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Original Article

PHYTOCHEMICAL STUDY AND ANTIMICROBIAL PROPERTIES OF FILMY FERN (*HYMENOPHYLLUM IMBRICATUM* BLUME)

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ABSTRACT

Objective: The research aimed for phytochemical study and screening antimicrobial activities of filmy fern *Hymenophyllum imbricatum* Blume.

Methods: Antimicrobial assay used agar diffusion method against several pathogenic bacteria: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* ATCC 14028, *Salmonella typhosa* NCTC 786, *Bacillus subtillis* ATCC 6633, *Enterococcus faecalis* ATCC 29212, *Micrococcus luteus* ATCC 10240, *Salmonella typhi*, *Staphylococcus epidermidis* ATCC 12228, *Streptococcus mutans* ATCC 25175, *Vibrio cholera* Inaba and *Methicillin-Resistant Staphylococcus aureus* (MRSA), the isolation used silica column chromatography, purification by recrystallization, and identification by spectroscopic techniques (NMR, MS, UV-Vis, IR).

Results: Two compounds have been isolated from the aerial part of *Hymenophyllum imbricatum* Blume as 4-acetylphenyl ether (1) and genkwanin-4"-*O*-*β*-glucopyranose (2). The activity inhibition of isolated compounds against some human pathogenic bacteria *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *S. typhimurium* ATCC 14028, *S. typhosa* NCTC 786, *B. subtillis* ATCC 6633, *E. faecalis* ATCC 29212, *M. luteus* ATCC 10240, *S. typhi*, *S. epidermidis* ATCC 12228, *S. mutans* ATCC 25175, *V. cholera* Inaba, and Methicillin-Resistant *S. aureus* (MRSA) was inhibited at concentrations ranging from 0.6-10 µg/disc.

Conclusion: Flavonoid glycoside and dimer compounds were isolated from *Himenophyllum imbricatum*; both isolated compounds showed lower inhibition in some bacteria compared to the ethyl acetate fraction, which obtained moderate inhibition against all the testing bacteria.

Keywords: Filmy fern, *Hymenophyllum imbricatum* blume, Antimicrobial, Human pathogen bacterial

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INTRODUCTION

Hymenophyllaceae is one of the primary ferns growing in temperate rainforests on the slope of Mt. Singgalang (1987-2567 m above sea level), West Sumatra, Indonesia. In continuation of our study of Sumatran ferns [1-3], the collecting trips were carried out in various places in the mountainous and hilly areas of West Sumatra. In a collecting trip on the slope of Mt. Singgalang, one fern identified as *H. imbricatum* Blume was found. In a previous survey of Sumatran ferns, *Trichomanes javanicum* from Mentawai Island was found to be traditionally used by the local people to treat measles and inflammation of earache and was found to contain mangiferin, vitexin, and 4-*O*-*β*-D-glucopyranosylcaffeic acid [3, 4]. Mangiferin, as a primary compound, was reported to have antioxidant properties with an IC_{50} value of 3.89 μM and active antibacterial toward *M. luteus* ATCC 10240, *S. aureus* ATCC 25923, *B. subtillis* ATCC 6633, and V. cholera Inaba [3]. Phytochemical study of another *Hymenophyllum* species from Japan yielded 24 secondary metabolites: hymenosides and hemiterpene glycosides [5-7]. *H. imbricatum* is distributed around Malesia to the Pacific; it is epiphytic and commonly grows in the tropical wet mountains. Its rhizome is more or less filiform, creeping; fronds ascending or pendent; blade glabrous, 2-pinnate pinnatifid, rachis terete, winged, the wings and undulate segments of the blade flat or undulate, entire. Sori marginal, at ends of veins, the involucres bivalvate, rounded [8, 9]. There is no information about the constituents and traditional use of *H. imbricatum* found in the literature, so following our previous study on *Hymenophyllum,* the study of its chemical constituents and antimicrobial properties against human pathogenic bacteria and secondary metabolites of this fern was carried out. Following our previous main interest to find antimicrobial from Sumatran lower plants it was desirable to obtain an antimicrobial constituents of *H. imbricatum* particularly against common infection of gastrointestinal and skin infection which also known as neglected tropical deseases. Antimicrobial study was started by screening the antibacterial activities of nonpolar, semi-polar, and polar fraction of *H. imbricatum* then, the most potential fraction continued to isolation.

MATERIALS AND METHODS

General experimental procedures

The melting point was measured with SYBRON thermolyne®. UV-Vis spectra were measured with Shimadzu® Pharmaspec 1700. IR spectra were recorded on a Spectrometer Frontier Perkin Elmer. Mass spectra were obtained with an ESI-TOF Waters® LCT Premier XE mass spectrometer in either positive or negative mode. NMR spectra, including ^{1}H-^{1}H COSY, HMQC, HMBC and NOESY experiments, were recorded on JEOL ECA500 operating at 500 MHz (^{1}H) and 125 MHz (^{13}C) using deuterated solvent ($CDCl_3$ and DMSO). Silica gel 60 (Merck®, 70-230 mesh and 230-400 mesh) was used for column chromatography. TLC was performed using Merck® precoated silica gel F_{254} plates. Spots were detected on TLC under UV light.

Plant materials

The aerial parts of *H. imbricatum* Blume were collected on the slope of Mt. Singgalang at an altitude of 1987-2567 m above sea level in January 2015 and identified by botanist Hernawati from Universitas Andalas Herbarium (ANDA); the specimen was deposited in the Herbarium with voucher specimen number H52.

In sunny weather, the field collection was carried out with the assistance of a botanist. To identify its species and prevent contamination with other filmy fern species, the ferns *H. imbricatum* were separated in the field. Prior to extraction, the fern was allowed to air dry after collecting.

Extraction and isolation

The air-dried aerial parts of *H. imbricatum* (406 g) were extracted

with MeOH at room temperature for 3 x 5 d and filtered. The combined filtrate was evaporated under reduced pressure to give a MeOH extract (130 g). The methanolic extract as usual after evaporation was kept in refrigerator before being used. In order to isolate the antimicrobial constituents, the methanolic exstract was fractionated into their polarities to simpilify the mixture using hexane, EtOAc and botanol. In this way the non polar constituents will soluble in hexane, semipolar in EtOAc and polar in butanol. The methanolic extract suspended in water (1000 ml) and then consecutively partitioned with *n*-hexane, EtOAc and *n*-BuOH, respectively, yielding 9.0, 27.0, and 50.0 g The EtOAc soluble fraction (25 g) was chromatographed on silica gel (200 g), eluted with step gradient polarity solvents consisting of *n*-hexane 100%, *n*-hexane: EtOAc 9:1, 4:1, 7:3, 3:2, 1:1, 2:3, 3:7, 1:4, 1:9, EtOAc 100%, EtOAc: MeOH 4:1, and MeOH 100% with volume 500 ml each. Based on the similarity of their TLC profiling, the sub-fractions were combined, which yielded seven sub-fractions (A-G). Sub-fraction B was combined and recrystallized with a mixture of EtOAc in *n*-hexane to gain compound 1 (96 mg) (fig. 2). Sub-fraction F was recrystallized in MeOH to obtain compound 2 (169 mg) (fig. 2).

Antimicrobial assay

Agar diffusion method [1, 10] was used to preliminary screening and determine the inhibition of extract and fractions (*n*-hexane, EtOAc, *n*-butanol), compounds 1 and 2 towards *S. aureus* ATCC 25923*, E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *S. typhimurium* ATCC 14028, *S. typhosa* NCTC 786, *B. subtillis* ATCC 6633, *E. faecalis* ATCC 29212, *M. luteus* ATCC 10240, *S. typhi*, *S. epidermidis* ATCC 12228, *S. mutans* ATCC 25175, *V. cholera* Inaba and *Methicillin-Resistant Staphylococcus aureus* (MRSA). The extract and fractions were prepared at 200, 100, 50, 25, 12.5, and 6.25 mg/ml concentrations. The isolated compounds were prepared at concentrations 2, 1, 0.5, 0.25, 0.125, and 0.0625 mg/ml. Chloramphenicol at a concentration of 3 mg/ml was used as the positive control, and DMSO as the negative control. Each disc contained 10 µl of the sample. The assay was done in triplicate of each sample.

RESULTS

The filmy fern that was collected and continued in this study is depicted in fig. 1.

Fig. 1: Picture of *Hymenophyllum imbricatum* Blume. In life form (A) growth on the tree, frond part (B), sori of ferns (C, D)

Antimicrobial assay

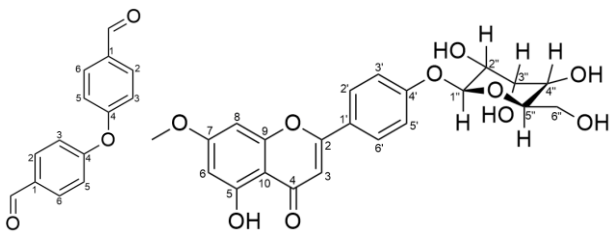
The agar diffusion method was selected because of its simplicity, as well as its relative reliability for preliminary work to identify the active compounds. The antimicrobial assay was started by testing *n*-hexane, ethyl acetate, and butanol fractions against 13 pathogenic bacteria, as shown in table 1. Then, the compounds from more active fractions were isolated and tested against the same bacteria as the extract (table 2). The inhibition data in tables 1 and 2 were the average inhibition zone from the triplicate assay of each sample. The diameter inhibition can be used to classify following scale: weak activity for diameter zone ≤12 mm, moderate activity for diameter zone range>12 and<20 mm, strong activity for diameterzone ≥20 mm [11]. The inhibition zone indicated the sample having potential to inhibit bacterial membrane.

Characterization of compound 1

Compound 1 was colorless crystal with melting point value 101-103 °C. The spectrospic measurement resulted for IR (KBr) ν_{max} cm^{-1}: 3305, 2895, 1662, 1353; UV (MeOH) λ_{max} (log ε) 219 nm (4.840), 268 nm (6.365); and ESI-MS *m/z* 256.2809 $[M+2H]^+$; (calcd for $C_{18}H_{14}O_3$, 254.0943). The ^1H and ^{13}C NMR data of compound 1 is compared in table 3. Based on the available spectrscopic data, the main isolated compound clearly belongs to an aromatic compound having ethereal linkage eventhough with limited data available in literature to compare. Then, compound 1 was identified as 4-acetylphenyl ether [12, 13].

Characterization of compound 2

Compound 2 had a colourless crystal with a meeting point value of 230 °C. The spectroscopic result for IR (KBr) ν_{max} cm^{-1}: 3221, 1559, 1441, 1353, 1164; UV (MeOH) λ_{max} (log ε) 211 nm (11.621), 268 nm (7.450), 336 nm (6.201); and ESI-MS *m/z* 285.1027 $[M+H]^+$, 447.1306 $[M+H]^+$; (calcd for $C_{22}H_{22}O_{10}$, 446.1213). The ^1H and ^{13}C NMR data of compound 2 were compared in table 4. Compound 2 was identified as genkwanin-4*"-O-β*-glucopyranose.



1 **2**

Fig. 2: Structures of compounds 1 and 2

Table 1: The result of the antimicrobial assay of extracts is *Hymenophyllum imbricatum* Blume

Testing bacteria	Zone diameter value (mm)*																						
	n-hexane extract (mg/disc)								EtOAc extract (mg/disc)								*n*-butanolic extract (mg/disc)							
	2.0	1.0	0.5	0.25	0.13	0.06	(+)[1]	(-)[2]	2.0	1.0	0.5	0.25	0.13	0.06	(+)[1]	(-)[2]	2.0	1.0	0.5	0.25	0.13	0.06	(+)[1]	(-)[2]
S. aureus	9.3	9.0	8.0	7.3	6.8	nd	30.0	nd	19.8	19.3	17.3	14.7	13.3	12.0	30.0	nd	14.3	13.7	12.0	10.3	9.3	nd	33.0	nd
E. coli	14.0	13.3	13.0	12.3	11.3	10.3	33.0	nd	20.0	18.3	16.3	15.3	14.0	12.7	30.0	nd	17.3	16.0	14.7	13.0	11.7	11.0	31.0	nd
P. aeruginosa	8.0	7.3	7.3	7.0	nd	nd	25.0	nd	19.7	17.7	16.3	14.7	12.7	9.7	25.0	nd	14.7	13.3	12.0	10.3	9.3	nd	24.0	nd
S. typhimurium	9.0	8.5	7.0	7.0	6.17	6.17	32.0	nd	19.3	18.3	16.5	16.0	15.0	13.7	33.0	nd	14.7	12.7	10.7	9.7	7.7	nd	30.0	nd
S. typhosa	11.3	10.6	10.3	9.7	9.3	9.0	32.0	nd	19.5	18.0	16.7	15.3	13.7	10.3	30.0	nd	14.0	12.7	11.5	11.0	9.0	8.0	26.0	nd
B. subtillis	13.3	12.7	11.3	10.0	9.0	8.0	29.0	nd	20.0	19.0	17.3	15.7	14.7	13.3	30.0	nd	16.7	15.7	14.3	12.3	11.0	9.7	30.0	nd
E. faecalis	10.0	8.3	8.0	7.3	7.0	7.0	31.0	nd	19.7	18.3	16.7	15.3	13.7	12.0	30.0	nd	15.0	13.0	nd	nd	nd	nd	32.0	nd
M. luteus	11.0	8.6	8.3	7.6	7.3	7.0	31.0	nd	20.0	18.0	16.3	14.0	12.0	10.0	30.0	nd	14.7	13.3	11.7	9.7	nd	nd	30.0	nd
S. typhi	17.0	15.3	15.0	13.3	12.0	10.6	32.0	nd	20.3	19.7	18.7	18.0	16.7	15.5	32.0	nd	15.7	14.0	13.7	12.7	11.0	10.0	30.0	nd
S. epidermidis	14.0	13.3	13.0	12.3	11.3	10.3	33.0	nd	20.3	19.8	18.2	16.0	15.2	12.7	32.0	nd	15.3	14.5	12.3	10.0	nd	nd	32.0	nd
S. mutans	14.7	14.0	12.7	11.3	10.3	9.0	30.0	nd	17.0	16.7	15.3	13.3	12.0	9.7	29.0	nd	13.7	12.3	10.7	9.3	8.0	nd	31.0	nd
V. cholera	11.0	11.0	10.5	10.0	10.0	10.0	25.0	nd	18.3	16.7	15.3	14.2	13.8	10.0	26.0	nd	15.0	11.7	10.0	nd	nd	nd	26.0	nd
MRSA	13.3	12.7	11.3	10.0	9.0	nd	30.0	nd	19.3	18.3	17.7	15.7	14.0	12.3	30.0	nd	15.3	13.3	11.3	9.0	nd	nd	30.0	nd

1 = positive control (chloramphenicol, 30 µg/disc), 2 = negative control (DMSO 10 µl/disc). nd = not detected. *Each test was done in triplicate.

Table 2: Result of antimicrobial assay of compounds 1 and 2

Testing bacteria	Zone diameter value (mm)*															
	Compound 1 (µg/disc)								Compound 2 (µg/disc)							
	20	10	5	2.5	1.25	0.63	(+)[1]	(-)[2]	20	10	5	2.5	1.25	0.63	(+)[1]	(-)[2]
S. aureus	7.7	6.7	6.7	6.7	6.3	nd	31.0	nd	7.0	6.5	nd	nd	nd	nd	31.0	nd
E. coli	7.7	7.0	6.0	nd	nd	nd	30.0	nd	nd	nd	nd	nd	nd	nd	30.0	nd
P. aeruginosa	nd	nd	nd	nd	nd	nd	25.0	nd	nd	nd	nd	nd	nd	nd	25.0	nd
S. typhimurium	nd	nd	nd	nd	nd	nd	33.0	nd	nd	nd	nd	nd	nd	nd	34.0	nd
S. typhosa	7.7	7.3	6.8	6.8	6.8	6.8	30.0	nd	7.7	7.5	7.5	7.0	7.0	7.0	29.0	nd
B. subtillis	nd	nd	nd	nd	nd	nd	32.0	nd	8.0	8.0	7.0	nd	nd	nd	30.0	nd
E. faecalis	8.0	7.0	7.0	7.0	6.8	6.7	32.0	nd	nd	nd	nd	nd	nd	nd	33.0	nd
M. luteus	nd	nd	nd	nd	nd	nd	30.0	nd	nd	nd	nd	nd	nd	nd	30.0	nd
S. typhi	7.7	6.7	nd	nd	nd	nd	32.0	nd	6.0	6.0	6.0	nd	nd	nd	32.0	nd
S. epidermidis	nd	nd	nd	nd	nd	nd	29.0	nd	nd	nd	nd	nd	nd	nd	29.0	nd
S. mutans	nd	nd	nd	nd	nd	nd	29.0	nd	nd	nd	nd	nd	nd	nd	33.0	nd
V. cholera	nd	nd	nd	nd	nd	nd	26.0	nd	nd	nd	nd	nd	nd	nd	26.0	nd
MRSA	nd	nd	nd	nd	nd	nd	30.0	nd	nd	nd	nd	nd	nd	nd	30.0	nd

1 = positive control (chloramphenicol 30 µg/disc), 2 = negative control (DMSO µl/disc). nd = not detected. *Each test was done in triplicate.

Table 3: ^{1}H and ^{13}C NMR spectroscopic data for compound 1 and reference [13]

Position	Compound 1*		References [13]**	
	^{1}H NMR (δ_H, multiplicity, *J* in Hz)	^{13}C NMR (δ_C, type)	^{1}H NMR (δ_H, multiplicity, *J* in Hz)	^{13}C NMR (δ_C, type)
1	-	115.5, C	-	115.4, C
2,6	7.92, d, 8	131.2, CH	7.86, d, 8.4	131.0, CH
3,5	6.95, d, 8.5	129.7, CH	6.89, d, 8.4	129.9, CH
4	-	161.3, C	-	160.9, C
CH_3	2.59, s	26.3, CH_3	2.53, s	26.2, CH_3
C=O	-	198.5, C=O	-	197.4, C=O

*$CDCl_3$, ^{1}H NMR 400 MHz, ^{13}C NMR 100 MHz, δ_H and δ_C in ppm, **$CDCl_3$, ^{1}H NMR 300 MHz, ^{13}C NMR 75 MHz

Table 4: ^{1}H and ^{13}C NMR spectroscopic data for compound 2 and reference[14]

Position	Compound 2*		References [14]**	
	^{1}H NMR (δ_H, multiplicity, *J* in Hz)	^{13}C NMR (δ_C, type)	^{1}H NMR (δ_H, multiplicity, *J* in Hz)	^{13}C NMR (δ_C, type)
2	-	163.4, C	-	163.5, C
3	6.95	104.8, CH	6.97	104.0, CH
4	-	182.0, C=O	-	182.1, C=O
5	-	161.2, C	-	161.3, C
6	6.38	98.1, CH	6.39, d, 2.3	98.0, CH
7	-	165.2, C	-	165.3, C
8	6.80	92.8, CH	6.82, d, 2.3	92.7, CH
9	-	157.3, C	-	157.3, C
10	-	104.0, C	-	104.8, C
OCH_3	3.86, s	56.1, OCH_3	3.87, s	56.0, OCH_3
$C_{1'}$	-	123.8,C	-	123.9, C
$C_{2'}$, $C_{6'}$	8.07, d, 6.8	128.3, CH	8.07, d, 8.9	128.3, CH
$C_{3'}$, $C_{5'}$	7.20, d, 6.4	116.6, CH	7.19, d, 8.9	116.6, CH
$C_{4'}$	-	160.4, C	-	160.5, C
$C_{1''}$	5.03, d, 5.6	99.8, CH	5.03, d, 7.1	99.9, CH
$C_{2''}$	3.18-3.72, m	73.2, CH	3.11-3.90, m	73.2
$C_{3''}$	3.18-3.72, m	76.6, CH	3.11-3.90, m	76.6, CH
$C_{4''}$	3.18-3.72, m	69.7, CH	3.11-3.90, m	69.7, CH
$C_{5''}$	3.18-3.72, m	77.2, CH	3.11-3.90, m	77.1, CH
$C_{6''}$	3.18-3.72, m	60.7, CH_2	3.11-3.90, m	60.7, CH_2

*DMSO-d_6, ^{1}H NMR 400 MHz, ^{13}C NMR 100 MHz, δ_H and δ_C in ppm, **DMSO-d_6, ^{1}H NMR 250 MHz, ^{13}C NMR 62.5 MHz, δ_H and δ_C in ppm

DISCUSSION

Twelve species of ferns were collected during the collection trip on the slope of Mt. Singgalang. Ten species were characterized as belonging to the genus *Hymenophylum*. One was *H. imbricatum* (fig. 1), which showed significant antimicrobial screening against human pathogenic bacteria (table 1). The potential effects of antimicrobial inhibition of *H. imbricatum* extracts exhibited by the ethyl acetate fraction, which was chosen for isolation work. Compounds 1 and 2 (fig. 2) were elucidated by ^{1}H, ^{13}C NMR spectra and MS methods and then compared to data reported in the literature [12-14]. The monomer of compound 1 was previously isolated from a higher plant, *Ficus beecheyana* Hook. and Arn., which gave a melting point of 108-110 °C [13]. Compound 1 had a lower melting point than its monomer, 101-103 °C. The value of the melting point compound 1 was supported by an experimental melting point from several vendors which sell this compound [15-17]. The LC-ESI-MS data indicated that (1) gave the value of 2 molecules of its monomer M^{+}-18 (H_2O) at *m/z* $[M+2H]^{+}$256.2809. A ferric chloride reagent was used to confirm the presence of the phenolic group. However, the result was negative. Compound 2 had been isolated previously from the fern *Equisetum arvense* [14]. Both isolated compounds were known, and their existence was reported for the first time from *H. imbricatum.*

The antibacterial properties of both compounds against human pathogenic bacteria are displayed in table 2. Compound 1 showed inhibition towards *S. aureus*, *E. coli*, *S. typhosa*, *E. faecalis*, and *S. typhi*, and compound 2 exhibited inhibition against *S. aureus*, *S. typhosa*, *B. subtilis*, and *S. typhi*. Compound 1 has aldehyde groups reported as antibacterial activity by damaging bacterial cell walls and cytoplasmic membranes [18]. Genkwanin aglycone (2) reported many biological activities, but there was limited information on its antimicrobial activities, including for genkwanin glycoside [19]. Structure-activity relationships study on flavonoids reported that flavonoids with high hydrophobicity could interact more with membrane cells of bacteria, especially for *E. coli* [20]. The critical position for the antimicrobial activity of flavonoids is the positive charge of C-3 of flavonoids and hydroxyl substitution at C-5 and C-4' in rings A and B, at C-3 and C-8 substitute with the methoxyl group [20, 21]. Compound 2 lacks hydroxyl substitution, causing less interaction with membrane bacteria. The interaction cell membranes of bacteria and compounds can inhibit bacteria; ethyl acetate extract (table 1) showed much inhibition against pathogenic bacteria since it consisted of varying secondary metabolites in extract working synergically on membrane cells [22]. Compared with isolated compounds, there was lower inhibition against fewer bacteria. The complexity of extract and mechanism of bacteria against membrane cell of bacteria is potential in the extract than isolated compounds [21, 22]. Based on the structure of isolated compounds, the antibacterial mechanism was through damaging membrane cells of bacteria. However, the degree of inhibition sensitivity against tested bacteria is still uncertain.

Little research has been conducted on the phytochemistry and bioassay of *Hymenophyllum* species, particularly *Hymenophyllum imbricatum*. A number of identified compounds from *H. imbricatum* that include flavonoid glycoside is corroborated by secondary metabolites from *H. barbatum* that identified compounds with a sugar moiety [5-7]. Other *Hymenophyllum* species in West Sumatra like *H. javanicum,* that contains sugar linked to flavonoid and caffeic acid structures, also supported the occurance of glycoside compounds.[3] Furthermore, those compounds exhibited antibacterial repression against a few pathogenic bacteria. Because of the availability of *H. imbricatum* in the field, the bioassay screening of antimicrobial was limited to antibacterial. Small quantities of natural product's isolated compounds get produce by the plants [23]. Still, there is an opportunity that antimicrobial screening may be broaden to involve ferns' antifungal and antiviral properties. Only two secondary metabolites were identified in this study from the ethyl acetate fraction; other fractions and the EtOAc fraction posses various minor chemical substabce that could potentially have antimicrobial activities. Following the first findings, a more thorough expanded investigation might be executed in the future.

CONCLUSION

Two secondary metabolites were isolated from EtOAc fraction, 4-acetyl phenyl ether and Genkwanin-4"-*O*-*β*-glucopyranose. When compared to kloramphenicol, which revealed weak inhibition, both compounds significantly inhibited some tested bacteria. the active compounds can be discovered in the future applying bioassay guided by TLC-bioautography to find the responsible compounds for antimicrobial activities. The bioactivity of *H. imbricatum* is currently remain unexplored; further study on its biological activity and the determination of its secondary metabolites will be needed.

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AUTHORS CONTRIBUTIONS

NS performed collection and design and supervised the project. YPS performed the isolation, structure elucidation, and antimicrobial assay. H collected and identified the plant. DA designed and supervised the project. NS wrote the manuscript, which DA finalized. All authors read and approved the final manuscript.

CONFLICT OF INTERESTS

The author declares that there is no conflict of interest to influence the report on this research.

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