# HASIL CEK_Malassezia sp., phenolic compound, water spinach (Ipomoea aquatica) herbs, antidandruff

*by* Damaharyuningtyas, Kintoko, Endang Darmawan Fractionation Of A Phenolic Compound From Water

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# Fractionation of a phenolic compound from water spinach (*Ipomoea aquatica*) herbs as anti-dandruff against *Malassezia sp.*

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## ABSTRACT

Dandruff is a scalp disorder caused by a fungus (*Malassezia sp*.). Water spinach (*Ipomoea aquatica*) contains phenolic compounds which have antifungal activity. The purpose of this research is to know the active fraction of water spinach (*Ipomoea aquatica*) herbs that have an anti-dandruff activity against *Malassezia sp*. The study used a true experimental design for an anti-dandruff activity test. Fractionation of water spinach (*Ipomoea aquatica*) methanol extracts used column chromatography which stationary phase with silica gel 60 powder and mobile phase with chloroform: ethanol: acetyl acetate (8:2:0,1). In vitro anti-dandruff activity based on minimum inhibitory concentration (MIC) and minimum antifungal concentration (MFC) against *Malassezia sp*. of human dandruff isolate. The average of MIC and MFC among groups fraction of water spinach (*Ipomoea aquatica*) methanol extracts compared used Friedman test ($p \leq 0{,}05$). Fractionation produces 12 fractions, and fraction number 7 of water spinach (*Ipomoea aquatica*) herbs have the best anti-dandruff activity against *Malassezia sp*. with MIC 125 $\mu$g/mL and MFC 250 $\mu$g/mL identified as a phenolic compound. Based on the results, a fraction of phenolic compounds from water spinach (*Ipomoea aquatica*) herbs have anti-dandruff activity against *Malassezia sp*.

**Keywords:** *Malassezia sp*., phenolic compound, water spinach (*Ipomoea aquatica*) herbs, antidandruff

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## INTRODUCTION

Dandruff is an inflammatory disorder affecting the hair follicle's sebaceous glands. Prevalence of dandruff a lot of the post-pubertal population, irrespective of ethnicity and gender suffered from dandruff. Along with the discomfort, this illness makes the sufferer feel self-condition in public and lowers their self-esteem. *Malassezia sp*. is the pathogenesis of dandruff (Isaiah & Karthikeyan, 2015; Rudramurthy et al., 2014).

Various natural product extracts have anti-dandruff activities, such as *Camellia sinensis*, *Rosmarinus officinalis*, *Glycyrrhiza glabra*, *Glycyrrhiza inflata*, *Mentha piperita*, *Thymus vulgaris*, *Zingiber officinalis*, *Sesamum indicum*, and *Ipomoea aquatica* (Borda & Wikramanayake, 2015; Chhavi et al., 2011; Schweiger et al., 2013). Plant extracts are evaluated and they can be utilized successfully with chemical agents in a variety anti dandruff formulations. In the present study, it is confirmed that the ethanol or water extracts of water spinach herb (*Ipomoea aquatica*) have antibacterial activity, but methanol extract has a maximum zone of antibacterial activity with MIC 1 mg/mL (Sivaraman et al., 2010). Water spinach (*Ipomoea aquatica*) contains phenolic compounds, such as caffeoyl-quinic acid, N-cis-feruloyl tyramine, N-trans-feruloyl tyramine, 7-O-β-D-glucopyranosyl-dihydroquercetin-3-O-α-D-glucopyranoside, quercetin 3'-methyl ether, and quercetin 4'-methyl ether (Malakar & Choudhury, 2015; Meira et al., 2012). Therefore, this study was to examine the anti-dandruff effects of a phenolic component from water spinach (*Ipomoea aquatica*) herbs fractions.

## MATERIALS AND METHODS

### *Plant material*

Fresh water spinach (*Ipomoea aquatica*) herbs were obtained from Jombor, Piyungan, and Bantul in October 2016. Water spinach (*Ipomoea aquatica*) herbs were adequately washed with water, dried, and ground to a 60-mesh powder.

### *Chemicals*

Chloroform, methanol, and acetyl acetate used for fractionation and column chromatography analysis have grade pro analysis (p.a) from PT. Brataco, Yogyakarta, Indonesia (Millipore®). Chemicals used an anti-dandruff activity from PT. Brataco, Yogyakarta, Indonesia (Sigma Aldrich®) were sodium chloride, penicillin, streptomycin, ketoconazole, sabouraud dextrose agar (SDA), sabouraud dextrose broth (SDB), polyethyleneglycol-400 (PEG 400), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and distilled grade water. Folin – Ciocalteu reagent was used to identify a phenolic compound from PT. Brataco, Yogyakarta, Indonesia (Millipore®).

### *Isolation of Malassezia sp.*

Two people – a man and a woman – from Dr. Kariadi Hospital, Semarang, as dandruff sample subjects. The research protocol was approved by The Ethics Commission of Medicine and Health Sciences Faculty, Yogyakarta Muhammadiyah University, on December 13th, 2016. Skin scraping followed by dissolving of the scales in 10% KOH solution. The dandruff scales of the sample were inoculated into a modified Dixon's agar plate were incubated at 32 $^0$C for 3-4 days. Identification of *Malassezia sp*. with methylene blue staining, urease test, and catalase test (Khosravi et al., 2009; Nakabayashi et al., 2000; Pisal & Mane, 2015).

### *Extraction and fractionation of a bioactive compound*

Water spinach (*Ipomoea aquatica*) herbs powder 20 g macerated with methanol for 24 hours. The extract was filtered through Whatman No.41 filter paper to remove suspended and concentrated under vacuum at 40 $^0$C. Fractionation of crude extract with n-hexane and methanol. After that, the methanol fraction was separated to column chromatography using silica gel 60–120 mesh which has eluted with a linear gradient of chloroform, methanol, and acetyl acetate (8:2:0,1).

All fractions were collected, divided into 12 groups, concentrated, and analyzed by thin layer chromatography (TLC) and tested for their anti-dandruff activity against *Malassezia sp*.

***Anti-dandruff activity***

The anti-dandruff activity was determined as MIC used microdilution with 96-well microplates and MFC used agar diffusion. There was Ketoconazole as a positive control and PEG 400 as a negative control. The microplates were incubated at 37 $^0$C for 24 hours. After that, MTT 25 µl of 2 mg/mL was added to all wells on the 96-well microplates and incubated for three hours. The purple color of MTT formazan crystals indicates the presence of *Malassezia sp*. as live cells, as MIC was obtained in yellow wells indicating no live cell activity. Sample of microdilution scratched in agar to determine the MFC of samples against *Malassezia sp*. (Balouiri et al., 2016; Mapfunde et al., 2016).

***Identification of phenolic compounds***

Colorimetric identification: 500 $\mu$L sample diluted with 1,5 mL Folin – Ciocalteu reagent 17-fold with distilled water and kept at room temperature for 5 minutes before 1,2 mL sodium hydrogen carbonate 75 g/L was added to the mixture. Thin layer chromatography (TLC): 5 $\mu$L of each column fraction was spotted on a silica gel TLC plate 10 × 10 cm. The plates were developed in ascending direction with chloroform, methanol, and acetyl acetate (8:2:0.1) as mobile phase and scanned with $UV_{366}$ (Simonovska et al., 2003).

## RESULTS AND DISCUSSION

***Isolation of Malassezia sp***.

A characteristic white scale is a sign of a fungus that causes dandruff. Modified Dixon's agar plates were used to inoculate the scales (Pisal & Mane, 2015). *Malassezia sp*. colonies of creamy consistency were seen after 5 days of incubation at 32 $^0$Celsius, stained with methylene blue, and viewed with an oil immersion lens. The *Malassezia sp*. bottle cells have characteristic blue coloration (Pisal & Mane, 2015). The isolated fungus showed specific bottle-shaped blue-colored cells that indicated *Malassezia sp*. A drop of 1% percent solution of benzalkonium chloride was used to test for the presence of urease, and a pink coloration was considered to be a positive reaction (Khosravi et al., 2009). A drop of 3 % hydrogen peroxide solution was used to test for the presence of catalase, and the positive reaction was shown by the formation of gas bubbles (Khosravi et al., 2009). The isolated fungus, which has a positive reaction with methylene blue, urease test, and catalase test identified as was Malassezia.

***Fractionation of anti-dandruff compound***

The dry powder from water spinach (*Ipomoea aquatica*) herbs was extracted with methanol. Crude extract of methanol was fractionated using n-hexane and methanol to separate nonpolar compounds. The result fractionation of n-hexane and methanol extract was analyzed with TLC on UV366, which showed in Figure 1. The anti-dandruff activity of the methanol fraction had compared with the n-hexane fraction. The methanol fraction was chosen for column separation because it has anti-dandruff activity better than the n-hexane fraction. The methanol fraction was selected for chromatography analysis on a silica gel column which was eluted with chloroform, methanol, and acetyl acetate (8:2:0,1), compound collected as a fraction, and anti-dandruff activity determined with MIC and MFC. The result was analyzed with TLC on UV366, which showed in Figure 2.

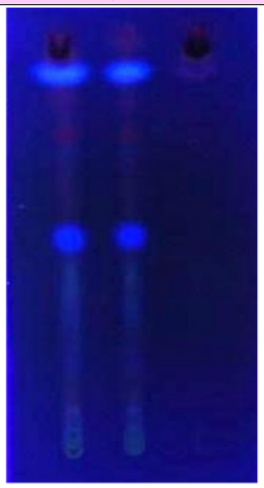


**Figure 1. TLC with mobile phase chloroform, methanol, and acetyl acetate (8:2:0,1), (A) methanol extract, (B) methanol fraction, and (C) n-hexane fraction in $UV_{366}$**

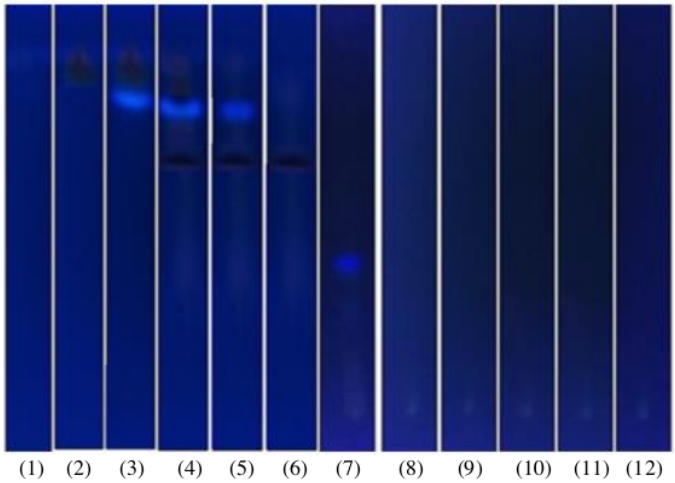


**Figure 2. TLC with mobile phase chloroform/methanol/ acetyl acetate (8/2/0,1 v/v/v) of fraction on $UV_{366}$, point (1) to (12) are number of column fraction**

The fraction of methanol extract from water spinach (*Ipomoea aquatica*) has anti-dandruff activity. Table 1 summarizes of anti-dandruff activities of the extract and fractions. The anti-dandruff activity of fraction 7 has MIC 125 µg/mL and MFC 250 µg/mL better than other fractions. Ketoconazole was used as the control drug and had MIC 10 µg/mL. PEG 400 as an organic solvent did not affect anti-dandruff activity.

**Table 1. Anti-dandruff activities from extract and fraction of *Ipomoea aquatica* herb.**

| Sample | MIC | | MFC | |
|---|---|---|---|---|
| Methanol extract | 4 | mg/mL | 8 | mg/mL |
| Methanol fraction | 2 | mg/mL | 4 | mg/mL |
| n-Hexane fraction | >10 | mg/mL | >10 | mg/mL |
| Fraction 1 | >2 | mg/mL | >2 | mg/mL |
| Fraction 2 | 1 | mg/mL | 2 | mg/mL |
| Fraction 3 | 250 | µg/mL | 500 | µg/mL |
| Fraction 4 | 250 | µg/mL | 500 | µg/mL |
| Fraction 5 | 250 | µg/mL | 500 | µg/mL |
| Fraction 6 | 250 | µg/mL | 500 | µg/mL |
| Fraction 7 | 125 | µg/mL | 250 | µg/mL |
| Fraction 8 | 500 | µg/mL | 1 | mg/mL |
| Fraction 9 | 500 | µg/mL | 1 | mg/mL |
| Fraction 10 | 500 | µg/mL | 1 | mg/mL |
| Fraction 11 | 500 | µg/mL | 1 | mg/mL |
| Fraction 12 | 250 | µg/mL | 500 | µg/mL |

***Identification of bioactive compound***

Identification of a phenolic compound of fraction using a Folin-Ciocalteu reagent reduced to a blue-colored complex in alkaline solution (Robbins, 2003). Phenolics are present in the crude methanol extract, methanol fraction, and fraction 7. Fraction 7 has the best anti-dandruff activity than crude methanol and methanol fractions, increasing blue intensity can improve anti-dandruff activity. TLC screening (Figure 2.G) of *Ipomoea aquatica* herb showed the presence of a phenolic compound. A phenolic compound showed blue fluorescence in $UV_{366}$ in Retention factor (Rf) 0,56 (Simonovska et al., 2003).

## CONCLUSION

A fraction of water spinach (*Ipomoea aquatica*) herb have an anti-dandruff activity against *Malassezia sp*., the anti-dandruff activity of fraction 7 has MIC 125 µg/mL and MFC 250 µg/mL identified as a phenolic compound.

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