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Research Article

PHYTOCHEMISTRY AND ANTIBACTERIAL ACTIVITY OF THE WATER EXTRACT FROM *PARASERIANTHES FALCATARIA*

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ABSTRACT

Chemotaxonomic and phylogenetic studies show that sengon wood (Paraserianthes falcataria) has the potential to produce secondary metabolites with diverse structures and are bioactive. This research aims to study the chemical composition of the water extract and the bioactivity for inhibiting bacteria of Sengon (Paraserianthes falcataria). The method involves extraction by maceration, and analysis the extract by LCMS/MS methodology. In addition, the extract was evaluated to inhibit the Escherichia coli and Staphylococcus aureus. It was found that the water extract gives a viscous brown liquid, and the identified compound indicate the present of glycosidic compound, amino-alcohol structure, and fatty acid ester. Moreover, the antibacterial evaluation was able to inhibit the growth of E. coli 8.685 ± 4.876 mm, and toward the S. aureus bacteria 14.120 ± 4.418 mm, respectively.

Keywords: *Paraserianthes falcataria*, secondary metabolite, antibacterial

1. Introduction

Indonesia's biodiversity is estimated at around 30,000 plants found in tropical rainforests, there are 1260 species, some of which have medicinal properties [1], [2], [3]. Which may contain chemical compounds (chemodiversity). This has the potential to carry out research and identification of chemical compounds, especially secondary metabolites contained in plants, in line with advances in science and technology, such as separation techniques, analytical methods and pharmacological tests. The isolated compounds obtained from plants function as medicinal raw materials [2], [4], [5].

The Sengon plant of *Paraserianthes falcataria* is a plant that is spread throughout the world, especially Indonesia.[6], [7] The sengon plant has many benefits, especially the wood which is used for housing and furniture. In Indonesia, this plant is spread across Java, Kalimantan, Sumatra and Maluku. The plantation is also reported in the regions of Philippine[8].

Recently, we have reported the cellulose composition isolated from the wood of *Paraserianthes falcataria* [9] and modifying their structure as their ester. Similar research was also reported for cellulose isolated fraction [10], [11]. However, the remaining investigation is the secondary metabolite [12]. Previously, the n-hexane extract was applied for lowering the glucose level in the model rats[13] and anthelmintic activity [14]. The secondary metabolites of *Paraserianthes falcataria*, with detail also have been the subject of numerous studies. A study on *P. falcataria* bark waste found that the bark waste contains tannins, flavonoids, alkaloids, saponins, and steroids in both aqueous and ethanol extracts [14]. An additional investigation of Sengon bark extract revealed the presence of alkaloid, flavonoids, and triterpenoid compounds [13]. Sengon leaves were shown to possess alkaloids and saponins in a study [15].

The aqueous fractions of many plants mostly have antibacterial activity, black alder (*Alnus glitunosa L.*) [16], roselle (*Hibiscus sabdariffa*), rosemary (*Rosmarinus officinalis*), clove (*Syzygium aromaticum*), and thyme (*Thymus vulgaris*) [17]. Morover, Canarium patentinervium leaf water fraction shown bactericidal activity against eight clinical pathogens, including Enterococcus faecalis, Klebsiela species, Candida parapsilosis, oxacillin-resistant CONS, and oxacillin-sensitive CONS. [18]. So far, no one has reported the antibacterial activity of the water fraction of crude ethanol from the Sengon plant. This

research is reported the secondary metabolite composition of Sengon wood composed in the water extract of the wood part, and their antibacterial activity on *Escherichia coli* and *Staphylococcus aureus*.

2. Materials and Methods

2.1 Materials

The sample of Sengon wood was collected from local plantation in Malang, Indonesia. The chemicals used include distillated water, dimethyl sulfoxide (Merck), ethanol (Smart Lab, n-hexane (Merck) and chloroform (Merck).

2.2 Sample extraction

A 300 g sample of Sengon wood was weighed, then macerated with 1,5 L ethanol for 3x24 hours at room temperature. The extracts were obtained after in vacuum evaporation. It was further partitioned with n-hexane, and chloroform. The residue was partitioned with ethyl acetate and water. The evaporated water fraction was collected and named as the water extract. It was further dried, and analyzed and antibacterial testing.

2.3 LCMS/MS analysis

A 5.0 mg of water extract was dissolved in acetonitrile (10 mL). It was further applied for LC-MS/MS (Acquity H UPLC Class/UPLC Xevo G2-S Qtof) analysis. Operating parameters using the C18 RP-column (1.8 µm, 2.1 x 150 mm), flow rate 0.2 mL/min, mass spectra acquisition using Quadrupole Time of Flight (QToF) applied using collision energy of 4 Volt, and voltage range of 25–70 Volt. The chromatogram resulted is further analyzed for mass spectra detected. The Masslinx software given from the provider is used to calculate the similarity of the observed molecular weight and formula of the mass spectra. The data is further linked to the library database from PubChem (https://pubchem.ncbi.nlm.nih.gov/) and ChemSpider (https://chemspider.com/), respectivelly. The highest value similarity percentage from the library is chosen for determining the prediction the molecules.

2.4 Antibacterial analysis

Antibacterial evaluation follows previously reported procedure[19]. A commercial antibiotic ceftriaxone 300 mL/mg used as positive control (P0) and DMSO 1% used as negative control (P1). Sample of water extract was prepared in 300 mg/mL using DMSO 1% as solvent. Antibacterial tests on samples were carried out 5 times (P2-P6) with the same concentration. The antibacterial test was carried out using the disc diffusion method. Samples, negative control, and positive control were all dropped onto disc paper, and they were let to stand for a minute to ensure that the test solution diffused perfectly. The test media's surface was covered bacteria test. For twenty-four hours, the test medium was incubated at 37°C. After 24-hour incubation period, the antibacterial activity was observed by measuring the clear zone that developed around the disc paper. The inhibitory zone width of the clear zone indicates how sensitive the bacteria are to antibiotics or other antibacterial agents used as test material.

3. Result and Discussion

3.1 Phytochemical analysis

Secondary metabolite composed in *Paraserianthes falcataria* previously screened, contained of 5 different types of secondary metabolites. The different solvent polarity such as hexane [13], ethanol [14], methanol [20], and aqueous [14] solvent provide contrast composition. In hexane extract indicates alkaloid, flavonoid and triterpenoid/saponin. Meanwhile extraction in ethanol, methanol, and water provide all type secondary metabolite (Table 1). This profile indicates the capability the hexane solvent slightly more selective than that extracted with methanol, ethanol, and aqueous solvents.

Table 1. Phytochemical screening of secondary metabolite previously reported

Secondary metabolite	**Hexane extract [13]**	**70% Ethanol extract [14]**	**Aqueous extract [14]**	**Methanol extract** [20]
Alkaloid	+	+	+	ND
Tannin	-	+	+	+
Flavonoid	+	+	+	+
Triterpenoid/saponin	+	+	+	+
Steroid	ND	+	+	+

Analysis of the composition of the aqueous extract by using the LCMS/MS methodology provide the chromatogram depicted in Figure 1. Similar chromatogram pattern was reported previously from n-hexane extract by using GCMS analysis [20]. Three detected compound was classified as triterpenoid steroid. In contrast, the water extract of the study gives minimum 10 compounds detected from the chromatogram peaks. Further scanning process of each peak chromatogram

provide the mass spectra data. Tabulation of each predicted molecular structure, formula and mass weight is summarized in Table 2.

According to Table 1, the aqueous extract mostly composed of all secondary metabolite. The identified compound in agreement to this finding. The alkaloid type structures are identified from molecule number **1**. The structure contained nitrogen heteroatom. The phenolic compound as identified from molecule number **6**. In addition, type of free fatty acid and its ester secondary metabolites are identified for molecule number **2**-**4**, **7**-**10** (Table 2). However, no steroid and triterpenoid were found composed in the water extract.

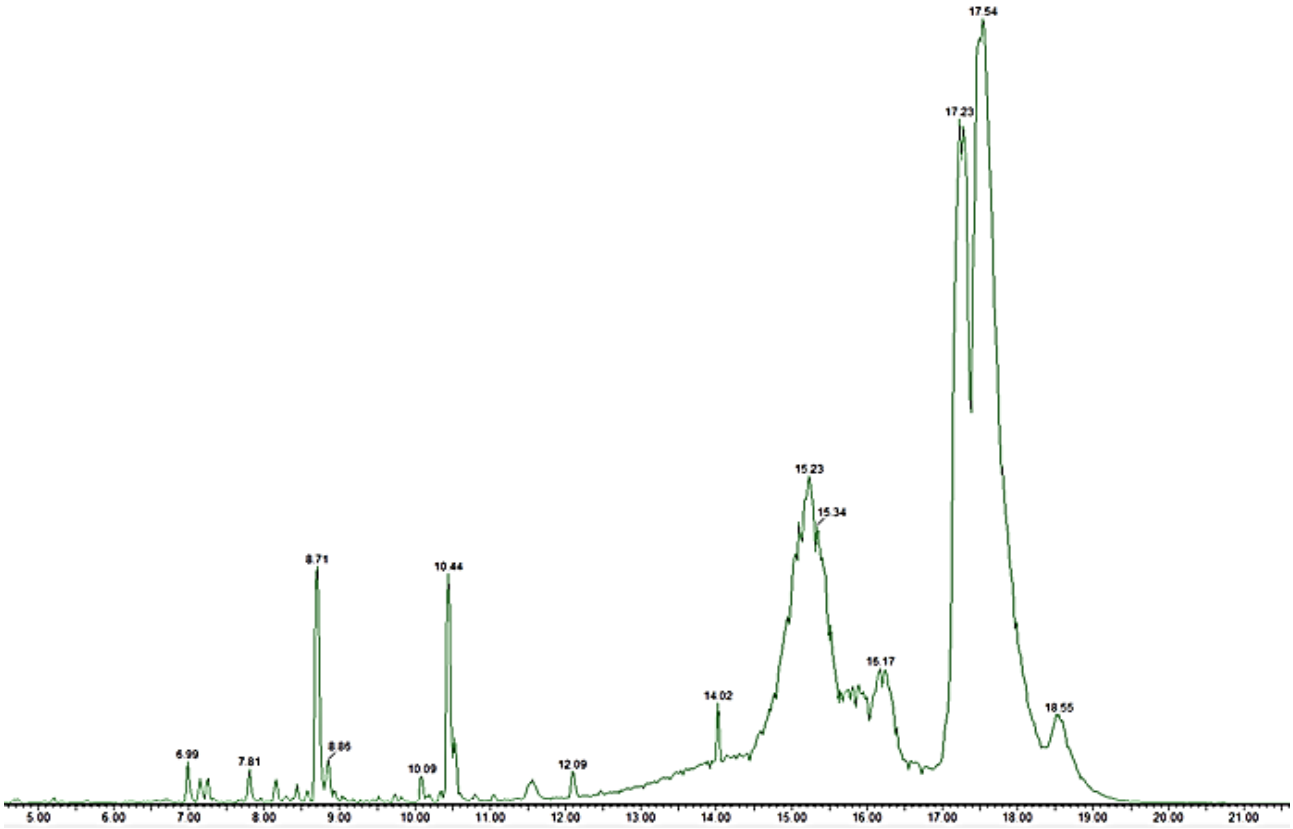
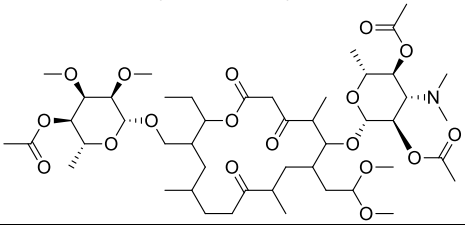
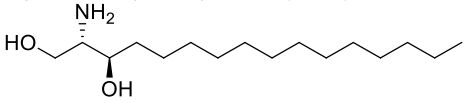
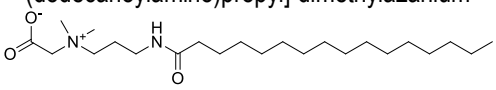
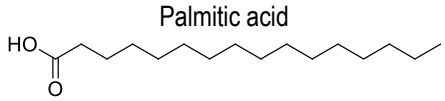
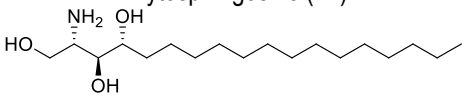
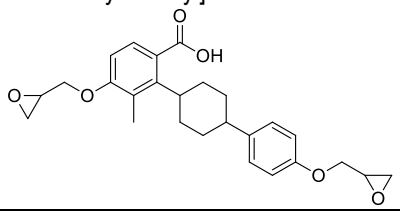
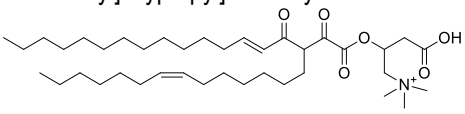
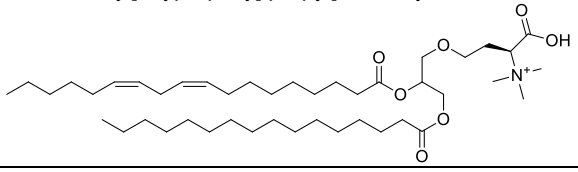
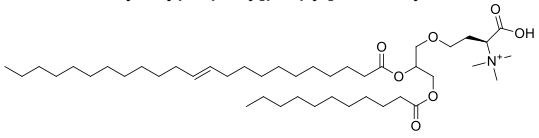
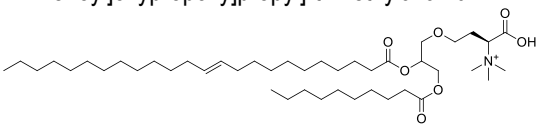


Figure 1. LCMS/MS-Chromatogram of the water extract from *Paraserianthes falcataria*

Table 2. Prediction of several compound extracted from water fraction of *Paraserianthes falcataria*

No.	Predicted compounds dan structure	Molecular Formula	Retention time (min.)	Molecular weight (m/z)
1.	[12-{[2,4-Di-O-acetyl-3,6-dideoxy-3-(dimethylamino)-β-D-glucopyranosyl]oxy}-11-(2,2-dimethoxyethyl)-2-ethyl-5,9,13-trimethyl-8,14,16-trioxooxacyclohexadecan-3-yl]methyl 4-O-acetyl-6-deoxy-2,3-di-O-methyl-β-D-altropyranoside 	$C_{47}H_{79}NO_{18}$	6.990	946.5343
2.	Hexadecasphinganine or [(2*S*,3*R*)-1,3-dihydroxyhexadecan-2-yl]azanium 	$C_{16}H_{36}NO_2$	8.685	274.2746

No.	Predicted compounds dan structure	Molecular Formula	Retention time (min.)	Molecular weight (m/z)
3.	Lauramido-propyl-betaine or carboxymethyl-[3-(dodecanoylamino)propyl]-dimethylazanium 	$C_{19}H_{39}N_2O_3$	10.09	343.2961
4.	Palmitic acid 	$C_{16}H_{36}NO_2$ ($M+NH_4^+$)	10.44	274.2737
5.	Phytosphingosine (1+) 	$C_{18}H_{40}NO_3$	12.09	318.2989
6.	3-Methyl-4-(oxiran-2-ylmethoxy)-2-[4-[4-(oxiran-2-ylmethoxy)phenyl] cyclohexyl] benzoate 	$C_{26}H_{29}O_6$	14.02	437.1944
7.	[3-carboxy-2-[(E)-3-oxo-2-[(Z)-tetradec-7-enyl]octadec-4-enoyl]oxypropyl]-trimethylazanium 	$C_{39}H_{72}NO_5^+$	15.23	634.5477
8.	[(1S)-1-carboxy-3-[3-hexadecanoyloxy-2-[(9Z,12Z)-octadeca-9,12-dienoyl]oxypropoxy] propyl]-trimethylazanium 	$C_{44}H_{82}NO_7$	17.23	736.6108
9.	[1-carboxy-3-[2-[(E)-tricos-11-enoyl]oxy-3-undecanoyloxypropoxy]propyl]-trimethylazanium 	$C_{44}H_{84}NO_7$	17.54	738.6281
10.	[1-carboxy-3-[2-decanoyloxy-3-[(E)-tetracos-11-enoyl]oxypropoxy]propyl]-trimethylazanium 	$C_{44}H_{84}NO_7$	18.55	738.6248

3.2 Antibacterial evaluation

The result of antibacterial test the water extract of *Paraserianthers falcataria* is sumarized in Table 3. Antibacterial evaluation of the extract is measured to evaluated capability of the extract in inhibiting the growth of *E. coli* and *S. aureus*. P0 is the positive control (ceftriaxone 300 mg/mL), P1 is the negative control (DMSO 1%), and P2-P6 are the test samples with 5 repetitions. The antibacterial test results show that the samples have the ability to inhibit *E. coli* and *S. aureus* bacteria. The diameter of the sample inhibition zone against *E. coli* bacteria (± 4.876) ranged from 0.00 to 0.71 mm and against *S. aureus* (± 4.418) ranged from 0.10 to 6.34 mm. According to theory, antibacterial activity is categorized as strong if its diameter is greater than 20 mm, moderate if it is between 5 and 10 mm, strong if it is between 19 and 20 mm, and weak if it is less than 5 mm [21]. Thus, the water extract from the *Paraserianthes falcataria* is classified as weak in inhibiting *E. coli* and *S. aureus* bacteria.

Table 3. Diameter inhibition on bacteria test

Bacteria	inhibition diameter (mm)						
	P0	P1	P2	P3	P4	P5	P6
E. coli (± 4.876)	21.34	7.98	8.69	8.17	7.49	5.86	7.45
S. aureus (± 4.418)	20.33	7.78	14.12	9.51	7.85	8.44	7.68

Note: P0 is a commercial antibiotic ceftriaxone 300 mL/mg, P1 is DMSO 1%, and P2-6 is sample 300 mg/mL.

4. Conclusion

In short, the water extract of *Paraserianthes falcataria* contains minimum 10 secondary metabolites, and predicted from the class of alkaloid, phenolics, fatty acid and its ester structure. The water extract also displays the capability for inhibiting the bacterial growth.

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