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Antibacterial activity of *Magnolia alba* flower extracts on *Staphylococcus epidermidis* and *Staphylococcus aureus*

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Abstract. The white champaca (*Magnolia alba*) plant has been reported possess antioxidant and antimicrobial activity. The aim of this study was to investigate the antibacterial activities of *n*-hexane, ethyl acetate, and methanolic *Magnolia alba* flower extracts on *Staphylococcus epidermidis* and *Staphylococcus aureus*. In this study, we also determined the secondary metabolites of the extracts by the phytochemical screening assay. The antibacterial activity of the *Magnolia alba* flower extracts was determined by the Kirby-Bauer diffusion method. The phytochemical screening assay showed that *n*-hexane extract contained of flavonoids, terpenoids, and steroid, while the ethyl acetate and methanolic extracts contains of alkaloids, flavonoids, terpenoids, and steroid. The antibacterial activity of the *n*-hexane, ethyl acetate, and methanolic *Magnolia alba* flower extracts was determined at four different concentrations of 5, 10, 20, and 50%. Results indicated that *n*-hexane extract had no activity against *Staphylococcus epidermidis* and *Staphylococcus aureus*. Meanwhile, ethyl acetate and methanolic extracts had antibacterial activities against *Staphylococcus epidermidis* and *Staphylococcus aureus*. The diameter zones of inhibition exhibited by the ethyl acetate extract against *Staphylococcus epidermidis* and *Staphylococcus aureus* ranged between 10.45 - 21.03 mm and 10.26 - 26.13 mm respectively. Meanwhile, the diameter zones of inhibition exhibited by the methanolic extract against *Staphylococcus epidermidis* ranged between 11.96 - 18.01 mm and against *Staphylococcus aureus* ranged between 7.23 - 13.9 mm. In conclusion, the ethyl acetate *Magnolia alba* flower extracts gave higher antibacterial activity against *Staphylococcus epidermidis* and *Staphylococcus aureus*.

1. Introduction

Natural antimicrobial substances, especially from plant origin, have attracted much attention in recent years [1]. A lot of efforts have been made to search for novel natural antimicrobials from various plant sources or natural products. Previous researchers revealed that Magnoliaceae family such as *Magnolia champaca* possesses antimicrobial activity [2, 3, 4]. The antimicrobial activities of Magnoliaceae family are due to containing secondary metabolites and their essential oil composition including alkaloids, sesquiterpene and terpenoids [5]. The extract of the bark of *Magnolia champaca* has been known contains sesquiterpene lactone, alkaloids parthenolide, and beta-sitosterol, while the flower and fruit extracts contain essential oil [6, 7]. Nursa'adah reported that *n*-hexane extract of *Magnolia champaca* contains of phenols, terpenoids, and steroids. Although, the antimicrobial activity of *Magnolia*



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champaka has been reported, studies pertaining to the antimicrobial activity of the flowers of white champaka (*Magnolia alba*) are still limited [8].

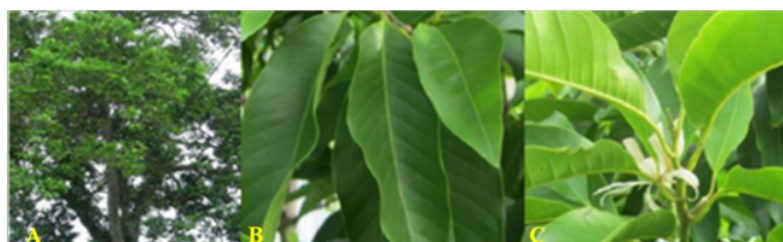


Figure 1. The plant of white champaka (*Magnolia alba*); A: tree; B: leaf; and C: flower

Magnolia alba also known as white champaka belonging to the Magnoliaceae family, have fragrant flowers (Fig. 1). *Magnolia alba* is widely used for medicine, all parts of this plant such as root, bark, leaves and flowers provide various benefits as medicine. The flowers of *Magnolia alba* are used for treatment of headache, sinusitis, cough, inflammation, flatulence, nausea, and vaginal discharge [5]. The flowers and leaves of *Magnolia alba* contain the essential oils that are used to relieve ophthalmic and gout [9]. Hsing mentioned that the extract of *Magnolia alba* leaves contain annonaine, a bioactive benzyloquinoline alkaloid compound. He also showed that, this compound has strong activity in inhibiting the growth of bacteria and fungi [10]. Besides that, Sree showed that *n*-butanol flower extract of *Magnolia alba* has strong antimicrobial activity against *Staphylococcus aureus* by a diameter of the inhibit zone formed was 17 mm [11]. In the present study, we investigated the phytochemical screening and antibacterial activity of various flower extracts (*n*-hexane, ethyl acetate, and methanolic extracts) of *Magnolia alba* on *Staphylococcus epidermidis* and *Staphylococcus aureus*.

2. Materials and Methods

2.1 Plant Material and Identification

Fresh flowers of white champaka (*Magnolia alba*) were obtained from Desa Sukaramai, Baiturrahman District, Banda Aceh, Indonesia. The white champaka was identified in the Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Darussalam, Banda Aceh, Indonesia.

2.2 Extracts Preparation

The flowers of *Magnolia alba* were first washed with tap water to remove the impurities and dried at room temperature for 26 days without exposure to the sunlight to produce a simplicial of flowers of *Magnolia alba*. The simplicial was weighed and broken down into small pieces by using a blender, then dried in the oven (Memmert thermostatic, universal oven UF55) at 50°C. The simplicial was then ground into fine powder, and macerated using multistage maceration method. The multistage maceration was performed by using three different solvents based on their polarity degree of solvents such as *n*-hexane, ethyl acetate and methanol. The extracts obtained were concentrated at reduced pressure to dryness using rotary evaporator (Rotaryvapor R-100, Buchi) until the solvent completed evaporation; the crude extracts obtained were dissolved and prepared in different concentrations: 5, 10, 20 and 50%. The concentrated extracts were stored at 4°C in labelled sterile screw-capped bottles till further analysis [12].

2.3 Characterization of Extracts

The crude flower extracts of *Magnolia alba* were characterized on the basis of water and ash contents, as well as solubility in water and ethanolic by using standard procedures [13].

2.4 Phytochemicals Screening

The crude flower extracts of *Magnolia alba* were used for phytochemical screening assay to detect the secondary metabolites such as alkaloids, flavonoids, saponins, tannins, and steroids/terpenoids by using standard procedures of phytochemical examination [14].

2.5 Antibacterial Assay

The antibacterial activity of *n*-hexane, ethyl acetate, and methanolic extract of *Magnolia alba* flower extracts was conducted using the agar disc diffusion (Kirby-Bauer) method. Mueller Hinton Agar (MHA) was poured in sterile Petri dishes and allowed to solidify, the test cultures of *Staphylococcus epidermidis* and *Staphylococcus aureus* were spread all over surface on the solidified MHA agar using a sterile cotton bud. The Whatmann paper disc of approximately 6 mm in diameter was placed on the surface of inoculated agar medium. Each disc was filled with 12 μ l of each extract, the paper disc of clindamycin was used as positive control, while the solvents such as *n*-hexane, ethyl acetate, and methanol were used as negative control. The plates were incubated at 37°C for 24 h, the inhibition zones surrounding the agar disc were measured in millimeters [15].

3. Results and Discussion

3.1 Characterization of the Extracts

The results showed that the percentage moisture content of *n*-hexane, ethyl acetate, and methanolic flowers extracts of white champaca were 20.3; 39.96; and 25.42% respectively (Table 1). It is important to analyse the moisture content of the extract because higher moisture accelerates the growth of the microbes and can destroy the secondary metabolites in the extract. Voight (1994) reported that the moisture content in the plant extracts should not exceed 30% [16]. Table 1 showed that the moisture content of *n*-hexane and methanolic extracts were less than 30%, while ethyl acetate extract was not qualified as predetermined requirements.

Table 1. Characterization of the *Magnolia alba* flowers extracts

<i>Magnolia alba</i> flower extracts	Moisture contents (%)	Solubility in water (%)	Solubility in ethanol (%)	Total ash (%)
<i>n</i> -Hexane extract	20.30 \pm 4.5	0 \pm 0	18.37 \pm 6.6	2.25 \pm 0.5
Ethyl acetate extract	39.96 \pm 0.4	15.00 \pm 3.00	56.33 \pm 9.0	0.03 \pm 0.0
Methanolic extract	25.42 \pm 1.9	65.66 \pm 1.58	70.00 \pm 2.6	10.65 \pm 2.1

The average of water soluble contents of *n*-hexane, ethyl acetate, and methanolic flower extracts of white champaca were 0; 15; and 65.66%, respectively. Meanwhile, the ethanol soluble content of *n*-hexane, ethyl acetate, and methanolic flower extracts of white champaca were 18.37; 56.33; and 79%, respectively. Determination of water and ethanol soluble contents was performed to determine the amount of the compounds soluble into a polar solvent. These results also indicated that ethyl acetate and methanolic extracts contain semi polar and polar compounds.

Determination of total ash was performed to determine the internal and the external of mineral contents of the extracts. The results showed that total ash of *n*-hexane, ethyl acetate, and methanolic flower extracts of white champaca were 2.25; 10.6; and 0.031%, respectively. These results showed that all extracts tested had low total ash contents. Total ash of the plant extracts is influenced by the physiological and environmental factors of plant [13].

3.2 Phytochemical screening of extracts

The phytochemical screening of *n*-hexane, ethyl acetate, and methanolic of *Magnolia alba* flower extracts are presented in Table 2. The preliminary results showed that phytochemicals including, alkaloids terpenoids, and steroids were present in the *n*-hexane of *Magnolia alba* flower extract, while flavonoids, saponins, tannins, terpenoids, and steroids were available in ethyl acetate of *Magnolia alba* flower extract. The phytochemical screening results also showed that all secondary metabolites tested were available or present in methanolic of *Magnolia alba* flower extract.

Table 2. Screening of phytochemicals in *Magnolia alba* flower extracts

Secondary Metabolites	<i>Magnolia alba</i> flowers extracts		
	<i>n</i> -Hexane extract	Ethyl acetate extract	Methanolic extract
Alkaloids	ve(-)	ve(+)	ve(+)
Flavonoids	ve(+)	ve(+)	ve(+)
Saponins	ve(-)	ve(+)	ve(+)
Tannins	ve(-)	ve(+)	ve(+)
Terpenoids	ve(+)	ve(+)	ve(+)
Steoids	ve(+)	ve(+)	ve(+)

3.3 Antibacterial Activity

Staphylococcus epidermidis and *Staphylococcus aureus* were grown on Manitol Salt Agar (MSA). MSA is a selective medium for strains of *Staphylococcus*, this media is used to distinguish between pathogenic bacteria such as *Staphylococcus aureus* and non-pathogenic species such as *Staphylococcus epidermidis*. MSA has high content of salt such NaCl until 7.5%, in addition, this media also contains of mannitol and phenol red as indicator of pH. Commonly, pathogenic *Staphylococcus* are able to ferment of mannitol and able to decrease of pH which changes the colour of the pH indicator in the medium from red to yellow.

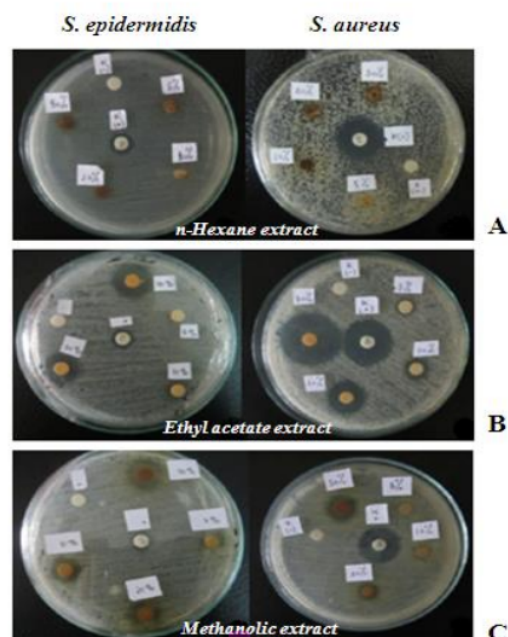


Figure 2. The activity of *n*-hexane (A), ethyl acetate (B), and methanolic (C) extracts of *Magnolia alba* flower on *S. epidermidis* and *S. aureus*.

Non-pathogenic *Staphylococcus* does not ferment mannitol and medium remains red [15]. This is in accordance with our results which revealed that *Shaphylococcus epidermidis* on MSA medium was unable to change the medium from red to be yellow, while *Staphylococcus aureus* was able to change the medium to be yellow on MSA. The antibacterial activity of *n*-hexane, ethyl acetate, and methanolic extracts of *Magnolia alba* flower towards *Staphylococcus epidermidis* and *Staphylococcus aureus* are

presented in Table 3 and Fig. 2. In this assay, the antibacterial assay of the extracts were performed at concentrations of 5, 10, 20 and 50%, the results showed that *n*-hexane of *Magnolia alba* flower (white champaca) extracts had no activity against *Staphylococcus epidermidis* and *Staphylococcus aureus* at concentrations used. This results were not in accordance with the results reported by Murniana which mentioned that *n*-hexane of *Magnolia alba* DC. flower (yellow champaca) extract has atibacterial activity against *Staphylococcus aureus* [17]. This is may be caused by difference in species of *Magnolia alba* that were used in this study.

From Table 3, it was evident that ethyl acetate extract of *Magnolia alba* flower had antibacterial activity against *Staphylococcus epidermidis* and *Staphylococcus aureus* at all concentrations tested. The data showed that the diameter of the inhibition zone increased by increasing the concentration of the extracts. According to Pelczar and Chan, the higher concentration has stronger activity in inhibiting the growth of microbes; this is because the secondary metabolites that penetrated into the cell of the bacterial at higher concentration will be greater [18].

Table 3. Antibacterial activity of *n*-hexane, ethyl acetate, and methanolic extracts of *Magnolia alba* flower on *S. epidermidis* and *S. aureus*

<i>Magnolia alba</i> flowers extracts	[Conc.]	Diameter of Inhibition Zone (mm \pm SD)	
		<i>S. epidermidis</i>	<i>S. aureus</i>
Clindamycin: positive control	2 μ g	11.98 \pm 0.5	22.51 \pm 2.3
<i>n</i> -Hexane: negative control		0 \pm 0	0 \pm 0
Ethyl acetate : negative control		0 \pm 0	0 \pm 0
Methanol: negative control		0 \pm 0	0 \pm 0
<i>n</i> -Hexane extract	5%	0 \pm 0	0 \pm 0
	10%	0 \pm 0	0 \pm 0
	20%	0 \pm 0	0 \pm 0
	50%	0 \pm 0	0 \pm 0
Ethyl acetate extract	5%	10.45 \pm 0.4	10.26 \pm 0.7
	10%	11.93 \pm 0.6	14.26 \pm 0.5
	20%	15.03 \pm 0.6	18.78 \pm 0.8
	50%	21.03 \pm 2.2	26.13 \pm 2.4
Methanolic extract	5%	11.96 \pm 0.8	07.23 \pm 0.1
	10%	14.11 \pm 0.2	08.45 \pm 0.6
	20%	16.13 \pm 1.4	10.46 \pm 0.4
	50%	18.01 \pm 1.0	13.90 \pm 0.8

Ethyl acetate extract at concentration of 50% had higher antibacterial activity against *Staphylococcus epidermidis* and *Staphylococcus aureus* with diameter of inhibition zone were 21.03 and 26.13 mm respectively. Meanwhile, the antibacterial activity of positive control (2 μ g of clindamycin) showed diameter of inhibition zone at 11.98 mm on *Staphylococcus epidermidis* and 22.51 mm on *Staphylococcus aureus*. The results also revealed that methanolic extract of *Magnolia alba* flowers can inhibit *Staphylococcus epidermidis* and *Staphylococcus aureus*. At concentration of 50%, the methanolic extract can inhibit *Staphylococcus epidermidis* at 18.01 mm and *Staphylococcus aureus* at 13.90 mm. Methanol as negative control showed no antibacterial activity, these results indicated that the antibacterial activity of the methanolic extract caused by the compounds from the extract.

Based on the antibacterial activity above, we could conclude that the greatest antibacterial activity was obtained from ethyl acetate extract, and followed by methanolic extract, while *n*-hexane extract did not show antibacterial activity to both of the bacteria tested. The antibacterial activity of ethyl acetate and methanolic extracts may be caused by the secondary metabolites contained in both extracts such as flavonoids, saponins, tannins, terpenoids and steroids. The antibacterial activity of ethyl acetate extract was greater than methanolic extract probably because the concentration of active compounds extracted in ethyl acetate extract was higher, and therefore more active to inhibit the growth of the bacteria. Further

research should investigate the specific compounds that inhibit growth or kill the bacteria. In addition, the secondary metabolites contained in ethyl acetate extract are thought work synergistically to inhibit the growth of both bacteria tested.

Alkaloids act by interfering with peptidoglycan layer of the cell, so the cell wall layer of the bacteria is not fully formed which causes cell death. The flavonoids act as antimicrobial by forming complex compounds with extracellular proteins, so the bacterial cell membrane will be destroyed due to discharge of the intracellular contents from the cell. While, the saponins act by reducing surface tension of the cell membrane, the cell permeability will increase and leakage, followed by release of intracellular compounds. Tannins act by shrinking the cell wall or cell membrane thus disrupting the cell permeability of the bacteria [19]. Terpenoids interact with purine or transmembrane protein in the outer membrane of the bacterial cell wall, forming a strong polymer bond resulting in damage to the purine [20]. Meanwhile, the steroids act by inhibiting bacterial growth by destroying bacterial cell membranes that can cause bacteria to become lysed [21].

4. Conclusion

This study has demonstrated the antimicrobial activity of *n*-hexane, ethyl acetate, and methanolic of *Magnolia alba* flower extracts against *Staphylococcus epidermidis* and *Staphylococcus aureus*. It was conclusive that ethyl acetate and methanolic extracts possessed antibacterial effect towards *Staphylococcus epidermidis* and *Staphylococcus aureus* at concentrations tested, while *n*-hexane extract had no antibacterial activity against these bacteria. This results suggest that *Magnolia alba* flower can be used to develop bioactive substances that may have promising effect on the treatment of some diseases caused by *Staphylococcus epidermidis* and *Staphylococcus aureus*.

Acknowledgements

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