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Antifungal activity of the extracts and fractions of dahlia tuber (*Dahlia variabilis*) against pathogenic skin fungi

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Abstract. *Saryono, Octarina N, Yuharmen, Pratiwi NW, Ardhi A. 2017. Antifungal activity of the extracts and fractions of dahlia tuber (Dahlia variabilis) against pathogenic skin fungi. Nusantara Bioscience 9: 146-151.* Dahlia plant (*Dahlia variabilis*) can be easily found in the highlands in Indonesia and its tuber has been reported to have some bioactive compounds which are potential to be utilized as an antimicrobial agent. This study aimed to determine antifungal activity and minimum inhibitory concentration (MIC) of n-hexane and methanol extracts and fractions of red-flowered dahlia tubers against pathogenic skin fungi *Candida albicans* and *Microsporum gypseum* using disc diffusion method. The n-hexane and methanol extracts and fractions of dahlia tuber showed their antifungal activity against *C. albicans* and *M. gypseum*, with the larger activity, was found in both extracts and fractions of methanol. The best fractions of n-hexane and methanol were chosen and determined their minimum inhibitory concentration; which the F5 n-hexane fraction and the F2 methanol fraction gave the MIC of 1.50% and 0.50% against *C. albicans* respectively, whereas both F1 n-hexane and F2 methanol fraction gave the MIC of 0.50% against *M. gypseum.* The high antifungal activity of F2 of methanol extract against *C. albicans* and *M. gypseum* allowed this fraction to be utilized as a medicinal drug for candidiasis and other fungal skin infections.

Keywords: Antifungal activities, pathogenic skin fungi, dahlia tuber, minimum inhibitory concentration

INTRODUCTION

The frequency of life-threatening infections caused by pathogenic microorganisms, including fungi, has increased in worldwide and become an important cause of morbidity and mortality in developing countries (Ara et al. 2009). Besides, humid and tropical Indonesian environment is a good environment for the growth and proliferation of fungi. One of the fungi associated with skin disease in human is dermatophyte. This disease leads to serious problems such as dysfunction of the skin if it does not receive good treatments. People who have been infected dermatophytosis cannot be completely healed (Sayuti et al. 2006). Dermatophytosis is often connected with epidemiological circumstances promoting reinfection. For example, *Microsporum canis* commonly indicates a cat as a persistent inoculums source, while *Microsporum gypseum* causing similar lesions, and indicates contact with contaminated soil. In various cases, sources of potentially reinfective inoculums must be dealt within dermatophytosis or else therapy runs a high risk of proving futile (Barisic-Drusko et al. 2003).

An important group of the skin pathogens is fungi, among which dermatophytes and *Candida* spp. are prominent (Fan et al. 2008; de Toledo et al. 2011). Candidiasis is a common infection of the nails, skin, oral cavity, esophagus, and vagina, caused by genus of *Candida*. *Candida albicans* is the most common pathogen causing that fungal opportunistic infection. Additionally, it can adhere to the host surfaces or to prosthesis leading to the formation of biofilms which further facilitate adhesion, infection, and resistance to the antifungals (Parahitiyawa et al. 2006). *C. albicans* infections faces a number of problems including limited number of effective antifungal agents, the toxicity of the available antifungal agents, the resistance of *Candida* to commonly used antifungals, relapse of *Candida* infections and non-cost effective antifungal agents (Sasidharan et al. 2008). As much as 3.6% of *C. albicans* vaginal isolates were found to be resistant to fluconazole, and 16.2% were resistant to itraconazole (Sobel et al. 2003; Richter et al. 2005).

To overcome the fungal infections, synthetic chemicals are used as raw material for antifungal medications. However, although a large number of antimicrobial agents have been discovered, pathogenic microorganisms are constantly developing resistance to these agents (Al-Bari et al. 2006). There are many antifungal drugs which have undesirable side effects such a high toxicity, producing recurrence, showing drug-drug interactions or leading to resistance, and showing ineffectiveness (Muschietti et al. 2005). Therefore it is necessary to search for more effective and less toxic novel antifungal agents that will overcome these disadvantages.

Dahlia (*Dahlia variabilis*) is one of the plants that can be easily found in the highlands in Indonesia. However, people only utilize Dahlia as an ornamental plant. Dahlia is

used as cut flowers while the tubers are discarded into waste. Dahlia tubers are known to contain compounds which have high bioactivity. In some regions in Sumatera with the mountainous topography, for instance, in the province of Sumatera Barat, Sumatera Utara, Bengkulu, and Sumatera Selatan, dahlia can naturally grow. Nevertheless, Dahlia is relatively difficult to find in Kalimantan due to environmental factors affecting the growth of Dahlia. The leaves, stems, tubers of Dahlia, and even secondary metabolites produced by its endophytic microbes contain a number of potential bioactive compounds that can be functioned as anti-microbial compounds (Lorenita et al. 2013; Sikumbang and Hindersah 2009; Saryono et al. 2015a, b). Suryadi (2007) reports that red-flowered dahlia tubers of plants contain a class of flavonoids, phenolics, and saponins which are reported to use as a potential antimicrobial agent. Because less information is known about the antifungal properties of organic extracts prepared from Dahlia, this study was performed to assess the efficacy of the extracts and fractions of dahlia tuber against pathogenic skin fungi. Departing from this point as well, the minimum inhibitory concentration of n-hexane and methanol extract of red-flowered dahlia tubers fractions against *C. albicans* and *M. gypseum* were determined.

MATERIALS AND METHODS

Dahlia and antagonism fungi

Dahlia tubers used in this research were harvested from red-flowered dahlia (*Dahlia variabilis*) with the age of 1-year old. The sample was taken from Payakumbuh, Province of Sumatera Barat. To confirm its antifungal activities, two antagonism fungi were used. *Candida albicans* (associated with vaginal discharge) and *Microsporum gypseum* (associated with tinea versicolor) were obtained from Microbiology Laboratory of the School of Pharmacy Institut Teknologi Bandung (ITB), Bandung. The fungi were prepared in Potato Dextrose Agar medium until used for the step of antifungal activities test.

Extraction of bioactive compound from dahlia tubers

Red-flowered dahlia tubers were treated with cleaning, cutting into pieces, sun-drying, and grinding to a powder. As much as 1,8 kg of dried dahlia tubers in the form of a fine powder was macerated with n-hexane for ± 24 hours and continued with ultrasonication. The extract was collected and evaporated with a rotary evaporator to obtain a concentrated n-hexane extract. The residue from the extraction process with n-hexane was macerated one more time, in the same way, using methanol, which was a more polar solvent. The extract was evaporated with a rotary evaporator to obtain a concentrated methanol extract.

Fractionation of n-hexane and methanol extract using Vacuum Liquid Chromatography

A column of vacuum liquid chromatography was created using silica gel 60 GF_{254} (230-400 mesh) without solvent wetted or made in a dry state. Vacuum method was selected to attempt the maximum packing density of silica gel. The ratio of sample with silica gel used was 1: 5. Extract with antifungal activity was separated first through the process of preadsorbsion. Approximately 3-4 g preadsorbed samples were incorporated into a silica gel column. Separation began by adding 150 mL of eluent with the lowest polarity; ranging from 100% n-hexane, n-hexane: ethyl acetate, up to ethyl acetate: methanol (5: 5) for n-hexane extract. As for the methanol extract, the ratio of eluent used was ranging from n-hexane: ethyl acetate (5: 5), ethyl acetate: methanol, up to 100% methanol. The results from the separation process were accommodated in numbered erlenmeyers and evaporated.

Verification of Vacuum Liquid Chromatography separation result using Thin Layer Chromatography

A plate that had been spotted with separation result of vacuum liquid chromatography was inserted into a developing vessel, saturated with a mixed eluent of hexane: ethyl acetate (7: 3), ethyl acetate: methanol (9: 1), and ethyl acetate: methanol (5: 5). The eluent was propagated to the upper plate boundary in accordance with the force of capillarity. Stains on the plate were calibrated with UV spectrophotometry at λ = 254/366 nm and sprayed with stain marker cerium sulfate. Rf of each stain appeared was measured, the Rf with almost the same value was combined into one fraction.

Antifungal activities confirmation of extract and fractions of n-hexane and methanol extract of Dahlia tubers

As much as 2 mL antagonism fungi suspension in peptone water media was inserted into petridish and added with sterilized Potato Dextrose Agar (PDA). After solidified, a paper disc with a diameter of 6 mm was laid above the surface of the medium. Each n-hexane and methanol extract was absorbed in a paper disc with a concentration of 2%, 4%, 6%, 8%, and 10% (w/v) by dissolving the extract in absolute ethanol. While each fraction of n-hexane and methanol dissolved in absolute ethanol at a concentration of 2% w/v. Paper disc that has been absorbing absolute ethanol was used as a negative control. Petridish were incubated for 48 hours at 35-37ºC in a reverse way so that moisture did not fall into the media. Clear zone appeared around the paper disc indicated a positive result. The diameter of clear zone obtained was measured using a caliper and compared with the standard compound of 2% ketoconazole as a positive control. The test was carried with three replications. The fraction with the highest activity was used for determination of minimum inhibitory concentration.

Determination of Minimum Inhibitory Concentration of active fractions against *Candida albicans* and *Microsporum gypseum*

Determination of minimum inhibitory concentration was performed on the fraction of n-hexane and methanol yielded from vacuum liquid chromatography which exhibited the highest activity against the antagonism fungi. As much as 2 mL suspension of the fungi in peptone water

media was inserted into petridish and added with 15 mL sterilized Potato Dextrose Agar medium. A series concentration of 0.5%; 0.75%; 1.0%; 1.25%; 1.5%; and 1.75% w/v was prepared by dissolving the fraction with absolute ethanol. Afterward, the sample was adsorbed in a paper disc with a diameter of 6 mm. After getting dried, the paper disc was placed in a petri dish that contained antagonism fungal culture. Ketoconazole was used as a positive control at a concentration of 2% w/v and paper disc adsorbed with absolute ethanol was used as a negative control. Petridish were incubated for 48 hours at 35-37ºC in a reverse way. Clear zone appeared around the paper disc was measured using a caliper. The test result indicated the minimum concentration of the fraction which could inhibit the fungal growth.

RESULTS AND DISCUSSION

Results

Extraction began with maceration using n-hexane, it was intended that nonpolar compounds contained in the sample could dissolve well. The residue of extraction with n-hexane solvent was macerated back with methanol which was a more polar solvent. Extraction using n-hexane produced 56.80 g of extract or equal to 3.16%, whereas methanolic extraction produced 234.04 g or equal to 13.00%. Test of thin layer chromatography (TLC) was made to n-hexane and methanol extracts of dahlia tuber to determine the most effective eluent ratio which was suitable to the fractionation process through vacuum liquid chromatography (VLC) regarding the number of compounds presented in the extract. Stains on the TLC plate eluted in the developing vessel were measured under UV light with λ = 254/366 nm and stained with cerium sulfate. The retention factor (R_f) of each extract can be seen in Table 1.

The best eluent which produced more stains were n-hexane: ethyl acetate (7: 3) for n-hexane extract and ethyl acetate: methanol (95: 15) for methanol extract. This eluent ratio was then used in the TLC verification of fractionation process using VLC. TLC verification showed that the fractionation of n-hexane and methanol extract using VLC yielded six fractions obtained for n-hexane and six fractions of methanol (Table 2).

It was noticed that the total extract of n-hexane and methanol were able to inhibit the growth of *C. albicans* and *M. gypseum*. Nevertheless, the ability of n-hexane and methanol extract to inhibit the growth of both fungi was still not comparable to the positive control. It can be seen from the size of the diameter of growth inhibition (Figure 1 and Table 3).

At a concentration of 2% w/v, n-hexane and methanol extract could inhibit the growth of *Candida albicans* with a diameter of inhibition of (7.33 ± 0.58) mm (8.83 ± 0.29) mm respectively. The results of data analysis also showed that the extract of n-hexane and methanol showed the smaller diameter of inhibition and significantly different ($p<0.05$) from positive control. Meanwhile, at the same concentration, the methanol extract was able to inhibit the growth of *Microsporum gypseum* with a diameter of inhibition of (7.50 ± 0.00) mm which was not showed by the extract of n-hexane. Furthermore, at a concentration of 10% w/v, methanol extract had the highest antifungal activity with a diameter of inhibition of (11.33 ± 0.29) mm. On the next step, the antifungal activity of n-hexane and methanol fractions was performed at a concentration of 2% w/v. The highest antifungal activity of n-hexane and methanol fractions were picked to the determination of minimum inhibitory concentration against both antagonism fungi.

Fraction of n-hexane and methanol which showed the highest antifungal activity (F5 and F1 n-hexane fractions and F2 methanol fraction) were tested in various concentrations of 0.5%; 0.75%; 1.0%; 1.25%; 1.5%; and 1.75% (w/v) to obtain the minimum inhibitory concentration of each fraction. The result was analyzed using Duncan's multiple range test result as shown in Table 5.

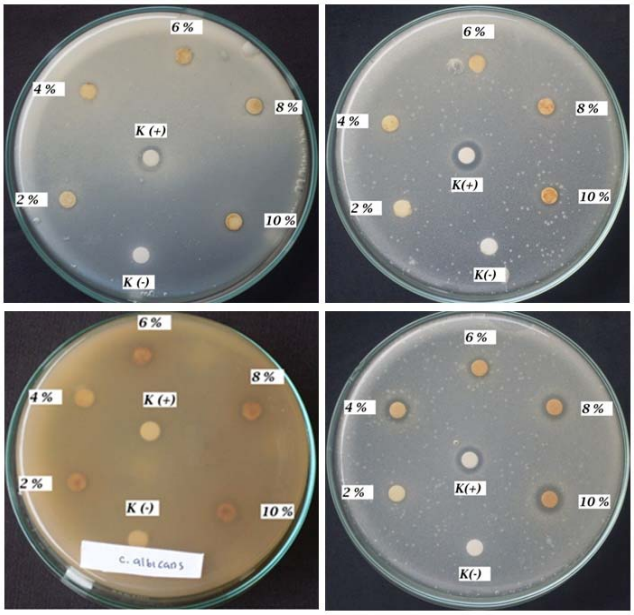

Figure 1. Clear zone of inhibition formed by n-hexane extract against *C. albicans* (A) and *M. gypseum* (B) and methanol extract against *C. albicans* (C) and *M. gypseum* (D)

Table1. Stains and retention factors obtained from TLC of n-hexane and methanol extract

No	Extract	Eluent	Number of stain	R_f
1	n-hexane	n-hexane: ethyl acetate (7: 3)	3	R_{f1}= 0.22 R_{f2}= 0.4 R_{f3}= 0.64
2		n-hexane: ethyl acetate (8: 2)	2	R_{f1} = 0.26 R_{f2}= 0.6
3	methanol	ethyl acetate: methanol (95: 15)	3	R_{f1}= 0.05 R_{f2}= 0.7 R_{f3}= 0.83
4		ethyl acetate: methanol (85: 25)	2	R_{f1}= 0.68 R_{f2}= 0.78

Table 2. TLC verification of n-hexane and methanol fractions

Extract	No. fraction	Number of Stains	R_f
n-hexane	F_1	1 stain	$R_f = 0.8$
	F_2	1 stain	$R_f = 0.74$
	F_3	1 stain	$R_f = 0.67$
	F_4	1 stain	$R_f = 0.48$
	F_5	1 stain	$R_f = 0.34$
	F_6	1 stain	$R_f = 0.12$
Methanol	F_1	2 stains	$R_{f1} = 0.76$ $R_{f2} = 0.6$
	F_2	2 stains	$R_{f1} = 0.76$ $R_{f2} = 0.6$
	F_3	4 stains	$R_{f1} = 0.76$ $R_{f1} = 0.6$ $R_{f3} = 0.46$ $R_{f4} = 0.2$
	F_4	3 stains	$R_{f1} = 0.6$ $R_{f2} = 0.46$ $R_{f3} = 0.2$
	F_5	stain did not move	$R_{f1} = -$
	F_6	stain did not move	$R_{f1} = -$

Table 3. A series concentration of n-hexane and methanol extract of dahlia tuber applied to inhibit the growth of *C. albicans* and *M. gypseum*

Concentration	Diameter of Inhibition Zone against *C. albicans* (mm)		Diameter of Inhibition Zone against *M. gypseum* (mm)	
	n-hexane extract	Methanol extract	n-hexane extract	Methanol extract
2%	$(7.33 \pm 0.57)^{g}$	$(8.83 \pm 0.29)^{cd}$	$(0.00 \pm 0.00)^{h}$	$(7.50 \pm 0.00)^{g}$
4%	$(7.50 \pm 0.00)^{g}$	$(8.50 \pm 0.50)^{de}$	$(8.00 \pm 0.00)^{f}$	$(9.00 \pm 0.00)^{cd}$
6%	$(7.50 \pm 0.00)^{g}$	$(9.00 \pm 0.00)^{cd}$	$(8.00 \pm 0.00)^{f}$	$(9.00 \pm 0.00)^{cd}$
8%	$(7.83 \pm 0.29)^{fg}$	$(9.33 \pm 0.58)^{bc}$	$(8.83 \pm 0.29)^{e}$	$(10.17 \pm 0.29)^{c}$
10%	$(8.17 \pm 0.29)^{ef}$	$(9.67 \pm 0.58)^{b}$	$(9.33 \pm 0.58)^{d}$	$(11.33 \pm 0.29)^{a}$
Control (+)	$(9.67 \pm 0.29)^{b}$	$(12.00 \pm 0.00)^{a}$	$(11.67 \pm 0.58)^{a}$	$(10.83 \pm 0.29)^{b}$
Control (-)	$(0.00 \pm 0.00)^{h}$	$(0.00 \pm 0.00)^{h}$	$(0.00 \pm 0.00)^{h}$	$(0.00 \pm 0.00)^{h}$

Note: Statistical analysis at 95% confidence level with same letters indicating no significant difference

Table 4. Antifungal activities of n-hexane and methanol fractions against *C. albicans* and *M. gypseum*

Fractions	Diameter of Inhibition Zone against *C. albicans* (mm)		Diameter of Inhibition Zone against *M. gypseum* (mm)	
	n-hexane fractions	Methanol fractions	n-hexane fractions	Methanol fractions
F1	$(7.33 \pm 0.29)^{ef}$	$(10.50 \pm 2.18)^{bc}$	**$(10.67 \pm 1.15)^{c}$**	$(10.67 \pm 1.15)^{c}$
F2	$(6.83 \pm 0.29)^{f}$	**$(17.00 \pm 2.29)^{a}$**	$(9.17 \pm 0.29)^{de}$	**$(22.00 \pm 1.73)^{a}$**
F3	$(6.67 \pm 0.29)^{f}$	$(7.67 \pm 0.29)^{ef}$	$(8.83 \pm 0.39)^{de}$	$(7.50 \pm 0.00)^{f}$
F4	$(9.17 \pm 0.29)^{cde}$	$(11.17 \pm 1.89)^{bc}$	$(8.50 \pm 0.50)^{ef}$	$(14.83 \pm 0.29)^{b}$
F5	**$(10.00 \pm 0.50)^{bcd}$**	$(7.33 \pm 0.58)^{ef}$	$(8.33 \pm 0.29)^{ef}$	$(8.83 \pm 0.76)^{de}$
F6	$(7.00 \pm 0.00)^{ef}$	$(7.33 \pm 0.29)^{ef}$	$(8.50 \pm 0.00)^{ef}$	$(0.00 \pm 0.00)^{g}$
Control (+)	$(8.17 \pm 0.29)^{def}$	$(11.67 \pm 2.89)^{b}$	$(9.83 \pm 0.29)^{cd}$	$(10.50 \pm 0.87)^{c}$
Control (-)	$(0.00 \pm 0.00)^{g}$	$(0.00 \pm 0.00)^{g}$	$(0.00 \pm 0.00)^{g}$	$(0.00 \pm 0.00)^{g}$

Note: Statistical analysis at 95% confidence level with same letters indicating no significant difference.

Table 5. Minimum inhibitory concentration of n-hexane and methanol fractions against *C. albicans* and *M. gypseum*

Concentration	Diameter of Inhibition Zone against *C. albicans* (mm)		Diameter of Inhibition Zone against *M. gypseum* (mm)	
	F5 n-Hexane Fractions	F2 Methanol Fractions	F1 n-Hexane Fractions	F2 Methanol Fractions
0,50%	$(0.00 \pm 0.00)^{i}$	$(9.00 \pm 0.00)^{fg}$	$(7.50 \pm 0.50)^{g}$	$(12.17 \pm 0.76)^{d}$
0,75%	$(0.00 \pm 0.00)^{i}$	$(9.00 \pm 0.00)^{fg}$	$(7.83 \pm 0.29)^{g}$	$(20.33 \pm 1.53)^{b}$
1,00%	$(0.00 \pm 0.00)^{i}$	$(9.00 \pm 0.00)^{fg}$	$(8.17 \pm 0.29)^{g}$	$(17.33 \pm 2.52)^{c}$
1,25%	$(0.00 \pm 0.00)^{i}$	$(9.00 \pm 0.00)^{fg}$	$(10.00 \pm 1.00)^{f}$	$(20.67 \pm 0.58)^{b}$
1,50%	$(7.00 \pm 0.00)^{h}$	$(9.67 \pm 0.58)^{f}$	$(8.33 \pm 0.29)^{fg}$	$(21.83 \pm 1.61)^{ab}$
1,75%	$(8.33 \pm 0.58)^{g}$	$(10.67 \pm 0.58)^{ef}$	$(10.33 \pm 0.58)^{ef}$	$(23.00 \pm 0.00)^{a}$
Control (+)	$(8.00 \pm 0.00)^{g}$	$(7.00 \pm 0.00)^{h}$	$(11.83 \pm 0.29)^{de}$	$(10.17 \pm 0.29)^{f}$
Control (-)	$(0.00 \pm 0.00)^{i}$	$(0.00 \pm 0.00)^{i}$	$(0.00 \pm 0.00)^{i}$	$(0.00 \pm 0.00)^{i}$

Statistical analysis at 95% confidence level with same letters indicating no significant difference

The antifungal activities of F5 n-hexane and F2 methanol fractions indicated that at concentrations below 1.50%, F5 n-hexane did not inhibit the growth of *C. albicans*, whilst higher antifungal activity was shown by F2 methanol, in which the concentration of 0.50% could inhibit the growth of *C. albicans* with diameter of the inhibition of (9.00 ± 0.00) mm and showed significant differences ($p<0.05$) from F5 n-hexane fractions and the positive control. Against other pathogenic fungi, at concentrations of 0.50%, F1 n-hexane was able to inhibit the growth of *M. gypseum*. Similarly, the F2 methanol fraction showed higher antifungal activity with an average diameter of inhibition (12.17 ± 0.76) mm at the same concentration. These results indicated that antifungal activity of F2 methanol against *M. gypseum* at a concentration of 0.50% was higher and significantly different ($p <0.05$) from F1 n-hexane and the positive control.

Discussion

In order to provide the optimum fractionation process of the extract of dahlia tuber, the most effective eluent might be found out. Based on Table 1, separation processes of the stains were progressing well on the extract of n-hexane and the extract of methanol marked by the emergence of three stains, which successively occurred in the eluent of n-hexane: ethyl acetate (7: 3) and ethyl acetate: methanol (95: 15). The more stains appear, then the separation would be better. Fractionation using VLC was performed to determine the combined fractions of each extract which were separated. The merger of the fractions was based on retention factor. The stains that had almost the same retention factor might be combined into a single fraction. Separation of compounds made by vacuum liquid chromatography method to extract a total of n-hexane and methanol dahlia tubers obtained six fractions of both n-hexane and methanol fractions after being analyzed using thin layer chromatography.

The ability of methanol extract in inhibiting the growth of *C. albicans* was better than the n-hexane extract (Figure 1 and Table 3). The results also showed that the extract of n-hexane and methanol showed the smaller diameter of inhibition and significantly different ($p<0.05$) from positive control. Whereas the methanol extracts showed larger growth inhibition ability than n-hexane extract against *M. gypseum*. The ability of n-hexane and methanol extract of dahlia tuber and their fractions in inhibiting the growth of both pathogenic fungi could be caused by the presence of bioactive compounds such as flavonoids, phenols, and saponins contained in dahlia tubers (Suryadi 2007). Phytochemical constituents such as alkaloids, flavonoids, tannins, steroids, saponins, and terpenoids are secondary metabolites of plants that serve a defense mechanism against prediction by many microorganisms, insects, and other herbivores (Bonjar et al. 2004). In other reports, antifungal activity has been associated with polar compounds such as glycosylated flavonoids and saponins isolated from polar extracts (Kim et al. 2010; Lanzotti et al. 2012), and in non-polar compounds, like terpenoids (Wang et al. 2011; Singh et al. 2011). The activity of phenolic compounds against microorganisms is by degrading lipids in the plasma membrane of microorganisms. The plasma membrane is semi-permeable and serves to control the transport of various metabolites into and out of the cell. Disruption to structures on the plasma membrane will result on inhibiting or impair the ability of the plasma membrane as a barrier osmosis and disrupt a number of processes required for membrane biosynthesis (Pratiwi 2008).

Interestingly, the overall results of antifungal activity test indicated that the methanol extracts and its fraction provided a greater antifungal activity than n-hexane extract and its fraction (Table 4). In general, phenolic compounds and flavonoids are secondary metabolites which belong to polar compounds and to obtain these compounds; the source plant has to be extracted with semipolar up to polar solvents such as chloroform, ethanol, or methanol (Sirait 2007). Pambayun et al. (2007) reported that extracting phenolic compounds using more polar solvent obtained greater flavonoids. Methanol is the most widely used solvent in the process of isolation of organic compounds of natural ingredients as it can dissolve almost all of secondary (polar) metabolites, such as flavonoids (Sulaiman et al. 2011). Beatriz et al. (2012) report that methanol extract of *Psidium guajava* leaf contains flavonoids, coumarins, saponins, sesquiterpene lactones, carbohydrates, and terpenoids while the n-hexane extract of *P. guajava* leaf contains fewer flavonoids, coumarins, sesquiterpene lactones, and terpenoids. Therefore, the result obtained from methanol extraction was more amount than that of from the n-hexane extraction. Therefore, the contents of phenolic compounds and flavonoids contained in extracts and fractions of methanol were more than that those contained in the extract and fraction of n-hexane. Moreover, other result shows that there is a noticeable difference in the antimicrobial activity of the extract of ginger when the methanol is the solvent than the extract when n-hexane is the solvent (Hasan et al. 2012). This was thought to lead to differences in growth inhibition of extracts and fractions of n-hexane and methanol against both pathogenic fungi.

Determination of MIC that has been made to the n-hexane and methanol fraction showed that the lowest concentration (0.50% w/v) shown by F2 methanol fraction gave the diameters of inhibition against *C. albicans* and *M. gypseum* which were larger than positive control. The different behaviors were shown by n-hexane fractions, which could only inhibit the growth of *C. albicans* when the concentration of the F5 n-hexane fraction applied was higher than 1.50%, while the antifungal activity of the F1 n-hexane fraction against *M. gypseum* was shown at a concentration of 0.50%. Anyanwu (2012) reports the MIC values for the honey against some pathogenic fungi, including *C. albicans* and *M. gypseum*, ranges between 12.5 and 50% (v/v). The ability of methanol extract of dahlia tuber has been proved to act as a growth inhibitor for *Escheria coli* and *Staphylococcus aureus* with MIC 0.65% and 0.50% (w/v) respectively (Haryani et al. 2013).

The conclusions obtained were the n-hexane and methanol extracts and fractions of dahlia tuber exhibited their antifungal activity against *C. albicans* and *M. gypseum*, with the larger activity was found in both extracts and fractions of methanol. The best fractions of n-hexane and methanol were chosen and determined their minimum inhibitory concentration; which the F5 n-hexane fraction and the F2 methanol fraction gave the MIC of 1.50% and 0.50% against *C. albicans* respectively, whereas both F1 n-hexane and F2 methanol fraction gave the MIC of 0.50% against *M. gypseum.* This preliminary research gave new information about the potency of the extracts and fractions of n-hexane and methanol extract of dahlia tuber to be utilized as an alternative source for fungal skin infection medication. Further works are needed to investigate and determine the bioactive molecules that play a role as an antifungal agent.

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