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ORIGINAL RESEARCH

Phytochemical screening and antibacterial activity of the *Barringtonia asiatica* seed's *n*-hexane extract

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Abstract. *Barringtonia asiatica*, a marine plant belonging to the Lecythidaceae family, exhibits bioactivity in all parts of the plant. In the Simeulue region, the seeds of this plant are used for fish trapping. The polar extracts of *B. asiatica* seeds has shown antibacterial activity, but the non-polar extracts has not been studied yet. Therefore, this study aimed to determine the secondary metabolites of *B. asiatica* using a non-polar solvent (n-hexane) and evaluate its activity against Gram-positive and Gram-negative bacteria. The extraction method employed using soxhlet extraction, and the antibacterial activity was determined using the Kirby-Bauer method. The presence of secondary metabolites in the *B. asiatica* seed extract was assessed using phytochemical tests, and its chemical composition was analyzed using Gas Chromatography Mass Spectrophotometry (GC-MS). The yield of n-hexane extract from *B. asiatica* seeds was 1.7%, and the phytochemical analysis revealed that the *B. asiatica* seed's *n*-hexane extract contains only terpenoids compounds. The *B. asistica* seed's n-hexane extract at concentration of 10% (w/v) showed the highest zone of inhibition against *Staphylococcus aureus* (9.3 mm) followed by *Escherichia coli* (9,9 mm). The GC-MS analysis showed that *B. asiatica* seed's n-hexane extract contains Guaiene, Guaiene, Ledene, 9,12-Octadecadienoic acid, and Ethyl linoleate. Based on these findings, it can be concluded that *B. asiatica* seed's n-hexane extract exhibited antibacterial activity against *S. aureus* and *E. coli*.

Keywords: *Barringtonia asiatica*, Phytochemicals screening, *Staphylococcus aureus*, *Escherichia coli*

INTRODUCTION

Every ethnic group or region have traditional medicinal remedies derived from plants. One plant with numerous medicinal properties is *B. asiatica*, commonly known as the sea putat. This plant is found in tropical and subtropical regions such as Africa, Southeast Asia, Australia, and several Pacific islands [1]. *B. asiatica* is a flowering tree belonging to the Lecythidaceae family [2]. In the Simeulue region, the plant is used for fish trapping, where its seeds are grated and dispersed on the water surface to cause the fish unconscious thus facilitating the fishing.

Barringtonia asiatica seeds contain saponin compounds, evident by the formation of foam reaching to 6-9 cm when reacted with water [3]. Decoctions of *B. asiatica* leaves are commonly used to treat stomach pain. Additionally, the leaves are employed as remedies for wounds, scabies, stomachaches, and rheumatism [4]. The bark of *B. asiatica* exhibits biological activities such as antioxidant, cytotoxic, antibacterial, and antifungal properties due to the presence of secondary metabolites such as alkaloids, lignins, flavonoids, terpenoids, steroids, and saponins [5]. Mouthwash formulation derived from the aqueous extract of *B. asiatica* seeds displays antibacterial activity against the oral bacterium *Streptococcus mutans*, exhibiting an inhibition zone of 20.33 mm at a concentration of 15% [5, 6]. Methanolic extract of *B. asiatica* seeds from Sri Lanka exhibits antibacterial activity against *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, and *Escherichia coli*, as well as antifungal activity against *Candida glabrata*, *Candida albicans*, and *Candida parapsilosis* [7].

To date, there have been no reports on the antibacterial activity of non-polar extracts of *B. asiatica* seeds originating from the Aceh region. Based on this, the present study was aimed to evaluate the antibacterial activity of the *B. asiatica* seed's *n*-hexane extract against Gram-positive and Gram-negative bacteria. The potentially antibacterial chemical components were analyzed using GC-MS.

METHODOLOGY

Sample Preparation and Extraction

Samples were collected from the coastal area of Kampung Aie, Simeulue Tengah, Simeulue. Aceh Province. They were cleaned and air-dried indoors, then was roughly ground using a blender. Subsequently, the samples were extracted using the Soxhlet method with *n*-hexane as the solvent. The extracted solution was collected and concentrated using a rotary evaporator (Buchi).

Phytochemical Analysis of n-Hexane Extract from *Barringtonia asiatica* Seeds

The presence of secondary metabolites (alkaloids, steroids, terpenoids, saponins, and flavonoids) in the n-hexane extract of *B. asiatica* seeds was analyzed using the method described by Harbone [8], with slightly modifications.

Antibacterial Assay

The test bacteria used in the current study were *Staphylococcus aureus* and *Escherichia coli*, were obtained from the Biochemistry Laboratory, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Syiah Kuala University.

Preparation of Test Solution

Two grams of *B. asiatica* seed's *n*-hexane extract were dissolved in 10 mL of ethanol. This solution is prepared in several concentrations, namely 1%, 5%, 10% and 20%.

Preparation of Nutrient Agar (NA) and Mueller Hinton Agar (MHA)

To prepare NA (Merck) and MHA (Merck), a 28 g of NA powder was dissolved in 1 L distilled water, while 38 g of MHA was used to produce 1 L MHA media. Both media solutions were then sterilized using an autoclave at 121°C for 15 minutes.

Test Bacteria

The bacteria (*S. aureus* and *E. coli*) were grown in the NA media and incubated at 37°C for 16-24 hours.

Preparation of Test Bacterial Suspensions

Cell suspensions of *S. aureus* and *E. coli* were prepared by picking a single colony of each bacterium and suspending it in 10 mL of sterile distilled water. The suspension was homogenized using a vortex. The suspension was then adjusted to match the McFarland 0.5 standard solution, where the number of bacterial cells was equivalent to $1x10^8$ CFU/mL [9].

Antibacterial Activity Assay

The antibacterial activity of n-hexane extract from *B. asiatica* seeds was tested using the disk diffusion method (Kirby-Bauer test). A culture of bacteria at a concentration of $1x10^5$ CFU/mL was spread on MHA media using a cotton swab. A 20 µL of n-hexane extract with each concentration was placed on filter paper discs, which were then placed on the MHA media. Positive and negative controls were included, using chloramphenicol and the solvent, respectively. The plates were incubated at 37°C for 16-24 hours. The diameter of the inhibition zones was observed and measured using a caliper in millimeters.

GC-MS Analysis

Analysis of antibacterial compounds in the *B. asiatica* seed's *n*-hexane extract used Gas Chromatography-Mass Spectrophotometry (GC-MS). This GC-MS using TG-5MS column as a stationary phase and Helium as mobile phase with a gas flow of 10 mL/minute. One microlitre of *B. asiatica* seed's *n*-hexane extracts was injected in GC-MS system. The increase in temperature from the initial temperature (40°C) to final temperature (300°C) lasted for 52 minutes, where the temperature increase of 5°C/minute. In the ISQTM 7000 Thermo ScientificTM GC-MS, from sample injection to obtain GC peaks and compound fragmentation was carried out for 64 minutes.

RESULTS AND DISCUSSION

Phytochemical Analysis of *B. asiatica* seed's *n*-Hexane Extract

The extraction of a chemical compound using a specific solvent is performed based on the polarity properties of the desired compounds. The soxhlet method is used to separate non-polar compounds in *B. asiatica* seeds, because this method has several advantages that is using less solvent when compared with maceration method. Besides that, soxhlet method can be repeated and easily controleed. *n*-Hexane is chosen as the solvent because it

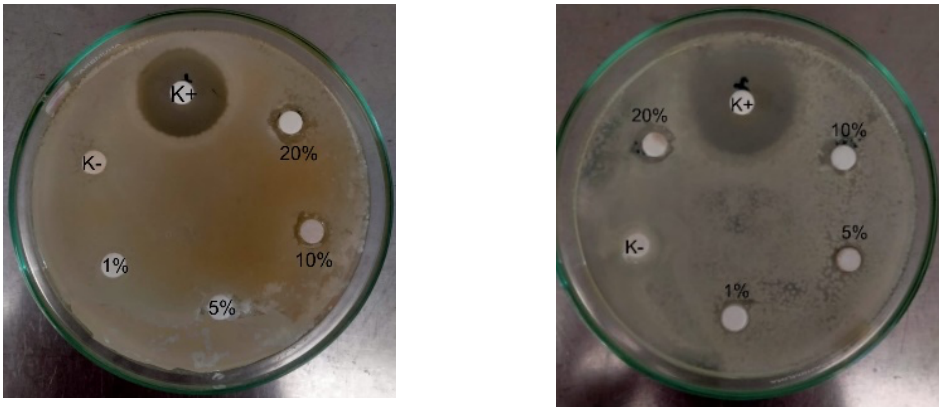
Table 1. The results of phytochemical analysis of the *B. asiatica* seed's *n*-hexane extract

Phytochemical analysis	Test method	Results
Alkaloid	Wagner reagent	–
	Mayer reagent	–
	Dragendorff reagent	–
Flavonoid	Mg + HCl	–
Terpenoid	Liebermann-Buchard reagent	+
Steroid	Liebermann-Buchard reagent	–
Saponin	Shaken with water	–
Phenolic compound	$FeCl_3$	–

Note: (+) presence of terpenoid compounds

Table 2. Antibacterial activity test results of the *B. asiatica* seed's *n*-hexane extract

Bacteria	Bacteria Sample Concentration (%) / Mean Inhibition Zone Diameter (mm ± SD)				Control	
	1	5	10	20	Positive	Negative
S. aureus	0.0 ± 0.0	0.0 ± 0.0	9.3 ± 0.3	12,8 ± 0.6	25.3 ± 0.6	0.0 ± 0.0
E. coli	6.3 ± 0.1	7.9 ± 0.4	9.9 ± 0.5	12,5 ± 2.7	27.8 ± 0.6	0.0 ± 0.0



(a) **(b)**

Figure 1. Antibacterial activity of *n*-hexane extract of *B. asiatica* seeds (a) against *S. aureus* (b) against *E. coli*.

is non-polar and has a lower boiling point compared to other non-polar solvents. The colour of *B. asiatica* seed's *n*-hexane extract is dark yellow and viscous liquid with its yield is 1.7%. This extract yield is low, this is in line with some research reports state that low yields of extracts if was used n-hexane as a solvent. For example, extract yield of kesum leaves (*Polygonum minus* Huds.) use *n*-hexane as solvent only 1,56% [10] and also reported that it's the volatile oil of lemon is only 1,52% [11]. Identification of compound groups in *B. asiatica* seed's *n*-hexane extract was carried out phytochemical analysis and these results are presented in Table 1.

Identification of Secondary Metabolite

From Table 1, it can be observed that the secondary metabolites present in the *n*-hexane extraction of *B. asiatica* seeds are solely terpenoids, as indicated by the formation of a red color in the *n*-hexane extract when treated with the Liebermann-Burchard reagent. Tanor and Meity [12] reported that the phytochemical compounds present in the methanolic extract of *B. asiatica* seeds are alkaloids, saponins, tannins, and terpenoids. Additionally, Rahmawati [13] also reported that the methanolic extract of *B. asiatica* seeds contains alkaloids, flavonoids, saponins, phenolic compounds, triterpenoids, and quinones, while the aqueous extract contains saponins (3-O-{[β-D-galactopyranosyl (1-->3)-β-D-glucopyranosyl(1-->2)]-β-D-glucuronopyranosyloxy}-22-O-(2-methylbutyroyloxy)-15,16,28-trihydroxy-(3β,15α,16α,22α)-olean-12-ene). Several factors that influence the composition of secondary metabolites include solvent variations, growth location, plant age differences, and climate [14].

Antibacterial activity test

The antibacterial activity of the n-hexane extract of *B. asiatica* seeds was tested against Gram-positive bacteria (*S. aureus*) and Gram-negative bacteria (*E. coli*). The antibacterial test was conducted using different concentrations (1%, 5%, 10%, and 20%). The data of the antibacterial activity test results for the n-hexane extraction of *B. asiatica* seeds are shown in Table 2. The positive and negative controls used were chloramphenicol and *n*-hexane, respectively.

Table 2 shows that the n-hexane extract of *B. asiatica* seeds exhibits antibacterial activity against *S. aureus* and *E. coli* (Figure 1). However, the extract does not display antibacterial activity against *S. aureus* at low concentrations. This may be due to its narrow spectrum, as it only inhibits the growth of Gram-negative bacteria. There are several mechanisms for antibacterial compounds, including inhibiting bacterial cell wall synthesis, impairing the integrity of bacterial cell wall permeability, inhibiting enzyme activity, and disrupting nucleic acid and protein synthesis [15]. In this case, the possible mechanism of the *n*-hexane extract from *B. asiatica* seeds is the inhibition of bacterial cell wall synthesis.

According to the Clinical Laboratory Standard Institute (CLSI), inhibition zone <14 mm is classified as resistant, while 15-18 mm is considered intermediate, and >19 mm is categorized as susceptible [9]. Based on this, the *B. asiatica* seed's *n*-hexane extract is classified as resistant since it exhibits antibacterial activity <14 mm.

Based on the previously, the *B. asiatica* seed's methanolic extract of fractionated with petroleum ether, chloroform, ethyl acetate, and butanol yielded fractions with antibacterial activity, with inhibition zones of 14

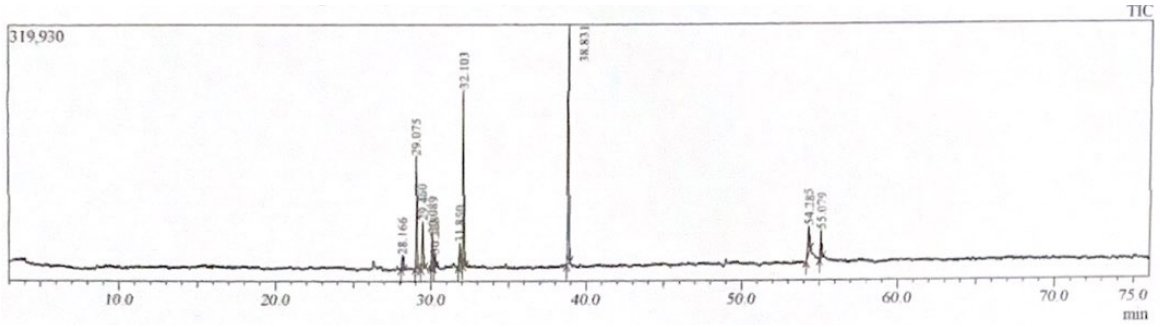


Figure 2. GC chromatogram of the *B. asiatica* seed's *n*-hexane extract

Table 3. Results of MS analysis of the *B. asiatica* seed's *n*-hexane extract

Peak	R. Time	Area %	Name	Similarity
1	28.166	2,46	trans-Caryophyllene	95
2	29.075	14,98	alpha-Guaiene	95
3	29.460	7,74	Seychellene (CAS) 1,6-methanonaphthale	93
4	30.089	4,8	alpha-Pachoulene	92
5	30.228	1,61	alpha-Gurjunene	88
6	31.850	3,23	alpha-Guaiene	88
7	32.103	23,01	delta-Guaiene	95
8	38.831	33,62	Ledene	85
9	54.285	4,95	9,12-Oktadecadienoic acid	92
10	55.079	3,59	Ethyl linoleat	94

mm, 16 mm, 18 mm, and 16 mm, respectively, at a concentration of 4 mg/disc [4]. This indicates that both polar and non-polar extracts of *B. asiatica* seeds possess antibacterial activity, although the activity of the non-polar extract is relatively smaller compared to the polar extract.

Based on the results of phytochemical screening, the *B. asiatica* seed's *n*-hexane extract of contains terpenoid compounds. Terpenoids have antibacterial activity, as they react with porins (transmembrane proteins) in the outer membrane of bacterial cell walls, forming strong polymer bonds that result in porin damage. Disruption of porin proteins reduces the permeability of the bacterial cell wall, leading to a shortage of nutrients and inhibition or death of bacterial growth [16].

However, in this study, the *n*-hexane extract of *B. asiatica* seeds exhibits greater (9.9 mm) activity against Gram-negative bacteria (*E. coli*) than that of Gram-positive bacteria (*S. aureus*). This is to Gram-positive bacteria contain teichoic acid that is covalently bound to peptidoglycan. Teichoic acid is hydrophilic and has a role as a medium for transporting of positively charged ions in and out of the cell wall. This solubility property causes the cell wall of Gram-positive bacteria to be more polar. The cell wall of Gram-positive bacteria contains teichoic acid associated with peptidoglycan through covalent bonds. This hydrophilic property causes the cell wall of Gram-positive bacteria to be polar [17]. This may explain why the non-polar n-hexane extract of *B. asiatica* seeds has more difficulty penetrating the cell wall of Gram-positive bacteria, resulting in its activity being more effective against Gram-negative bacteria.

GC-MS Analysis

The GC-MS analysis revealed that the *B. asiatica* seed's *n*-hexane extract contains 10 chemical compounds, as indicated by the 10 peaks in the GC chromatogram (Figure 2), with their Mass Spectrometry (MS) analysis results shown in Table 3.

In Table 4, three compounds with antibacterial activity are observed in the *B. asiatica n*-hexane extract: alpha-Guaiene, delta-Guaiene, and Ledene. According to Rialita [18], alpha-Guaiene, delta-Guaiene, and Ledene are sesquiterpene compounds that contribute to the aroma of essential oils. These compounds are commonly found in patchouli oil and cinnamon oil [19]. Sesquiterpene compounds in essential oils can disrupt cell membranes by binding to enzymatic proteins, thereby inhibiting bacterial growth [20]. Ledene is the most abundant compound in the n-hexane extract, with a concentration of 33.62%. Based on this, it can be stated that Ledene plays a role in the antibacterial activity.

Furthermore, compounds such as 9,12-Octadecadienoic acid and Ethyl linoleate, which are fatty acids present in the *B. asiatica* seed's *n*-hexane extract also exhibit antibacterial properties. The antibacterial activity of 9,12-Octadecadienoic acid has been reported by Karunia [21], and the antibacterial activity of Ethyl linoleate has

been demonstrated by Ko and Cho [22]. Thus, it can be concluded that the *B. asiatica* seed's *n*-hexane extract possesses antibacterial activity due to the presence of compounds such as alpha-Guaiene, delta-Guaiene, Ledene, 9,12-Octadecadienoic acid, and Ethyl linoleate.

CONCLUSION

The *B. asiatica* seed's *n*-hexane extract (10%) exhibits antibacterial activity against *S. aureus* (9,3 mm) and *E. coli* (9,9 mm). The identified chemical components in the *B. asiatica* seed's *n*-hexane extract that expected to its antibacterial activity are alpha-Guaiene, delta-Guaiene, Ledene, 9,12-Octadecadienoic acid, and Ethyl linoleate.

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