of inhibitory zone which had been distributed was normal because p-value > α (0.05). The next thing to do was doing homogeneity test to find out the variance of data whether it was homogenous or not. Homogeneity test used Lavene test with SPSS 22.00 software for windows with the result as follows:

Table 4.2 Result of Homogeneity Test (α = 0.05)

Test of Homogeneity of Variances			
Inhibitory Zone			
Levene Statistic	df1	df2	P-value
.500	2	6	.630

The result of homogeneity test above showed that the variance of homogenous data was at p-value > α (0.630 > 0.05) so that One Way ANOVA test could be done due to the assumption that normality and homogeneity had been fulfilled. One Way ANOVA test was used to find out whether there was the influence of the extract of kaffir lime leaves on the growth of *Pityrosporum ovale* fungi. The result of One Way ANOVA test, using SPSS 22.0 software for windows showed in the following Table:

Table 4.3 Result of One Way ANOVA Test (α = 0,05)

ANOVA					
Inhibitory Zone					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6.080	2	3.040	3.707	.089
Within Groups	4.920	6	.820		
Total	11.000	8			

The result of One Way ANOVA test above indicated that there was no influence of the extract of kaffir lime leaves on the growth of *Pityrosporum ovale* fungi. This was indicated by the fact that p-value > α (0.089 > 0.05) so that the next Post Hoc test was not continued, and it could be concluded that there was no mean difference among the treatments.

DISCUSSION

Based on the result of the research, it was found that the extract of bay leaves and kaffir lime leaves had the effectiveness of anti-fungi on the growth of *Pityrosporum ovale* fungi which was indicated by the establishment of inhibitory zone or limpid zone surrounding disc paper. The diameter of inhibitory zone was measured by using vernier calipers in order to find out the power of anti-fungi. The concentration consisted of 50%, 75%, and 95%.

The result of the research, based on the classification of Greenwood inhibitory zone, showed that the extract of bay leaves had the effectiveness of anti-fungi in inhibiting the growth of *Pityrosporum ovale* fungi with the concentration of 50%, 75%, and 95% at the mean diameter of inhibitory zone of 11.85 mm, 14.37 mm, and 18.42 mm respectively. Meanwhile, the extract of kaffir lime leaves had the stronger effectiveness in inhibiting the growth of *Pityrosporum ovale* fungi with the mean diameter of its inhibitory zone of 27.1 mm. Its negative control, using aquadest, did not have any inhibitory zone in *Pityrosporum ovale* fungi.

The result of phytochemical screening showed that the extract of bay leaves contains chemical compound such as flavonoid, tannin, glycoside, saponin, and triterpenoid/steroid.

Flavonoid content in bay leaves provides antioxidant in bay leaves. Phenol compound is the main and abundant antioxidant found in the extract of bay leaves. Flavonoid in bay leaves also plays an important role as anti-bacteria since it is able to interact with DNA bacterium. The interaction can cause the damage in permeability of bacterium cell wall. The content of active compound such as tannin, flavonoid, and essential oil which consist of eugenol and citral provide anti-bacteria in bay leaves. The content of active compound can inhibit the growth streptococcus sp. in oral cavity and can inhibit the growth of Candida albicans. Tannin which is phenol compound works by inhibiting the growth of bacteria by doing denaturation of protein and decreasing surface tension so that there is the increase in bacterium permeability. The process causes cell growth to be inhibited and can cause the death of bacterium cells.

Intan Fajar Ningtias, et. al, point out that medicine for decreasing uric acid which is often used as xanthenes oxidation inhibitor is allopurinol. Herbal medicines are used by many people as anti-hyperuricemia, and one of these herbal medicines is bay leaf plant (*Syzygium polyanthum Wight*). Besides that, to handle uric

acid, bay leaves can also be used as the medicine for high cholesterol, diabetes mellitus, hypertension, gastritis, and diarrhea. Bay leaf plant contains tannin, flavonoid, alkaloid, and essential oil which consist of citrate and eugenol. It is able to increase urine production (diuretic) so that it can decrease uric acid content. A research points out that the extract of bay Leaves can decrease IL-6 content and TNF-α serum in patients with hyperuricemia which is indicated by the decrease in uric acid more than allopurinol.

The extract of kaffir lime leaves contains chemical compound such as alkaloid, flavonoid, glycoside, saponin, tannin, and tritterpenoid/steroid in which the content of saponin is an active compound for strong surface and can cause foam which is shuffled with water. Some saponin works as anti-microbe. Saponin can increase permeability of bacterium cell membrane which causes denaturation of membrane protein so that the cell membrane can be lysis. Tannin in low concentration is able to inhibit the growth of microbes, but in its high concentration it works as anti-microbe by becoming coagulation or agglomerating bacterium protoplasm which establishes stable bond with bacterium protein. In digestive tract, tannin is able to eliminate toxin. Meanwhile, flavonoid has the activity of anti-fungi, anti-virus, and anti-bacteria. Some researches have studied on the correlation between flavonoid structure and anti-bacteria activity. Flavonoid can inhibit the function of cytoplasmic membrane and inhibit energy metabolism.

Esy Maryanti, et. al, point out that the extract of ethanol of kaffir lime leaves has the effect of larvicide on *Aedes aegypti* larvae. The concentration of ethanol extract of kaffir lime leaves is needed to kill 50% of the population of *Aedes aegypti* larvae.

CONCLUSION

- Extract of bay leaves (*Syzygium Polyanthum*) has the effect on inhibiting the growth of *Pityrosporum ovale* fungi at the inhibitory power of 18.42 mm;
- Extract of kaffir lime leaves (*Citrus Hystrix*) has the effect on inhibiting the growth of *Pityrosporum ovale* microbe (fungi) at the inhibitory power of 16.20 mm;
- Its positive control, using miconazole, has the better effect in inhibiting the growth of anti-fungi on *Pityrosporum ovale* at the inhibitory power of 27.1 mm.

SUGGESTION

- The next testing should be done on the effectiveness of the extract of bay leaves and kaffir lime leaves on other fungi or bacteria be done;
- Testing on guinea pig should be done by using the extract of bay leaves and kaffir lime leaves with different cases;
- The next testing on different extracts should be done on *Pityrosporum ovale* fungi.

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