



Research articles

Crude extracts of *Magnolia alba* flowers using n-hexane and methanol solvents and their anti-acne potential

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ABSTRACT

This study aimed to test the antibacterial activity of n-hexane and methanol extracts of white cempaka flowers (*Magnolia alba*) against *Propionibacterium acnes* bacteria. The extract was obtained by using the maceration method for 72 hours. Each extract was tested for its antibacterial properties using the disc diffusion method with the variant concentration of the extract used was 0.05%; 2%; 4%; 6%; and 8%. The results showed that the methanol extract had a higher inhibition zone than the n-hexane extract. In n-hexane extract, the lowest concentration of 0.05% showed an inhibition zone of 6.6 mm and the highest concentration of 8% had an inhibition zone of 9.6 mm. Whereas in methanol extract, the inhibition zone obtained was 7.3 mm at a low concentration of 0.05%, but at the highest concentration of 8% the inhibition zone obtained was 11.6 mm. The effect of extract concentration on the diameter of the inhibition zone formed is very significant (directly proportional), where the higher the concentration used, the larger the diameter of the inhibition zone produced. Antibacterial testing for both extracts showed a weak (< 10 mm) to moderate (> 10 mm) bacterial inhibition, which was similar with the inhibition zone obtained from the positive control (11-11.3 mm; moderate bacterial inhibition).

Keywords: *Magnolia alba*, antibacterial, *Propionibacterium acnes*, skin disease, anti-acne.

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INTRODUCTION

Acne is a skin disease that is often experienced by everyone, both teenagers and adults ^[1]. Acne skin problems often occur, although unlike other diseases, they can make a person experience dermatological and psychological problems. ^[2]. Although acne does not cause death, someone who has acne problems usually has a problem with self-confidence ^[1-3]. It was reported that as many as 85% of the human population had acne problems at the age of < 25 years (12-25 years) and 15-20% at the age of > 25 years ^[2]. There are several factors that cause teenagers and adults to experience acne problems, including stimulating natural oil (sebum) activity in the sebaceous glands and hair follicle areas on the face, stress, hormonal activity, irritation due to scratching and bacterial ^[4]. Generally, at the age of teenagers or adults, acne problems can be based on hormonal activity, stress and even bacteria such as *Propionibacterium acnes* bacteria ^[1]. *P. acnes* bacteria are gram-positive bacteria that have the ability to break down triglycerides into fatty acids in the oil glands ^[5]. Bacterial overgrowth can cause inflammation on the skin ^[3, 6]. Hence, before the *P. acnes* bacteria grow more and more on the facial skin, prevention should be done by treating of existing acne.

Currently, there are many problems experienced by people in the process of healing acne, including taking a lot of time and requiring a large amount of money ^[7]. There are several acne treatments that people often do, including using acne medications sold in the market. The availability of anti-acne drugs can be in the form of oral drugs such as antibiotics, creams/gels/ointments containing sulfur, and so on ^[8]. The use of antibiotics or other chemicals can have side effects that can be detrimental to health. Therefore, this research was carried out in order to find anti-acne drugs that are suitable for the skin and utilize local resource plants such as white cempaka flowers (*Magnolia alba*) for acne treatment using natural ingredients. Plant-based drugs are favorable due to their biocompatibility and effective biological activities ^[9, 10].

The use of *M. alba* as a medicinal plant is believed to have antiseptic, anti-inflammatory and antibacterial properties ^[11]. All parts of the *M. alba* plant can be used for the treatment of kidney stones, fever, cough because it can act as anti-inflammatory, antimicrobial and insecticidal ^[12]. There are several chemical compounds with considerable abundance in the essential oil of *M.*

alba including: linalool (85.78%), beta-elemene (3.82%), methyl eugenol (3.59%) and ethyl 2-methylbutyrate (3.14%) [13]. However, currently, research on the treatment of *M. alba* flower acne against *P. acnes* bacteria is still relatively scarce. Thus, this research was conducted to find more information about the use of natural resources of *M. alba* flowers as antibacterial.

MATERIAL AND METHOD

Materials and Bioindicator

Samples of *M. alba* flowers were taken fresh in Baiturrahman District, Banda Aceh. The sample was then identified at the Taxonomy Herbarium Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Syiah Kuala University, Banda Aceh, by Saida Rasnovi, M.Si.

The bioindicator used is the bacteria that causes acne: *Propionibacterium acnes* (ATCC 27853) which was obtained from the Microbiology Laboratory, Faculty of Medicine, Universities Sumatera Utara (USU), Medan, Indonesia.

Chemicals

The chemicals used were n-hexane solvent, ethyl acetate solvent, methanol solvent, aquades, ammonia, reagents for phytochemical tests (Dragendorff, Wagner, Mayer, Lieberman Burchard, FeCl_3), a blank disc, amoxicillin 25 g disc /L, 70% ethanol, 96% ethanol, sterile Lysogeny Broth (LB) 8 g/L and Mueller Hinton Agar (MHA) 38 g/L. All chemicals were analytical grade and purchased from Merck, Selangor, Malaysia.

Phytochemical Screening

The method used in phytochemical testing can be found in phytochemical analysis, Secondary Metabolite Analysis. [14]. Phytochemical testing usually analyzes the content of plant secondary metabolites including alkaloids, flavonoids, terpenoids, steroids and saponins.

Extraction of *M. alba* flower

Fresh *M. alba* flowers weighing 8 kg were cleaned first using running water and dried at room temperature. 2.1 kg of dried *M. alba* flowers were cut into small pieces and macerated using n-hexane solvent for 72 hours. Then filtered to separate the n-hexane filtrate and residue. The dried residue was then macerated again using ethyl acetate solvent for 72 hours, then filtered again to separate the ethyl acetate filtrate and residue. Then the residue was macerated again using methanol for 72 hours and filtered. The filtrate obtained for each solvent was evaporated using a rotary evaporator, in order to obtain n-hexane extract, ethyl acetate extract and methanol extract. Concentrated extracts from each solvent were tested for bacterial activity against *P. acnes* bacteria.

Antibacterial test against *P. acnes*

Antibacterial testing of *M. alba* flower extract against *P. acnes* bacteria was carried out using the disc diffusion method. Bacterial suspension was made by adding 1 to 2 oses of *P. acnes*

(ATCC 27853) bacteria into sterile LB liquid media and incubated for ± 12 hours using a shaker at 100 rpm. The results of bacterial regeneration were diluted with sterile NaCl and the absorbance of the solution turbidity was measured using a spectrophotometer at a wavelength of 600 nm until the OD value reached $0.5 \times 10^9 - 0.8 \times 10^9$ CFU/mL (according to the Mac-Farland rule).

The adjusted bacterial suspension was taken as much as 20 μL to be dripped into MHA media which had solidified in a petri dish and leveled using a spreader. The entire cup was left for a few moments so that the bacteria reached the logarithmic phase. The agar will be placed on a 6 mm diameter disc paper that has been dripped with a test solution with several concentrations of 0.5%; 2%; 4%; 6% and 8% (w/v). The negative control used the appropriate solvent for each extract. Positive control used amoxicillin 25 g/L antibiotic disc. Then the petri dishes were incubated at 37°C for 18 hours.

RESULT AND DISCUSSION

Phytochemical Profile of *M. alba* Flower Extracts

Phytochemical testing was conducted to determine the secondary metabolite compounds from *M. alba* flowers. The compounds tested included alkaloids, steroids, terpenoids, saponins, flavonoids and phenolics (Figure 1). The results of testing for secondary metabolites in each extract can be seen in Table 1 below.

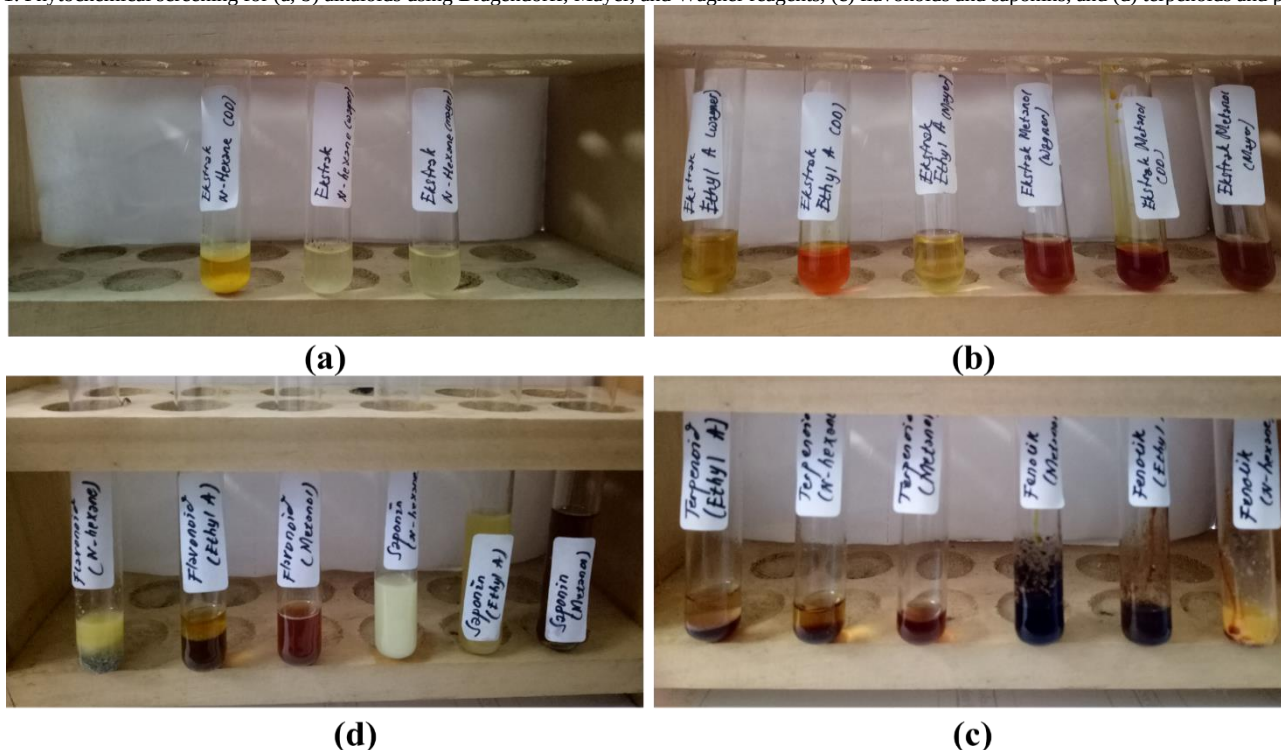
Table 1. Results of phytochemical screening of *M. alba* flower extracts

Metabolite Seconder	Ekstrak, n heksana	Ekstrak Metanol
Alkaloids		
• Mayer	Not detected	Detected
• Dragendorff	Not detected	Detected
• Wagner	Not detected	Detected
Steroids	Detected	Detected
Terpenoids	Detected	Detected
Saponins	Not detected	Detected
Flavonoids	Detected	Detected
Fenols	Detected	Detected

Based on Table 1 above, it can be seen that the n-hexane extract of *M. alba* flower contains secondary metabolites of steroids, flavonoid terpenoids and phenols. The positive test of steroid and terpenoid compounds is the formation of green (steroid) and purple (terpenoid) colors. [15]. Meanwhile, the ethyl acetate and methanol extracts of the positive *M. alba* flower contained secondary metabolites of alkaloids, steroids, terpenoids, saponins, flavonoids and phenols. Positive test of alkaloid compounds formed red precipitate (Dragendorff reagent), white precipitate (Mayer's reagent) and brown precipitate (Wagner's reagent). The positive test for steroid and terpenoid group compounds is the formation of green and red colors. The positive test of saponin compounds is the formation of stable foam for ± 30 minutes. This result is similar to the research conducted previously [16]. While the positive test of flavonoid and phenolic compounds was tested for all extracts (n-hexane, ethyl acetate and methanol) using concentrated HCL solution and Mg powder which produced yellow, orange or red colors. The results obtained for the two extracts are yellow. The

results of the phenolic compound test using FeCl_3 solution were marked by the formation of a purple or blue-black color in each

Figure 1. Phytochemical screening for (a, b) alkaloids using Dragendorff, Mayer, and Wagner reagents, (c) flavonoids and saponins, and (d) terpenoids and phenolics



Antibacterial Activities of *M. alba* Eextracts

Testing the antibacterial activity of *M. alba* flower extract against *P. acnes* bacteria was carried out to determine the ability of each extract to inhibit the growth of these bacteria. The selection of test bacteria bioindicators for *P. acnes* bacteria is that this bacterium is reported to be one of the causes of acne on human skin. Antibacterial activity testing was carried out by agar diffusion method using discs that had been dripped with extract solution in several concentrations before being placed on the test medium. The concentration of the extract solution used in this antibacterial activity test was 0.5%, 2%, 4%, 6% and 8% (w/v). The testing process was repeated three times. The results of the inhibition zone of *M. alba* flower extract against *P. acnes* bacteria can be seen in Figure 2 and the measurement of the diameter of the inhibition zone of each extract can be seen in Table 2 below. The results of the antibacterial activity of the ethyl acetate extract were reported separately.

Table 2. Antibacterial inhibition for n-hexane and methanol extracts from *M. alba* flowers

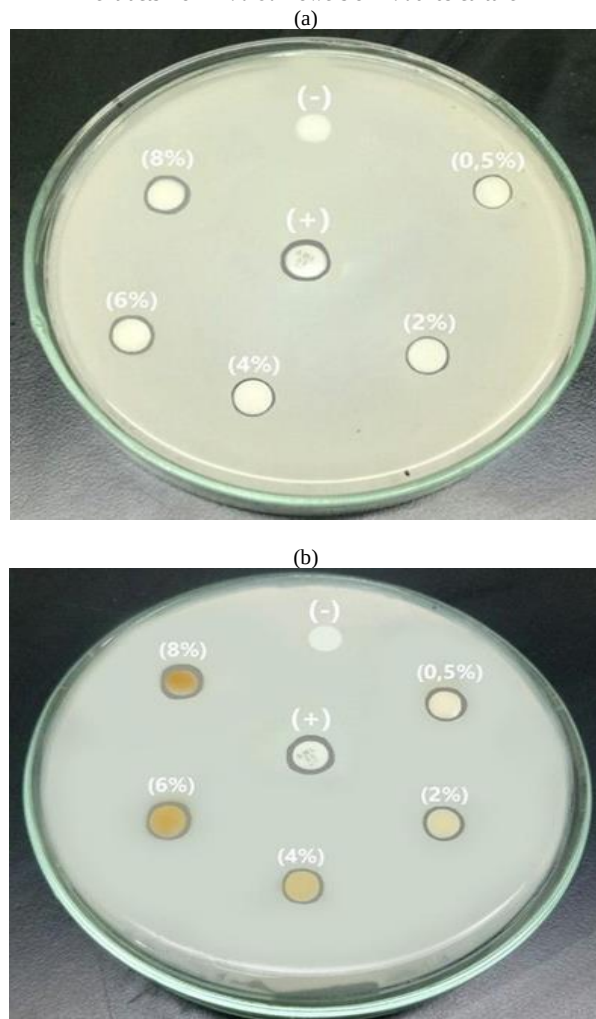
Antibacterial agent	Zona Hambat (mm) $\pm$ SD				
	0.5%	2%	4%	6%	8%
n-hexane extract	6.6 $\pm$ 0.5	7.3 $\pm$ 0.5	7.6 $\pm$ 0.5	8.6 $\pm$ 0.5	9.6 $\pm$ 0.5
Methanol extract	7.3 $\pm$ 0.5	7.6 $\pm$ 0.5	8.6 $\pm$ 0.5	9.6 $\pm$ 0.5	11.6 $\pm$ 0.5
Amoxicillin (25 $\mu\text{g/L}$)	11 $\pm$ 3.1				
Solvent	0.0 $\pm$ 0				

Based on above findings, it can be seen that the

extract.

Anti-bacterial activity against *P. acnes* bacteria for the inhibition zone of each extract is directly proportional to the concentration tested. It is evident that the greater the concentration used, the greater the clear zone formed around the disc. This indicates that the greater the concentration tested; the more active compounds diffuse into the bacterial cell wall. Of the two extracts tested, the n-hexane extract showed the lowest inhibition zone at 0.05% concentration of 6.6 mm and the highest inhibition zone at 8% concentration of 9.6 mm. As for the methanol extract, the lowest inhibition zone was at a concentration of 0.05% of 7.3 mm and the highest inhibition zone was at a concentration of 8% of 11.6 mm. Amoxicillin is one of the antibiotics used as a positive control in testing against several types of bacteria, the resulting inhibition zone is 11-11.3 mm. The negative control used was the solvent of each extract, where the negative control did not show an inhibition zone that affected the growth of bacteria. Of the two extracts that have been tested, the methanol extract showed a slightly higher inhibition zone than the n-hexane extract inhibition zone. The antibacterial activity test can be categorized based on the diameter of the inhibition zone formed, very strong inhibition zone > 20 mm, moderate inhibition zone (16-20 mm), weak inhibition zone (10-15 mm), very weak inhibition zone (< 10 mm). Generally, if the inhibition zone is < 10 mm, it indicates that it is resistant.

Figure 2. Inhibition zone after the exposure of (a) n-hexane and (b) methanol extracts from *M. alba* flowers on *P. acnes* culture



Inhibition of bacterial growth with a diameter of > 20 mm indicates very strong inhibition, if the inhibition zone is around 10-20 mm, it shows moderate inhibition, if the inhibition zone is around 5-10 mm, it shows weak inhibition and if the diameter of the inhibition zone is < 5 showed very weak inhibition [17]. In this study, it was shown that the positive control (amoxicillin) used by the given inhibition zone was still included in the weak category so that in the future it could be replaced with antibiotics that provide strong activity in inhibiting *P. acnes* bacteria. The n-hexane extract showed that from a concentration of 0.05-8% it had a weak zone of inhibition because it had a diameter of < 10 mm. Meanwhile, the methanol extract showed that from a concentration of 0.05-4% it had a weak zone of inhibition category (< 10 mm) and a concentration of 6-8% had a moderate zone of inhibition. For further studies, the application of these anti-acne agents could be further combined using topical drug delivery strategies including cosmetic masks prepared using various biopolymers [18-22] and lotions [23-25].

CONCLUSION

Phytochemical test results showed that the n-hexane

extract contained secondary metabolites of steroids, terpenoids, saponins, flavonoids and phenols. As for the methanol extract of secondary metabolites contained are alkaloids, steroids, terpenoids, flavonoids and phenols. Antibacterial testing for both extracts showed a weak inhibition zone (< 10 mm) for n-hexane extract while for methanol extract it was categorized as a weak inhibition zone < 10 mm (at a concentration of 0.05-4 mm) and moderate at a concentration of 6-8% (> 10mm).

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