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RESEARCH ARTICLE

Effects of Solvent Composition on Profile of Total Extracts, Phenolic Compounds, and Antifungal Activity of *Sterculia quadrifida* R.Br Bark

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Abstract

Faloak bark extract has antibacterial, antioxidant and anticancer potential but no optimal solvent has found to extract the active compounds of faloak bark. Therefore, it is necessary to optimize the solvent against the faloak bark. This study aims to determine the optimum solvent of methanol, ethyl acetate, petroleum ether, and water in order to produce an optimal response value in the extract, total phenolic, and inhibitory results in antifungal tests. Extracts obtained through reflux extraction for 2 hours and extract yield calculated. The extract results tested to determine the total phenolic content and inhibition of antifungal test. The determination of the optimum solvent composition carried out using Rstudio v.3.2.2 software with ANOVA parametric test or Kruskal-Wallis non-parametric test and using Design Expert v.11 software with Simplex Lattice Design (SLD) method. The results showed that the solvent composition of faloak bark extract (Sterculia quadrifida R.Br) in giving the yield of extract, total phenolic, and optimal antifungal inhibition was methanol: water 0,438:0,562.

Keywords: *Sterculia quadrifida R.Br; Optimization; Total Phenolic; Antifungal.*

Introduction

Faloak (*Sterculia quadrifida* R.Br) is an Indonesia's native plant has been used to treat hepatitis, gastroenteritis, diabetes, and rheumatoid arthritis in Nusa Tenggara Timur (NTT) [1]. Scientifically, a study was conducted by Rollando (2016) showed that the ethanolic extract if faloak bark has antibacterial activities on *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, and *Candida albicans*.

Moreover, the extract also showed an antioxidant activity as high as the ascorbic acid [2]. Nevertheless, the extraction that was done through maceration with ethanol gave a small yield causing an ineffective use of plant material [3]. Extraction is separation process of some targeted substances from a mixture by using an appropriate solvent.

The effectiveness of an extraction process is determined by several factors, mainly the particle size of the plant material [4]. A fine pulverized plant material allows an extensive contact with the solvent producing a more effective separation process [5].

The presence of heat also plays a role in determining the separation process [6]. Reflux is an extraction method that can be done by heating the mixture of plant material and solvent on its boiling point inside a flask that is connected to a condenser. The method can produce a higher yield of extract in a relatively short time [7]. The extract contains a lot of active substances, including phenolic substance. Phenol is one of the plant's secondary metabolite that has a function as a

self-protection agent through antimicrobial activity [2].

Phenol derivative compounds can interact with fungal cell through a hydrogen bond mediated adsorption causing a different effect based on the applied active substance concentration. There is a possibility that the active substance content in the extract can be affected by the chosen extraction method and the polarity of the solvent [8]. Thus, a study on the effect of solvent polarity to the yield of active substance needs to be done in order to find the optimal solvent composition for a better extraction process [9].

Method

The research was conducted through an experimental laboratory activity by using a complete randomized design. The research activities consist of an extraction process, determination of the extraction yield, determination of total phenolic content, and screening on antifungal activity.

Materials

The research was done by using oven, blender, micropipette (Socorex), paper dics, waterbath (labo-tech, Heraceus), analytical balance (BP221S, Scaltec SBC 22, BP 160P), Laminar Air Flow cabinet (FARRco), reflux extractor, rotary vacuum evaporator (Junke dan Kunkel), Autoclaft (AC-300AE, Tiyoda Manufacturing Co. Ltd.), UV-VIS spectrophotometer, dan several kinds of glassware (Pyrex). Faloak (*Sterculia quadrifida* R.Br) bark was collected from Kupang, NTT. The solvent used in extraction process are methanol (Merck, Germany), ethyl acetate (Merck, Germany), petroleum ether (Merck, Germany).

Chemical reagents used to identify the total phenolic content consist of Folin-Ciocalteu reagent, sodium carbonate, gallic acid, dan sodium chloride. Microbial activity test was conductend by using *Candida albicans* culture, *Sabouraud Dextrose Agar* (SDA), *Saboraud Dxtrose Broth (SDB),* and Ketoconazole (Dexa) as a positive control.

Procedures

Preparation of Plant Material

Plant material used in this research was taken from Faloak tree having a minimum size of 30 cm in diameter.

The barks were washed to remove the impurities, then dried on room temperature. The dryed barks were pulverized by using hammer mill and sieved by 40-60 mesh sieves. After that, the acquired powder was dried on 50°C until the moisture content of the powder is less than 10%.

Extraction

The extraction process of faloak bark was done through reflux method by using several combinations of water, methanol, ethyl acetate, and petroleum ether.

The solvent composition of each formula is shown in table 1. For each formula, as much as 25 grams plant material was extracted by using 250 mL solvent under reflux condition for 2 hours. The reflux product was filtered to separate the filtrate. An evaporation process was conducted by rotary evaporator on 40°C under vacuum condition until a thick extract was formed.

Determination of Total Phenolic Content

Total phenolic content of each extract was determined by using Folin-Ciocalteu reagent and gallic acid as the standard. The test solutions were made by dissolving 4 mg extract in a mixture of 2 mL of methanol, 1 mL of Folin-Ciocalteu reagent (50%), and 4 mL of Na_2CO_3 20%. Then, destilated water was added to the test solution until its volume reach 10 mL.

The final solution was incubated on the room temperature for 20 minutes and followed by an absorbance measurement at 765 nm. The same procedure was done to the standard with a series concentration of 30, 45, 60, 75, and 90 µg/mL.The equation from standard curve was used to calculate the total phenolic content on every extract (Rollando *et al*, 2017).

Antifungal Activity Test

Preparation of Culture Media

Agar medium was made by mixing 6, 5 grams of SDA powder and 100 mL of destiled water. Meanwhile, broth medium was made by adding 3 grams of SDB powder to 100 mL of destiled water. Both mixtures were heated to their boiling point so that a homogenous solution was formed. Sterilization of culture media was done through autoclave method on

121°C temperature and 2 atm pressure for 15 minutes.

Fungal Culture Rejuvenation of Candida albicans

SDA medium was being melted and poured on 3 glass tubes. Then, the tubes were tilted on room temperature to let the media to be solidified. Aseptically, a colony of Candida albicans was inoculated on the surface of the media. The inoculated media were incubated on 37°C for 24 hours.

Preparation of Fungal Broth Culture

The formed colonies on SDA media were taken with inoculating loop and are inoculated in 25 mL of fresh SDB medium. The inoculated medium was incubated on 37°C for 24 hours to get a broth culture suspension. Then, the suspension was diluted to get an absorbance of 0, 39 on spectrophotometer that is equal to 1, 25.10^7 cfu/mL microbial concentration.

Antifungal Activity Test on Candida albicans

Antifungal activity test of the natural extract was done through agar diffusion method by using a paper disc (7 mm diameter) as the carrier. Culture media were prepared by mixing 100 µl of Candida albicans broth culture and 10 mL of melted SDA medium inside a petri disc. The paper discs were soaked in the extract solution (0, 1 g/mL) for 30 minutes. The soaked paper discs were applied on the surface of solidified culture media, then it was incubated on 37°C for 24 hours. A 10% ketoconazole solution was used as the positive control, while a DMSO was used as the negative control. After a 24 hours incubation period, a clear zone around the paper disc indicates an antifungal activity.

Data Analysis

Data analysis was done by using Rstudio v.3.2.2 software. Solvent optimization for obtaining optimal solvent combination was determined through statistical parametric test that was ANOVA method. The response difference between extracts was determined with Tukey HSD method. If the data showed an asymmetric distribution model on normality test, Kruskal-Wallis and Dunn test method was done as an alternative method for the previous parametric test.

Bonferroni method was used to prevent a false positive error during the data interpretation in Dunn test method. The response data used for the optimization process were extract yield, total phenolic content, and antifungal activity from each solvent combination thas is shown in Table 1.

Table 1: Solvent Combination Formula for Extraction Processes

	Water	
Formula 1	100%	
	Methanol	Water
Formula 2	100%	0%
Formula3	50%	50%
	Ethyl Acetate	Water
Formula4	100%	0%
Formula5	50%	50%
	Petroleum Ether	Water
Formula6	100%	0%
Formula7	50%	50%

The most optimal solvent combination was analyzed with 2 factor 2 level simplex lattice design method on Design Expert v.11 software. The combination of solvent was done to increase the solubility of targeted substance and so increasing the extraction process efficiency. The solvent mixture was made by adding a fraction of second solvent (X_2) to a fraction of first solvent (X_1) so that $X_1 + X_2$ equal to 1.

The response data were proceed through equation [$Y = a (X_1) + b (X_2) + ab (X_1) (X_2)$] from the optimization process. Two solvent combinations gave 3 equations that were used to calculate extract concentration from each solvent through antifungal activity test.

Discussion

Extraction processes were done by reflux method with several solvent combinations of methanol, ethyl acetate, petroleum ether, and water for 2 hours. While the plant material-solvent mixture was being heated, the solvent vapor was condensed to its liquid phase to prevent the solvent loss during the extraction process. The low boiling point of the selected solvent, such as: methanol (64°C), ethyl acetate (77°C), and petroleum ether

(36°C), caused faster evaporation and better condensation process. The applied heating process increased the yield of extract since a high temperature of solvent destroy the cell of plant material so increasing the desorption of the active substance from the cell [10]. During the filtration process, the flowing rates of the water-containing combinations were slow due to the relatively high viscosity of water. Therefore, a white filter cloth was used to accelerate the filtration process [11].

The filtration processes were done 3 times for each formula to make sure that filtrate contained no particle. Rotary evaporator was used to thicken the extract so that it could be dried faster by using waterbath. The dried extract was weighted to determine the yield of extraction for each formula. Figure 1 shows that water (100%), methanol (100%), and methanol 50% produced higher yield of extract. The high value of standard deviation for the formulas was caused by a higher yield of extract in the first replication. The higher yield of extract was due to the different plant material used in the first replication of extraction process.

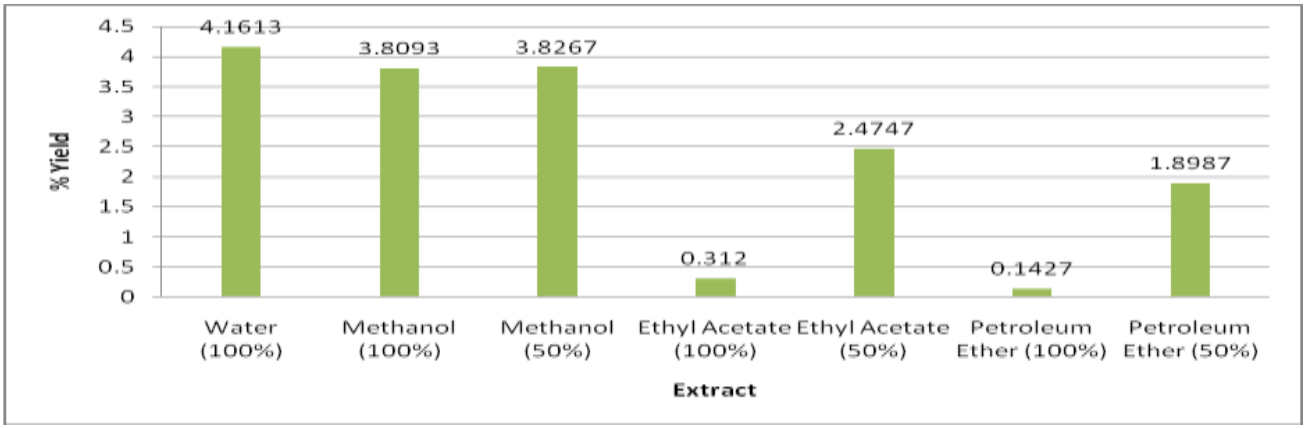

Figure 1: Yield of Faloak Bark Extract for Each Solvent Combination

Table 2: Yield of Faloak Bark Extract for Each Solvent Combination

Sample	Extract Weight (g)	Yield (%)	Standard Deviation
Water (100%)	1.0403	4.1613	±0.08
Methanol (100%)	0.9523	3.8093	±0.13
Methanol (50%)	0.9567	3.8267	±0.07
Ethyl Acetate (100%)	0.0780	0.3120	±0.05
Ethyl Acetate (50%)	0.6187	2.4747	±0.12
Petroleum Ether (100%)	0.0357	0.1427	±0.07
Petroleum Ether (50%)	0.4747	1.8987	±0.42

Table 2 shows that faloak bark (*S. quadrifida* R.Br) contains higher polar secondary metabolites. The yield of extract for each formula was calculated by comparing the weight of the extract with the weight of the plant material that is expressed in percentage. Water as the most polar solvent gave the higher yield of extract (4, 1613%) than pure methanol (3, 8093%). As the polarity of the solvent decrease (water> methanol > ethyl acetate > petroleum ether), the less yield of extract was produced dan vice versa.

This result is identic to the previous study that aqueous extract of faloak bark has 4,68% yield of extract [1]. The water containing formula produced a higher yield of extract compared to others. It is caused by the high content of polar substance inside the plant material that is easier to be extracted by water. Petroleum ether, has lowest polarity, extracted the non-polar substituent producing a little yield of extract (0.1427 %). Meanwhile, ethyl acetate extracted polar and non-polar substance having less yield of extract (0.312%) compared to polar solvent like methanol. The yield of extract shows the amount of substance that could be extracted. The result shows that faloak bark has a dominant amount of polar substance.

Determination of Total Phenolic Content

Phenolic compound works an antifungal agent by destroying the cell membrane, denaturating the protein, shrinking the cell wall, and distrubting the metabolic pathway of the fungi. Phenol is an oxygenated hydrocarbon derivative that has a strong antibacterial activity. Phenol derivative substance can interact with fungal cell

through an adsorption process by hydrogen bond.

At low concentration, phenol forms a complex with protein through a weak bond that degradates immediately. The free phenol penetrates to intracellular environment causing a protein precipitation and denaturation. At high concentration, phenol induces protein coagulation and lysis of cell membrane. Thus, total phenolic content needs to be determined.

The qualitative test was done at first by Folin-Ciocalteu reagent to identify the presence of phenolic compound in the faloak bark extract. Folin-Ciocalteu reagent consists of phophomolibdate acid and molybdenum-tungsten. It detects the presence of phenolic compound through reduction and oxidation reaction under acidic condition. The reaction will produce a blue solution whose intensity is proportional to with its phenolic content.

The total phenolic content was determined by spectrophotometry method with Folin-Ciocalteu reagent. It was expressed as gallic acid equivalent. Table 3 shows the result of absorbance measurement of gallic acid solution after being reacted with Folin-Ciocalteu reagent. The absorbance data were plotted to the concentration series resulting an equation y = 0.0103x + 0.3925 with a correlation coefficient of 0.9704. The correlation coefficient value is higher than the requirement that is 0,9587 (db = 3 and p = 0.01) [12].

Table 3: Gallic Acid Concentration and Its Absorbance Value after Reacting with Folin-Ciocalteu Reagent on 765 nm Wavelength

Gallic Acid Conc. (µg/mL)	Absorbance Value
75	1.0940
60	1.0808
45	0.8864
30	0.7335
15	0.4926

The previous equation was used to calculate the total phenolic content for each extract [13]. Table 4 shows that pure ethyl acetate was able to produce extract with higher phenolic content, that was equivalent to 206 mg of gallic acid per gram of extract. Meanwhile, petroleum ether 50% produced extract with lowest total phenolic content that was equivalent to 8, 27 mg of gallic acid per gram of extract.

Table 4: Total Phenolic Content of Faloak Bark Extract for Each Solvent Combination

Sample	Total Phenolic Content (mg of gallic acid per gram of extract) ± Std. Deviation
Water 100%	112.08 ± 0.40
Methanol 100%	123.94 ± 0.77
Methanol 50%	142.22 ± 0.57
Ethyl Acetate 100%	206.65 ± 0.29
Ethyl Acetate 50%	25.87 ± 0.42
Petroleum Ether 100%	150.75 ± 0.83
Petroleum Ether 50%	8.27 ± 0.88

The data of total phenolic content was visualized in figure 2. It shows that pure ethyl acetate solvent produced an extract whose total phenolic content was higher than others. Standard deviation value of the pure water, pure methanol, dan 50% methanol were high due to higher yield of extract in the first replication. The high yield of extract was caused by the used plant material variation. The first plant material was used up on the first replication, so that a new plant material was used for the other extraction process.

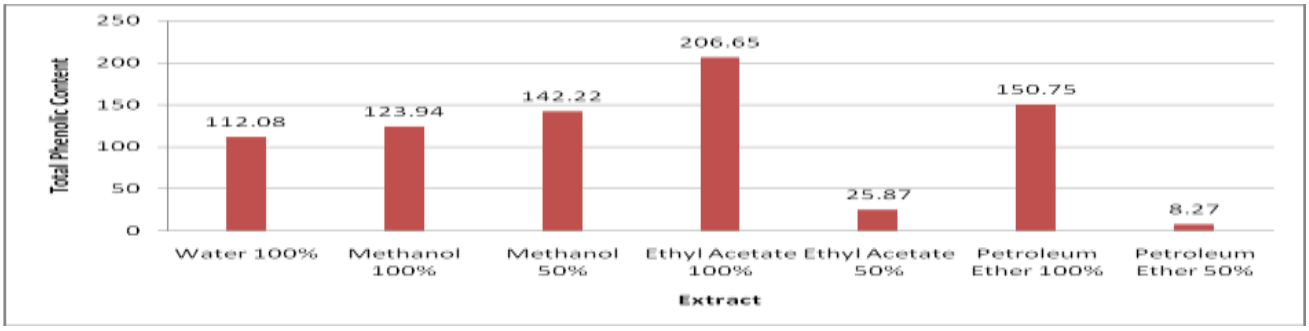

Figure 2: Total Phenolic Content of Faloak Bark Extract Chart

Result of Antifungal Activity Test

Antifungal activity test was done by using disc diffusion method. The samples were prepared by making a series concentration of faloak bark extract solution that consisted of 5.0 mg/mL, 4.0 mg/mL, 3.0 mg/mL, and 2.0 mg/mL extract solutions. A NaCl 0, 9% solution was used as negative control, while the positive control was made by using ketoconazole 10%.

Ketoconazole was chosen since it is a distictive azole antibiotic agent that inhibits cytochrome p450 enzyme activity by converting lanosterol to ergosterol (the main component of fungal cell wall) on *Candida albicans*. Water was used as the sample's solvent due to its absence of antifungal activity. The test was done in 3 replications.

The growth of *Candida albicans* was examined by comparing the SDA medium control and culture medium of *Candida albicans*. The fungi were inoculated to SDA medium by using a cottonbud. The cottonbud was soaked in inoculum, then being drained by pushing the cottonbud head to the reaction tube wall. The drained cottonbud was swabbed to the surface of SDA medium.

The inoculation process was done 3 times to make sure that the fungi had been spread all over the medium surface. Both of control medium and culture medium were incubated for 24 hours. The growth of *C. albicans* was showed by the presence of white colony on the surface of the culture medium. Figure 3 shows the comparation of control medium and culture medium after 24 hours of incubation process.

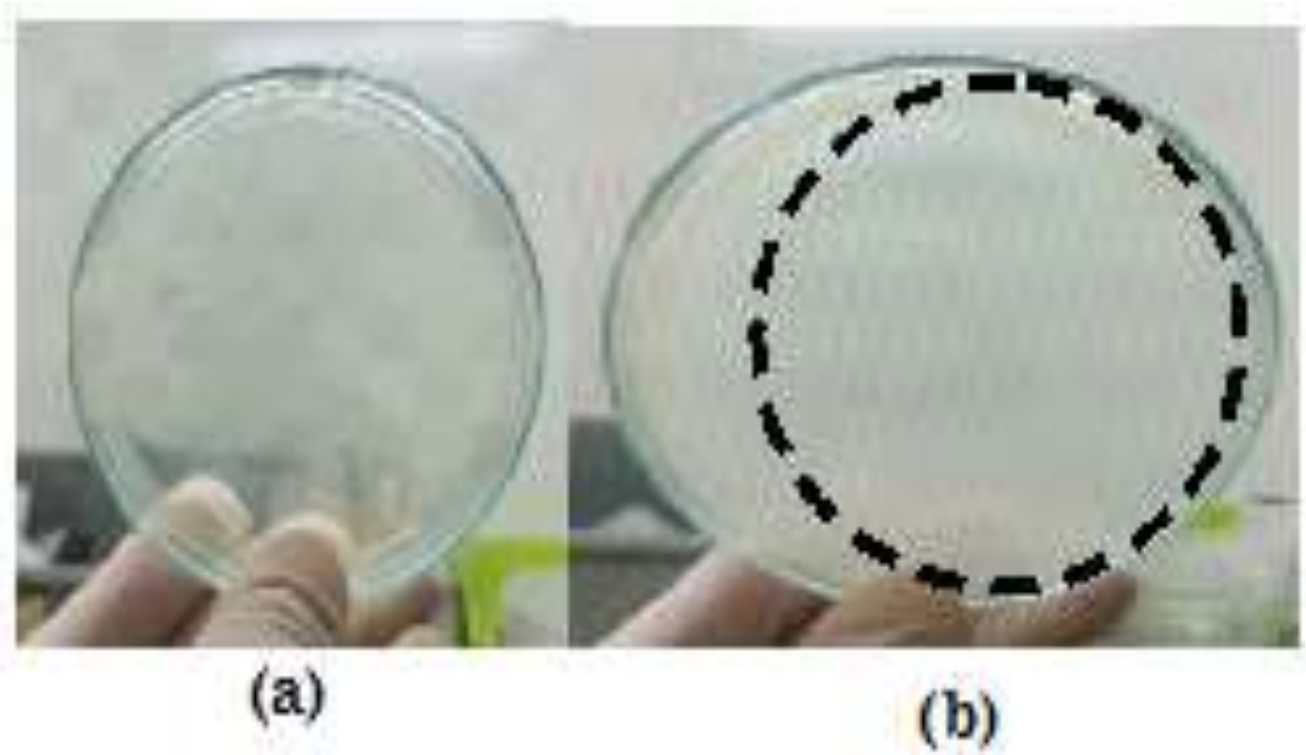

Figure 3: Examination Result on *Candida albicans* Growth: (a) Control Medium; dan (b) Culture Medium of *Candida albicans*

The antifungal activity can be classified as: (1) strong, if the inhibitory zone is 15-20 mm in diameter; (2) intermediate, if the inhibitory zone is 10 – 15 mm in diameter; and (3) weak, if the inhibitory zone is less than 10 mm in diameter (Imani, 2014). Based on those classifications, faloak bark extract has a weak antifungal activity on Candida albicans as shown in table 5. The weak antifungal activity was possibly caused by the presence of water as the solvent of the working solutions.

Water is a good growth medium for microorganisms, including C. albicans, so that reducing the antifungal potency during the test. Besides, the low antifungal activity was suspected due to the low concentration or the low potency of the secondary metabolites in the extract that can inhibit C. albicans's growth. The minimum amount of the effective amount of the secondary metabolite to inhibit fungal growth is still unknown, so that it could not be determined if the amount of secondary metabolite in each faloak bark extract was enough.

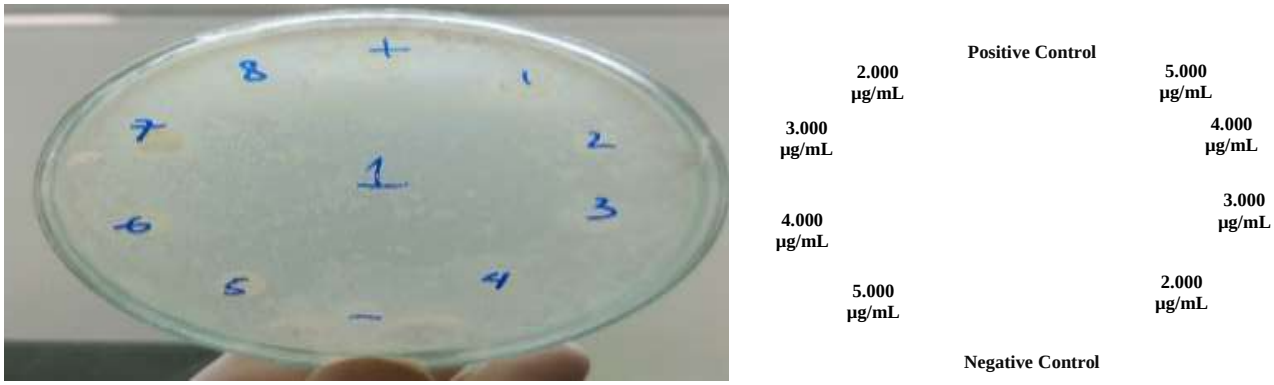

Figure 4: The Result of Antifungal Activity Test through Disc Diffusion Method

The antifungal activity increased as the concentration of the extract solution increased. The sample extracted from pure methanol showed the relationship between extract concentration and antifungal activity. The most concentrate solution (5.0 mg/mL) had a bigger inhibitory zone (0.63 cm) than the 4.0 mg/mL solution that had an inhibitory zone diameter of 0.616 cm and the inhibitory zone would be smaller as the concentration decreased.

Ethanolic extract of bay leaf also had a low antifungal activity with the inhibitory zone diameters of 7, 9, and 11 mm [14]. Meanwhile, the methanolic and ethanolic extract of banana pseudostem had a broad inhibitory zone indicating a strong antifungal activity [15].

Even if it was low, faloak bark extract still has an antifungal activity. The activity was due to the presence of phenolic, terpenoid, and flavonoid compounds. The amount of hydroxyl group on phenolic compounds gave a relative toxicity on the microorganism through an increasing in hydroxylation reaction. The higher its concetration, the higher antifungal activity detected.

Phenolic compound that has a C-3 side chain on the lower oxidation level and has no oxygen is classified as essential oil or be better known as antimicrobial. Flavonoid compounds have an antifungal activity by inhibiting the growth of fungi. It inhibits the diffusion process of nutrient that causes a stoppage on fungal growth or even death. The flavon containing isolate shows that the hydroxyl groups on ring-A have an important role in its antifungal activity [16].

Result of Data Analysis

Faloak bark extracts were tested to determine their yield of extract, total phenolic content, and antifungal activity on *Candida albicans*. The obtained data then being analyzed by ANOVA as parametric test and Kruskal-Wallis as non-parametric test in Rstudio v.3.2.2 software.Based on the nomality test, one of the respon in yield of extract showed an asymmetrical distribution (p-value < 0.05) so that the data could not be proceed through parametric test. Kruskal-Wallis analysis, as the alternative for ANOVA, showed that the yield of extract for each sample was statistically different (p-value < 0.05). Dunn test was done to compare one sample response to others in order to determine their difference significancy.

Based on the Dunn test table, sample from pure water solvent was statistically different from the response of the sample from pure petroleum ether. Besides, the response in the pure petroleum sample was not statistically different with the other samples implying that the optimal yield of extract was obtained from pure water formula.

The normality test, was done in total phenolic content responses, showed that the data were distributed asymmetrically (p-valeu < 0.05). It made the ANOVA method could not be applied. Kruskal-Wallis analysis, as the alternative for ANOVA, showed that the total phenolic content for each sample was statistically different (p-value < 0.05).

The further analysis was done by Dunn test showed that each extract contained statistically not different phenolic content. Thus, the extraction solvent selection did not a significant influence in total phenolic content of the extract. The normality test on antifungal activity responses showed that the data distributed asymmetrically (p-value <

0.05). Therefore, the futher analysis process was done by Kruskal-Wallis method and Dunn test. Kruskal-Wallis test result showed that there was a difference between the detected antifungal activities (p-value < 0.05), while the Dunn test result indicated that those differences were significant.

Based on the Dunn test table, the both samples that were extracted by using methanol had the most optimal antifungal activity. The antifungal activity of both extracts was significantly different from the one that was extracted by using pure petroleum ether (p-value < 0.05).

Meanwhile, the other extracts showed no significant difference to each other. Based on those responses, it could be concluded that methanol was the most optimal solvent for extracting the active substances from faloak bark. Both of pure methanol and methanol 50% were said to be the most optimal extraction solvent due to the statistically difference between their extracts and the other extracts. The antifungal activity was possibly affected by the phenolic residue from the used solvent.

Pure water was the most optimal solvent in producing highest yield of extract. However, the yield of extract from aqueous extraction and methanolic extraction process did not show a statistically different result. The data from pure methanol and methanol 50% were analyzed by using 2 factors 2 levels Simplex Lattice Design method in Design Expert v.11 software to determine the most optimal solvent formula. The solvent combinations that were used in the SLD consisted of methanol and water with a composition of 1:0 (pure methanol); 0.5:0.5 (methanol 50%); and 0.1 (pure water). The responses that were being used as the input to the SLD consist of yield of extract, total phenolic content, and antifungal activity.

The most optimal sample had the closest desirability value to 1. Figure 5 shows that a mixture of methanol-water with a ratio of 0.438:0.562 was the most optimal extraction solvent.Based on that solvent combination, it could predict a point prediction where the yield of extract would be 3.9543% (w/w), the total phenolic content would be equivalent to 120.2 mg of gallic acid per gram of extract, and the inhibitory zone diameter in antifungal activity would be 0.6674 cm.

Table 5: Result of Antifungal Activity Test on *Candida albicans*

Sample	Concentration	Replication (cm)			Average (cm) ± Std. Deviation
		1	2	3	
Water (100%)	5000 µg/mL	0.8	0.6	0.75	0.72 ± 0.10
	4000 µg/mL	0.7	0.6	0.6	0.63 ± 0.06
	3000 µg/mL	0.6	0.6	0.55	0.58 ± 0.03
	2000 µg/mL	0.6	0.6	0.55	0.58 ± 0.03
Methanol (100%)	5000 µg/mL	0.6	0.7	0.6	0.63 ± 0.06
	4000 µg/mL	0.6	0.65	0.6	0.62 ± 0.03
	3000 µg/mL	0.6	0.65	0.6	0.62 ± 0.03
	2000 µg/mL	0.65	0.6	0.6	0.62 ± 0.03
Methanol (50%)	5000 µg/mL	0.8	0.6	0.6	0.67 ± 0.01
	4000 µg/mL	0.7	0.6	0.6	0.63 ± 0.06
	3000 µg/mL	0.6	0.6	0.55	0.58 ± 0.03
	2000 µg/mL	0.6	0.6	0.55	0.58 ± 0.03
Ethyl Acetate (100%)	5000 µg/mL	0.7	0.7	0.65	0.68 ± 0.03
	4000 µg/mL	0.7	0.65	0.6	0.65 ± 0.05
	3000 µg/mL	0.65	0.6	0.6	0.62 ± 0.03
	2000 µg/mL	0.65	0.6	0.6	0.62 ± 0.03
Ethyl Acetate (50%)	5000 µg/mL	0.65	0.65	0.6	0.63 ± 0.03
	4000 µg/mL	0.6	0.65	0.6	0.62 ± 0.03
	3000 µg/mL	0.6	0.6	0.55	0.58 ± 0.03
	2000 µg/mL	0.6	0.6	0.55	0.58 ± 0.03
Petroleum Ether	5000 µg/mL	0.55	0.55	0.55	0.55 ± 0.00
(100%)	4000 µg/mL	-	0.55	-	0.55 ± 0.00
	3000 µg/mL	0.55	0.55	-	0.55 ± 0.00
	2000 µg/mL	-	0.55	-	0.55 ± 0.00
Petroleum Ether	5000 µg/mL	0.55	0.6	0.6	0.58 ± 0.03
(50%)	4000 µg/mL	0.6	0.6	0.6	0.60 ± 0.00
	3000 µg/mL	0.6	0.55	0.6	0.58 ± 0.03
	2000 µg/mL	0.55	0.55	0.6	0.57 ± 0.03
Control +	0,1 mg/100mL				0.766667
Control (-)	0,9% NaCl				0.577778

Note: (-) no antifungal activity test conducted

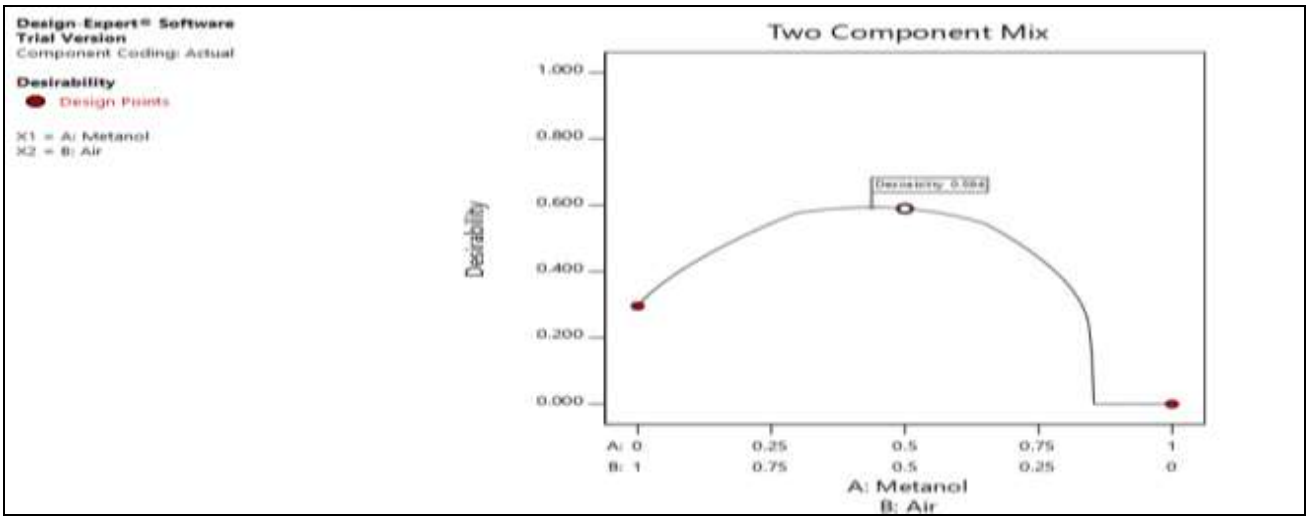

Figure 5: Response Desirability of Metanol-Water Solvent Combination

Conclusion

- The optimal solvent combination formulas for faloak bark that resulting the higher yield of extract, total phenolic content, and antifungal activity were methanol (100%) and methanol (50%).
- The result of 2 factor 2 level Simplex Lattice Design on methanol-water combination showed that a ratio of 0,438:0,562 gives highest desirability value with a predictive yield of extract of 3,9543% w/w; total phenolic content of 120,2 mg of gallic acid aquivalent per grams of extract; and an antifungal inhibitory potency of 0,6674 cm.

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