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Isolation and antibacterial activity of diffractic acid compound from lichen *Usnea blepharea* Motyka

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ABSTRACT

Lichen's rich natural resources have been utilized for traditional and herbal medicine. The secondary metabolite compounds of lichen genus *Usnea blepharea* Motyka have not been reported much. This research was to explore secondary metabolites of lichen *U. blepharea* Motyka from the mountains in South Sulawesi, Indonesia, and their bioactivity as antibacterials. The powder of lichen was extracted using an acetone solvent and continued separation using gravity column chromatography, preparative thin-layer chromatography, and radial chromatography. Determination of molecular structure was carried out by analyzing the spectral data of UV-Vis, Fourier transform infrared spectroscopy, liquid chromatography-mass spectrophotometer, ^{1}H-NMR (1D-nuclear magnetic resonance), ^{13}C-NMR, and NMR-two-dimensional, including heteronuclear multiple quantum correlation, heteronuclear multiple bond correlation, distortionless enhancement by polarization transfer, and correlated spectroscopy. The isolate of *U. blepharea* Motyka obtained a diffractic acid compound with molecular formula $C_{20}H_{22}O_7$. The antibacterial bioactivity test showed that the diffractic acid compound actively inhibited (very strongly) the growth of *Staphylococcus aureus* bacteria at concentrations of 750 and 1,000 ppm with inhibition zones of 14.27 and 17.25 mm, respectively. In addition, *Escherichia coli* was also actively inhibited (strongly) at concentrations of 750 and 1,000 ppm at 11.00 and 12.75 mm, respectively. This study is interestingly the latest reference source for natural compounds, especially the lichen *U. blepharea* Motyka, because there have been no reports related to diffractic acid compounds found from this lichen genus and also its strong activity as an antibacterial.

INTRODUCTION

The wealth of biological natural resources from lichen organisms is widely used for traditional and herbal medicine. Lichen is a symbiotic organism between fungi and algae or cyanobacteria (Maulidiyah *et al*., 2021b). Generally, these organisms are found in tree trunks, soil, rocks, shorelines, and mountain cliffs. Lichen is divided into three groups, namely crustose, foliose, and fruticose (Lumbsch, 2020). The mutualistic symbiosis of lichen has the potential to produce secondary metabolites for medicines of natural origin or as raw materials for the pharmaceutical industry. One of the several genera of fruticose is *Usnea*. Several types of lichen from the genus *Usnea* that have been widely used as traditional medicines are the species *U. articulate*, *U. dasypoga*, *U. longissima*, *U. sternaii*, *U. vrieseana*, *U. comosa*, *U. blepharea*, *U. baileyi*, *U. javanica*, and *U. flexuosa* (Dini *et al*., 2017; Maulidiyah *et al*., 2011; Sepahvand *et al*., 2020).

Secondary metabolites in *Usnea* sp. include benzofuran, depside, depsidone, benzyl ester, xanthone, aliphatic, and lactone groups (Huneck and Yoshimura, 1996). Several previous studies have reported various biological activities of lichen *Usnea*, including as an antiproliferative (Mitrovic *et al*., 2011), antibiotic (Shrestha and Clair, 2013), antifungal (Yu *et al*., 2016), anticancer (Zugic *et al*., 2016), antimalarial (Pamenta *et al*., 2020), antibacterial (Maulidiyah *et al*., 2020b; Siddiqi *et al*., 2018), antidiabetic (Maulidiyah *et al*., 2020a), and antioxidant (Maulidiyah *et al*., 2021a) agent. The use of lichen *Usnea* in the healthcare sector, especially medicinal substances, is related to the substances contained therein. Based on Kosanic and Rankovic's (2015) study, lichen has been used to treat diseases caused

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by the bacteria *Staphylococcus aureus* and *Escherichia coli*. *Staphylococcus aureus* has infected many humans, especially the mucosa of the nasal area, upper respiratory tract, gastrointestinal tract, and skin. *Escherichia coli* bacteria also can cause infections in the human digestive tract such as diarrhea (Karve *et al*., 2017).

Bacteria can be inhibited by secondary metabolites of lichen, such as lauric acid compounds from *Usnea barbata* (Rankovic *et al*., 2012), a usnic acid compound from *U. longissima* (Maulidiyah et al., 2016a), a protolichesterinic acid compound from *Usnea albopunctata* (Sasidharan *et al*., 2014), and atranorin from *Usnea rubrotincta* (Sasidharan *et al*., 2014). According to Sudarwanti *et al*. (2018), the acetone extract is more effective at inhibiting bacterial growth because it contains various types of secondary metabolite compounds such as flavonoids, alkaloids, tannins, and terpenoids. Some probable antibacterial mechanisms of lichen substances are inhibition of protein and nucleic acid synthesis, cell membrane alteration, cell wall, and inhibition of metabolism (Rankovic and Kosanic, 2019).

Overall, only a few lichens have been studied for their chemical content and biological activity and many have not been exploited, especially lichen *U. blepharea* Motyka. Lichen *U. blepharea* Motyka is a specific lichen that we have been studying in recent years. This lichen is an endemic lichen that grows in the mountainous region of Sulawesi, Indonesia. Several secondary metabolites have been reported for this species, including usnic acid (Maulidiyah *et al*., 2015a) and eumitrin A1 (Maulidiyah *et al*., 2015b). As another novelty of this study, we specifically studied the characteristics of the secondary metabolite obtained, namely diffractic acid. This has not been carried out before by other researchers.

EXPERIMENTAL METHOD

Materials

One of the materials used in this research is a sample of lichen *U. blepharea* Motyka which was obtained from Mount Bawakaraeng, Malino, Gowa Regency, South Sulawesi, Indonesia. Acetone, n-hexane, ethyl acetate, dichloromethane, chloroform, diethyl ether, ethanol, glacial acetic acid, deuteriochloroform, 95% ethanol, distilled water, dimethyl sulfoxide, H_2SO_4 10% in methanol, chloramphenicol (Bernofarm), and amoxicillin (Bernofarm) were obtained from Sigma-Aldrich. Nutrient agar, peptone 0.5%, agarose 3%, beef extract, NaCl, and $BaCl_2$ were obtained from Merck, Germany. Other materials used were silica gel 60 (Merck 7732) for column chromatography, silica gel 60 F254 no. 1.05554.0001 for aluminum and glass thin-layer chromatography (TLC) plates (Merck), and bacteria: *E. coli* and *S. aureus*.

Isolation of secondary metabolite from lichen U. blepharea Motyka

Lichen *U. blepharea* Motyka in powder as much as 500 g was extracted continuously using Soxhlet with an acetone solvent for 8 hours till a crude extract of acetone was obtained. The crude extract of acetone was evaporated in a vacuum rotary evaporator. Furthermore, the dry extract was tested using TLC with a plate size of 1 × 4 cm to determine the composition of the solvent that gave the best separation. The TLC plate was sprayed with a stain of 10% H_2SO_4 solution in methanol. The dry extract was separated by gravity column chromatography (GCC) with a column size of 50 ml and a diameter of 2 cm. The preparation of GCC was carried out by the stationary phase of 60 G silica gel eluted with a mixed solvent of n-hexane and ethyl acetate in a gradient. Each eluted was collected every 60–100 ml and these were grouped based on the similarity of the stain spots in TLC. The almost pure fraction was purified based on differences in polarity in the solvent, while the impure fraction was refined using column chromatography and preparative TLC.

Identification of the isolate structure

The structure of the pure isolates obtained was a determined compound using 1D-nuclear magnetic resonance [(NMR) (^{1}H and ^{13}C)] (JEOL JNM ECA 500), Fourier-transform infrared spectroscopy (FTIR) (Shimadzu IRAffinity-1S system), UV-Vis spectrophotometer (UV 3150 Shimadzu Corp., Japan), and liquid chromatography-mass spectrophotometer (LC-MS) (LCMS 8060 System). Based on the spectroscopic measurements data, each molecular structure was determined by comparing each spectrum with data from the literature and database (ChemOffice) and based on the basic theory of spectroscopy.

Antibacterial inhibitory activity test

Antibacterial activity was tested by the diffusion method using the well technique (Oh *et al*., 2018). The first stage was making the base of the test media using 10 ml nutrient agar (NA) media, which was poured into a sterile Petri dish and then left for 15 minutes until it became solid. After the solid NA media solidified, six anchors were placed as a mold for holes or wells. Semisolid media were prepared and homogenized by mixing 200 µl of bacterial suspension into 10 ml of liquefied NA media in a test tube. Semisolid media were poured into the Petri dish around the storage device and allowed to solidify. The backup tool was then removed using sterile tweezers to form a hole (well). A total of 30 µl of test isolate samples was dropped into the well with various concentrations of 1,000, 750, 500, 100, 50, and 25 ppm, respectively, followed by positive and negative controls. The positive control used 1,000 ppm chloramphenicol and amoxicillin, while the negative control used distilled water and acetone. Petri dishes containing the test compound were put into an incubator at 37°C for 24 hours. The results of the antibacterial power test are based on measuring the diameter of the inhibition area formed around the well hole.

RESULTS AND DISCUSSION

Isolation of lichen U. blepharea Motyka

Isolation and purification produced isolate compounds in the form of white needle crystals, with a melting point of 190°C–191°C, which dissolved in chloroform and acetone. Based on the liquid chromatography (LC) data, the high-intensity chromatogram (T2.4) showed the purity of the obtained isolated compound (Fig. 1A). This was confirmed by another chromatogram with a small intensity. Based on the mass spectrometry (MS) data of the isolate compounds (Fig. 1B), the compound with the addition of hydrogen shows the peak of the molecular ion $[M + H]^+$ at $m/z = 375.09$ and indicates a molecular weight of $[M]^+ = 374.09$. Based on the report of Huneck and Yoshimura (1996), a melting point with a range of 190°C–191°C and a molecular weight of

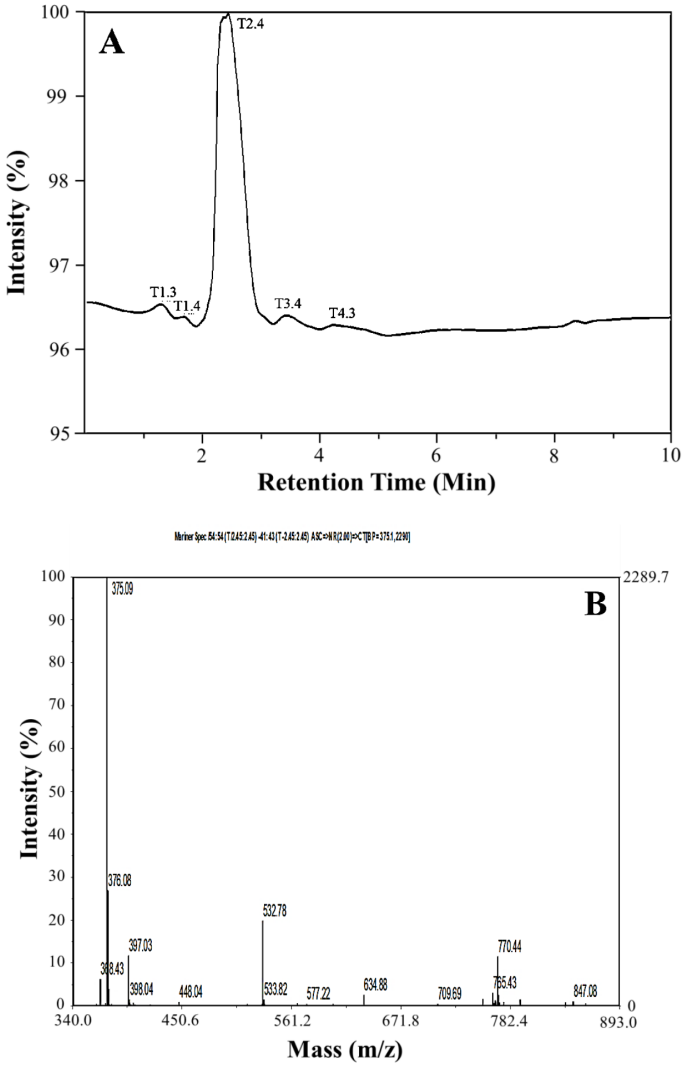

Figure 1. Chromatogram of data measurement. (A) LC and (B) MS.

$[M]^+$ = 374 indicates a diffractic acid compound ($C_{20}H_{22}0_7$). To strengthen this assumption, we continue to interpret the UV-Vis spectrophotometer, FTIR, and NMR data.

The UV-Vis spectroscopic data of the isolates were measured at a wavelength of λ = 300–500 nm in an acetone solvent which gave maximum absorption at a wavelength of λ_{max} = 324 nm (Fig. 2). Based on a study of the literature, this wavelength describes two electronic transition states for isolates, namely the $n \rightarrow \pi^*$ transition originating from the carbonyl group and the $\pi \rightarrow \pi^*$ transition originating from the benzene ring and carbon bond. This situation is strengthened by the high absorption intensity (Abid *et al*., 2017; Bayir *et al*., 2006; Belay and Gholap, 2009). Determination of the functional groups of isolated compounds used FTIR (Fig. 3). The FTIR spectrum of the isolate compound obtained the absorption band in the region of wavenumber (υ) 3,726 cm^{-1} indicating the presence of an OH group; the absorption band at wavenumber 2,954 cm^{-1} is the asymmetric stretching vibration C–H. The presence of an absorption band at wavenumber 1,735 cm^{-1} is the vibration of the carbonyl group, while the absorption band at wavenumber 1,612 cm^{-1} is the vibration of the C=C bond from aromatics. Furthermore, the absorption band of 1,134 cm^{-1} indicates the presence of C–O–C bonds. Based on the FTIR spectrum data, it can be estimated that the isolate compound has a hydroxy group (−OH), a carbonyl group (C=O), an aromatic group, and a CH_3 group.

Based on the ^{1}H-NMR spectrum, Figure 4 and Table 1 show the presence of two methoxy groups, namely the chemical shifts δ_H = 3.83 ppm (3H, s) and 3.89 ppm (3H, s). δ_H = 6.67 ppm (1H, s) and 6.75 ppm (1H, s) indicate the presence of two protons, each bound to the aromatic and isolated rings. The presence of four singlet methyl groups is seen in the chemical shifts δ = 2.13 ppm (3H, s), 2.12 ppm (3H, s), 2.44 ppm (3H, s), and 2.61 ppm (3H, s).

The ^{13}C-NMR spectrum (Fig. 5) accompanied by distortionless enhancement by polarization transfer (DEPT) (Fig. 6A) shows that the compound contains 20 carbon atoms consisting of 4 methyl groups, 2 methoxy groups, 2 methine groups, and 12 quaternary atoms. Four methyl groups are seen at δ_C = 9.11, 9.36, 20.04, and 24.01 ppm. Two methoxy groups were seen at C = 56.25 ppm and 62.39 ppm. Two methine (CH) groups were seen at δ_C = 117.18 and 109.10 ppm. The chemical shift C = 166.54 and 174.36 ppm is the carbon of the carbonyl group. Chemical shifts δ_C = 110.33, 117.85, 120.92, 136.05, 141.13, and 160.92 ppm are quaternary olefinic carbon; δ_C = 154.44, 157.89, 160.92, and 164.07 ppm are quaternary carbons bonded to oxygen atoms. The ^{13}C-NMR data is supported by the ^{1}H-NMR data.

^{13}C-NMR data show that there are two carbons which are carbons of the carbonyl group. This is reinforced by the FTIR spectrum data at a wavenumber of 1,735 cm^{-1}. A wavenumber of 1,134 cm^{-1} indicates the presence of C–O–C bonds strengthening the presence of carbon bonded to oxygen atoms. Based on comparing the spectroscopic data from the isolate compound with the compounds in the literature, the isolate compound was very similar to the diffractic acid compound. This is reinforced by the heteronuclear multiple quantum correlation (HMQC) spectrum (Fig. 6B). It can be seen that the position of C-8^1 (δ_C = 9.11 ppm) correlates with protons δ_H = 2.12 ppm. The position of C-8^1 (δ_C = 9.36 ppm) correlated with protons H = 2.13 ppm, C-9 (δ_C = 20.04 ppm) correlated with protons δ_H = 2.44 ppm, C-91 (δ_C = 24.01 ppm) correlated with protons H = 2.61 ppm, C-2-OCH_3 (δ_C = 62.39 ppm) correlated with protons δ_H = 3.83 ppm, C-4-OCH_3 (δ_C = 56.25 ppm) correlated with protons δ_H = 3.89 ppm, C-5 (δ_C = 109.10 ppm) correlated with protons δ_H = 6.75 ppm, and C-5^1 (δ_C = 117.18 ppm) correlated with protons δ_H = 6.67 ppm.

Based on correlated spectroscopy (COSY) (Fig. 6C), a proton with a chemical shift δ_H = 2.61 ppm correlates with a proton δ_H = 6.67 ppm. Protons δ_H = 2.44 ppm correlated with protons δ_H H = 6.75 ppm. Protons δ_H = 3.89 ppm correlated with protons δ_H = 6.75 ppm. A proton with H = 3.83 ppm a proton with H = 2.12 ppm. The heteronuclear multiple bond correlation (HMBC) (Fig. 6D) shows the correlation between proton H-5 at δ_H = 6.75 (s) and carbon C-4 (δ_C = 160.91), C-9 (δ_C = 20.04), and C-3 (δ_C = 120.92). Proton H-5^1 at δ_H = 6.67 (s) correlated with carbon C-4^1 (δ_C = 154.44), C-31 (δ_C = 117.52), C-11 (δ_C = 110.33), and C-91 (δ_C = 24.01). Proton H-8 at δ_H = 2.12 (s) correlated with carbon C-4 (δ_C = 160.92) and C-2 (δ_C = 157.89). Proton H-9 at δ_H = 2.44 (s) correlated with carbons C-1 (δ_C = 117.85), C-6 (δ_C = 136.05), and C-5 (δ_C = 109.11). The proton δ_H-8^1 is at H = 2.13 with carbons C-2^1 (δ_C = 164.06), C-5^1 (δ_C

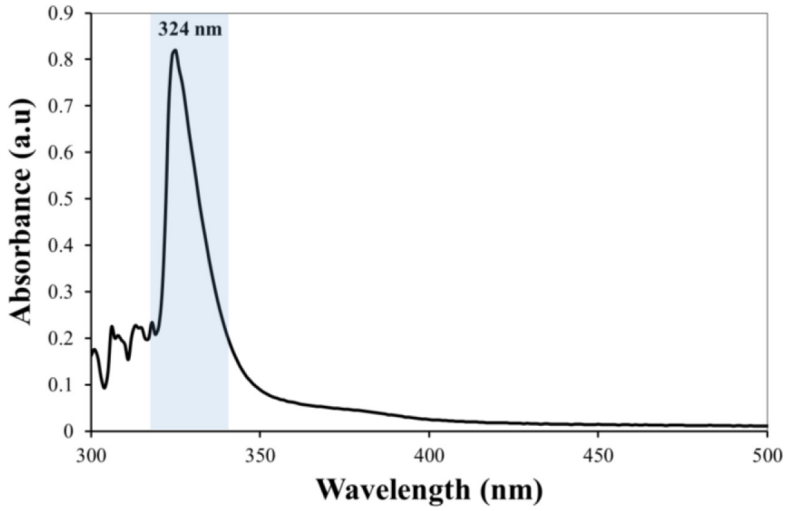

Figure 2. UV-Vis spectroscopic data of isolate compound.

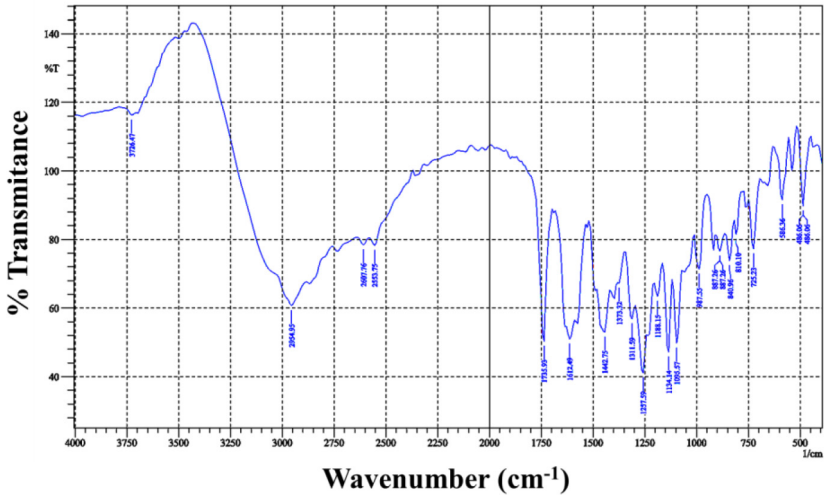

Figure 3. FTIR spectrum of isolate compounds.

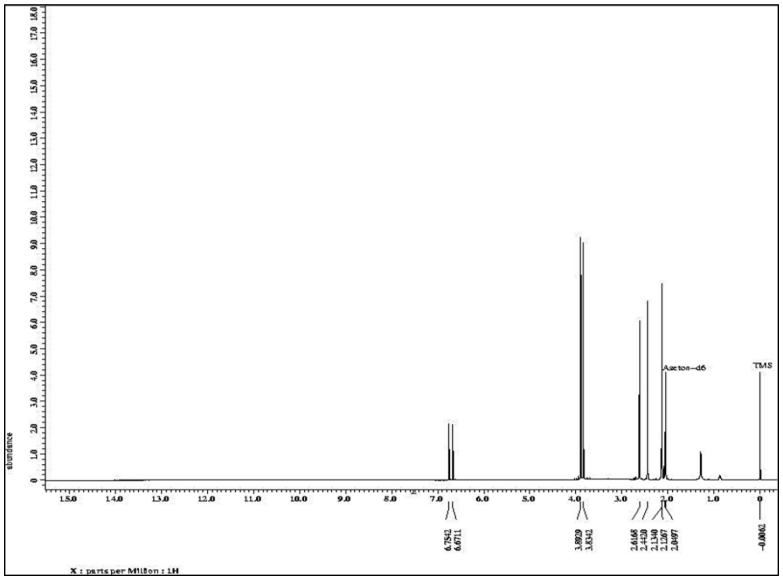

Figure 4. [1]H-NMR spectrum data of compound isolate.

Table 1. ^{1}H-NMR and ^{13}C-NMR data for isolate and diffractic acid compound.

H	*C*	Isolate compound		Diffractic acid[a]		HMBC isolate compound
		δ_H (ppm)	δ_C (ppm)	δ_H (ppm)	δ_C (ppm)	
	1		117.85		119.7	
	2		157.89		159.5	
	3		120.92		116.1	
	4		160.92		156.4	
H-5	5	6.75 (s)	109.11	6,75 (s)	108.4	4, 3, 9
	6		136.05		134.6	
	7		166.54		165.5	
H-8	8	2.12 (s)	9.11	2.18 (s)	8.8	2, 4
H-9	9	2.44 (s)	20.04	2.47 (s)	19.5	1, 5, 6
	1^1		110.33		116.5	
	2^1		164.07		161.7	
	3^1		117.52		111.0	
	4^1		154.44		152.4	
H-5^1	5^1	6.67 (s)	117.18	6.67 (s)	115.8	1^1,3^1,4^1,9^1
	6^1		141.13		139.3	
	7^1		174.36		173.4	
H-8^1	8^1	2.13 (s)	9.36	2.18 (s)	9.0	2^1, 3^1, 4^1
H-9^1	9^1	2.61 (s)	24.01	2.61 (s)	23.0	1^1, 5^1, 6^1
2-OCH_3	2-OCH_3	3.83 (s)	62.39	3.85 (s)	61.7	2
4-OCH_3	4-OCH_3	3.89 (s)	56.25	3.87 (s)	55.7	4
2^1-OH				11.7 (s)		

[a]Adapted from Huneck and Yoshimura (1996).

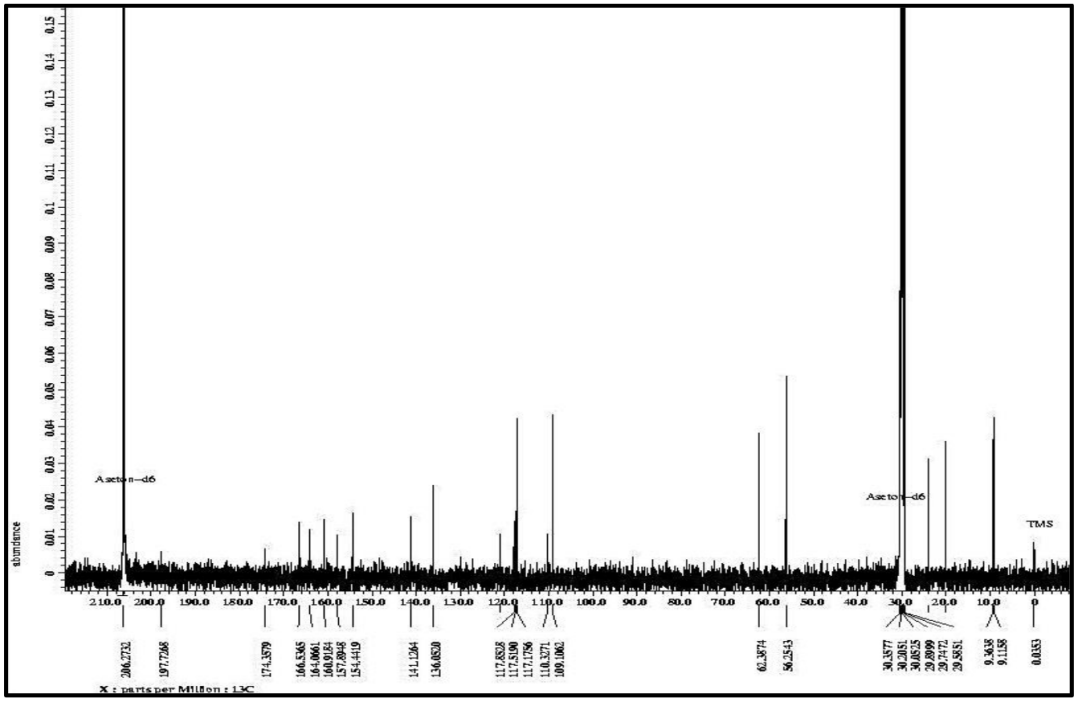

Figure 5. ^{13}C-NMR spectrum data of compound isolate.

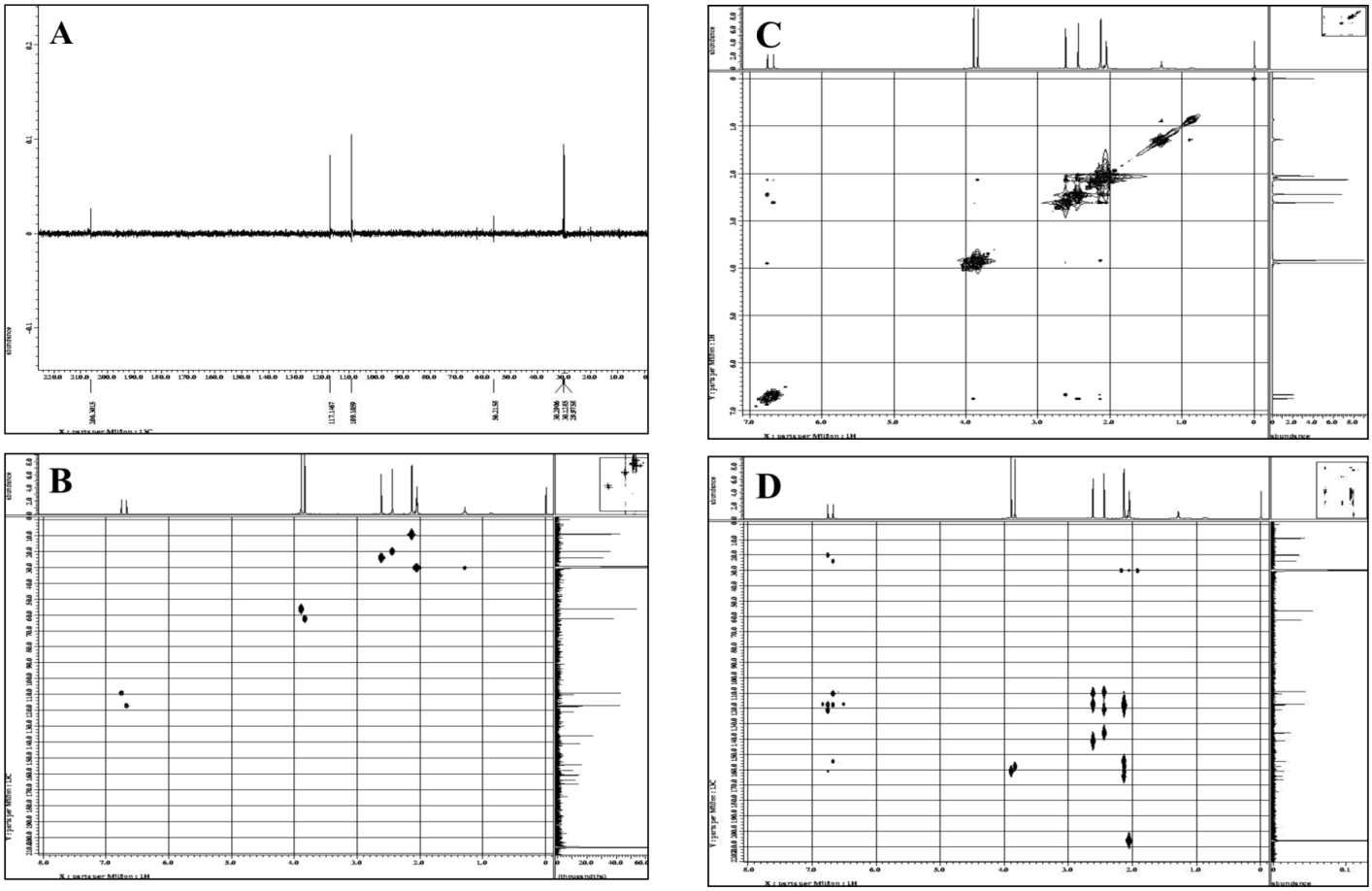

Figure 6. NMR-two-dimensional spectrum of (A) DEPT, (B) HMQC, (C) COSY, and (D) HMBC.

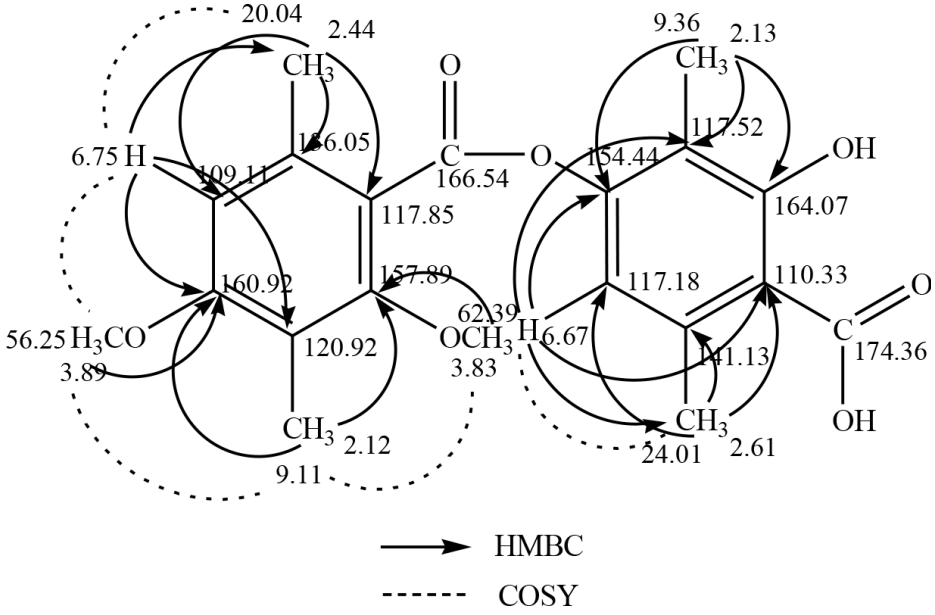

Figure 7. HMBC and COSY analysis of isolate compounds.

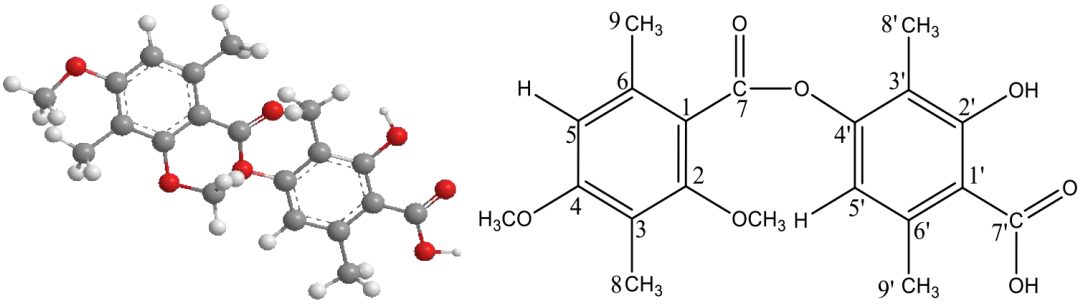

Figure 8. Structure of diffractic acid compound.

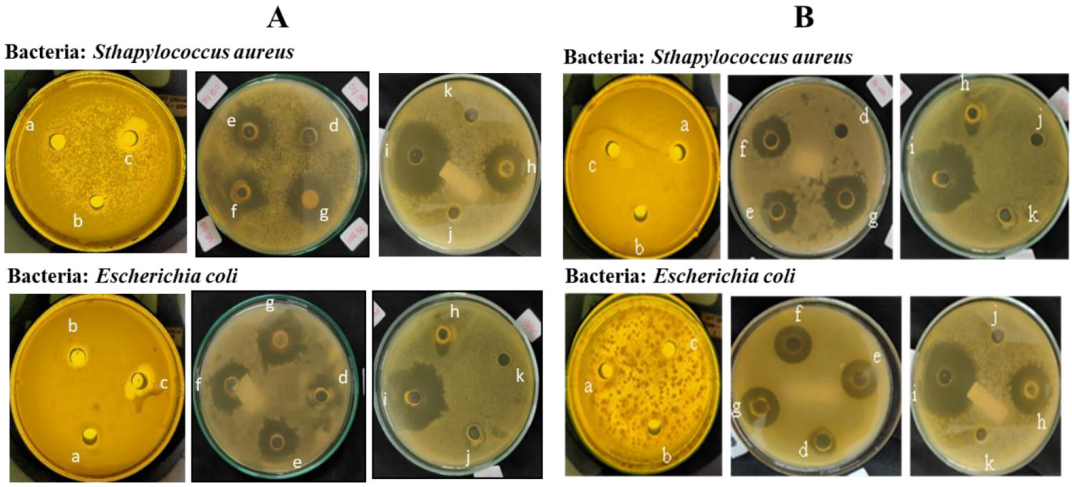

Figure 9. Inhibition zone of (A) acetone extract of lichen *U. blepharea* Motyka and (B) diffractic acid compound. (a) 12 ppm, (b) 25 ppm, (c) 50 ppm, (d) 100 ppm, (e) 500 ppm, (f) 750 ppm, (g) 1,000 ppm, (h) amoxicillin 1,000 ppm, (i) chloramphenicol 1,000 ppm, (j) water, and (k) acetone.

= 117.18), and C-4[1] (δ_C = 154.44). The proton H-9[1] is at δ_H = 2.61 with carbons C-1[1] (δ_C = 110.33), C-3[1] (δ_C = 117.52), and C-6[1] (δ_C = 141.13). Proton 2-OCH_3 in chemical shift δ_H = 3.83 correlated with carbon C-2 (δ_C = 157.89). Proton 4-OCH_3 in chemical shift δ_H = 3.89 correlated with carbon C-4 (δ_C = 160.92). HMBC and COSY analysis of isolate compounds can be seen in Figure 7.

Based on the measurement results of ^{1}H-NMR, ^{13}C-NMR, FTIR, UV-Vis, and LC-MS, the structure of the compound isolate is diffractic acid with the molecular structure formula $C_{20}H_{22}O_7$. Diffractic acid compound is a single compound that has a hydroxyl (OH), methyl (-CH_3), carbonyl (CO), and oxygen bridge (R-O-R) group connecting two benzenes (Fig. 8). Diffractic acid has been found in lichen *Usnea diffracta* Vain (Huneck and Yoshimura, 1996), *U. Heterodea muelleri* (Hampe) Nyl., Stictaceae (Hager *et al*., 2008), *U. longissima* (Culberson, 1969), and *Usnea* sp. (Maulidiyah *et al*., 2021a). Based on the literature search, this compound has never been found in *U. blepharea* Motyka.

Antibacterial activity test

Antibacterial activity testing aims to determine the sensitivity of bacteria to the test sample (Murugesan, 2020; Sisodia *et al*., 2013). In the test, we specifically compared the activity of diffractic acid to amoxicillin and chloramphenicol. The two compounds were used as a comparison, with the concentration 1,000 ppm. In addition to being commonly used in testing the activity of control compounds, the selection of concentrations of both amoxicillin and chloramphenicol is based on the standard dose of the drug and the repetition of daily doses, where the standard doses for both are 500 and 400 mg, respectively (Rocha *et al*., 2019; Yuxuan *et al*., 2019). The sensitivity of bacteria to the test sample is indicated by the formation of a clear zone around the well hole which indicates an area of inhibition of bacterial growth. The results of the inhibition zone measurement of the test sample against *S. aureus* and *E. coli* bacteria are shown in Figure 9A and B.

Based on Table 2, the largest diameter of the zone of inhibition was shown in the acetone extract of lichen *U. blepharea* Motyka at a concentration of 1,000 ppm against *S. aureus* bacteria which was 21.25 mm (very strongly) and *E. coli* bacteria which was 17 mm (strongly). The diffractic acid compound also actively inhibited the growth of *S. aureus* bacteria at a concentration of 1,000 ppm with inhibition zones of 17.25 mm (very strongly) and inhibited the growth of *E. coli* bacteria at a concentration of 1,000 ppm with inhibition zones of 12.75 mm (strongly). Although in general the activity of antibiotic compounds (amoxicillin and chloramphenicol) is better than diffractic acid, with a larger diameter of inhibition zone, the category shows a good potential of diffractic acid as a new antibiotic candidate.

Table 2. Antibacterial test results of acetone extract and diffractic acid from lichen *U. blepharea* Motyka.

Bacteria	Sample	Inhibition zone (mm)	Category
S. aureus (Gram positive)	Amoxicillin 1,000 ppm (+)	22	Very strong
	Chloramphenicol 1,000 ppm (+)	30.75	Very strong
	Acetone (−)	0	Not active
	Water (−)	0	Not active
	Acetone extract 12 ppm	0	Not active
	Acetone extract 25 ppm	2.25	Weak
	Acetone extract 50 ppm	6.5	Moderate
	Acetone extract 100 ppm	12.75	Strong
	Acetone extract 500 ppm	17	Strong
	Acetone extract 750 ppm	20	Very strong
	Acetone extract 1,000 ppm	21.25	Very strong
	Diffractic acid 12 ppm	0	Not active
	Diffractic acid 25 ppm	0	Not active
	Diffractic acid 50 ppm	2.5	Weak
	Diffractic acid 100 ppm	7.5	Moderate
	Diffractic acid 500 ppm	13.27	Strong
	Diffractic acid 750 ppm	14.27	Very strong
	Diffractic acid 1,000 ppm	17.75	Very strong
E. coli (Gram negative)	Amoxicillin 1,000 ppm (+)	15.75	Strong
	Chloramphenicol 1,000 ppm (+)	31.25	Very strong
	Acetone (−)	0	Not active
	Water (−)	0	Not active
	Acetone extract 12 ppm	0	Not active
	Acetone extract 25 ppm	1.25	Weak
	Acetone extract 50 ppm	6.75	Moderate
	Acetone extract 100 ppm	9	Moderate
	Acetone extract 500 ppm	12.5	Strong
	Acetone extract 750 ppm	15.25	Strong
	Acetone extract 1,000 ppm	17	Strong
	Diffractic acid 12 ppm	0	Not active
	Diffractic acid 25 ppm	0	Not active
	Diffractic acid 50 ppm	1.5	Weak
	Diffractic acid 100 ppm	5.2	Moderate
	Diffractic acid 500 ppm	11	Strong
	Diffractic acid 750 ppm	11	Strong
	Diffractic acid 1,000 ppm	12.75	Strong

The diameter of the inhibition zone formed increases with an increasing concentration of the test sample. This is supported by a research report from Maulidiyah *et al*. (2016b), Cankilic *et al*. (2017) and Nugraha *et al*. (2019) where the chloroform extract of lichen *U. blepharea* Motyka was found to increase with increasing concentration. The minimum concentration of each test sample used shows no inhibitory activity. The minimum inhibitory concentration has been shown in this test. The control of acetone and water did not show any inhibition zone formed. This proves that the presence of acetone in the extract of lichen *U. blepharea* Motyka did not affect the diameter size of the inhibition zone of the two tested bacteria. The water used as negative control also did not show the inhibition zone activity of the two test bacteria added to the antibiotic. The OH group in diffractic acid can damage the tertiary structure of the protein on the bacterial cell membrane by forming hydrogen bonds with the N and H atoms in the amino acids that make up the protein (Fig. 10). Furthermore, the damage to the cell membrane results in damage to the transport route for the entry and exit of substances into the cell (Shrestha *et al*., 2016).

The testing antibacterial activity of the acetone extract lichen *U. blepharea* Motyka and diffractic acid compounds against *E. coli* and *S. aureus* bacteria showed a difference in the test results on the average diameter of the resulting inhibition zone. *Escherichia coli* bacteria have an average inhibition zone diameter smaller than

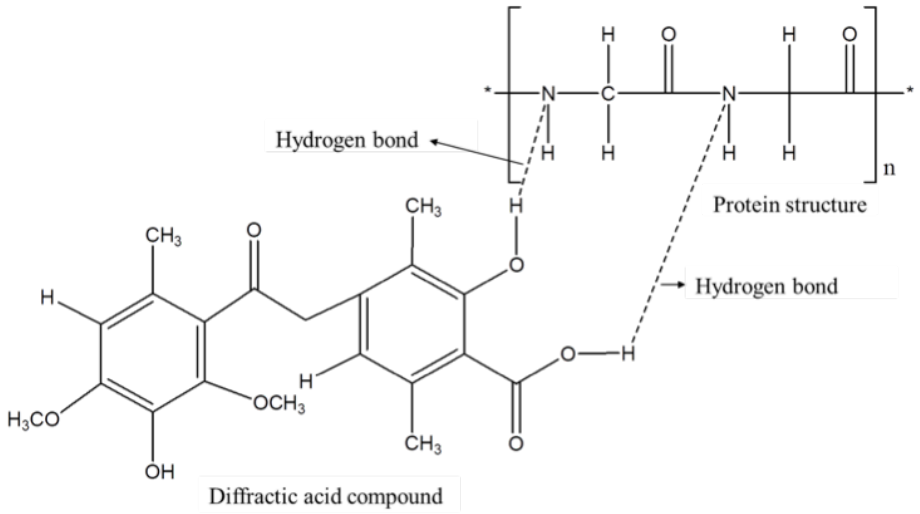

Figure 10. Hydrogen bond formation mechanism in diffractic acid compounds.

the average inhibition zone diameter in *S. aureus* bacteria. This is due to differences in the types between the two bacteria which affect the differences in the structure of the cell wall, plasma membrane, and the pathogenicity of the two bacteria. *Escherichia coli* bacteria belonging to Gram-negative bacteria have a complex cell wall structure (Maulidiyah *et al*., 2018; Sarowska *et al*., 2019). The structure of the Gram-negative cell wall, namely the outer layer, consists of lipoproteins, the middle layer is thin peptidoglycan, and the inner layer is thick lipopolysaccharides (Horvatic *et al*., 2019). This is thought to have caused the activity of the test sample against *E. coli* to be weaker than against the Gram-positive *S. aureus*.

CONCLUSION

The secondary metabolite isolated from *U. blepharea* Motyka is a diffractic acid compound with the molecular formula $C_{20}H_{22}O_7$. Antibacterial bioactivity test showed that the diffractic acid compound actively inhibited (very strongly) the growth of *S. aureus* bacteria at concentrations of 750 and 1,000 ppm with inhibition zones of 14.27 and 17.25 mm, respectively. In addition, *E. coli* bacteria were also actively inhibited (strongly) at concentrations of 750 and 1,000 ppm at 11.00 and 12.75 mm, respectively. This study is very interestingly the latest reference source for natural compounds, especially the lichen *U. blepharea* Motyka, because there have been no reports related to diffractic acid compounds found from this lichen genus and also its activity as a strong antibacterial. Positive results from antibacterial testing of *S. aureus* and *E. coli.* are very important for the development of the pharmaceutical field in the future, especially in the development of diarrhea and antibiotic drugs.

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AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

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