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ATRANORIN SECONDARY METABOLITE FROM LICHEN *Usnea* sp. AND ITS ANTIBACTERIAL ACTIVITY

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ABSTRACT

Isolation and identification of secondary metabolites from lichen *Usnea* sp. fraction of n-hexane and its activity against bacteria have been carried out. The isolation was done by maceration extraction with methanol, then performed a partition with n-hexane solvent. Fractions of n-hexane was then separated by gravity column chromatography (GCC) and thin layer chromatography (TLC) techniques using a gradient eluent mixture of n-hexane : ethyl acetate. The results of data analysis-1D NMR spectroscopy (^{1}H and ^{13}C-NMR), 2D NMR (HMQC, HMBC and ^{1}H-^{1}H COSY) were compared with similar data from the literature. The isolated compound was identified as atranorin. The antibacterial activities test were applied on the fraction of n-hexane and isolated compound. Test results of n-hexane fraction activity against *Escherichia coli* (ATCC 35218), *Staphylococcus aureus* (ATCC 25923) and *Salmonella typhi* (YCTC) with a diameter of inhibition zone on the concentration of 500 mg/mL were 9.33, 10.6 and 10.6 mm, respectively. While, test of isolated compound activity against the same three types of bacteria at concentrations of 500 mg/mL were 3.0, 4.0 and 5.3 mm, respectively.

KEYWORDS: **Isolation, identification, lichen, Usnea sp., antibacterial, atranorin**

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INTRODUCTION

Indonesia is a tropical country which is rich of medicinal plants that can be used as the raw substances in chemical and pharmaceutical industries [1-2]. One of the source of medicinal plants is lichen. Lichen is a kind of lower and a unique plant. Lichen is classified into two organisms that are fungi and algae [3-4]. Lichen produces very useful secondary metabolite substances which have bioactivity including antibiotic or antibacterial agent [5-7]. Antibacterial compounds has been widely cultivated either from plants or synthesis process [8]; however, one kind of antibacterial agent, usnic acid, which was invented by Huneck and Yoshimura in lichen from genus *Usnea* also possesses considerable effectiveness as antibacterial agent [9]. Several species of lichen are widely cultivated as traditional medicines in several countries [10-12]; for instance, the species of lichen called *Cetraria islandica* (L.) Ach is processed as cough medicine and *Loboria pulmonaria* (L.) Hoffm. is cultivated as the medicine for lung disease [13]. Similarly, *Usnea baileyi* Spirt. is one of the substances combined to produce the medicine for lung disease [14].Some compounds of lichen exhibits, activities as antibiotic and anticancer which can hinder the growth of pathogenic organisms. Eumitrin A1 which is isolated from *U. blpeharea* Motyka has potential cytotoxic activity towards murine P388 cell [15]. Usnic acid, which is isolated from genus *Usnea* has wide spectrum of antibacterial activity and can be used as a remedy for burn [16]. In addition to usnic acid, other secondary metabolite compounds which are isolated from genus *Usnea*, i.e. galbanic acid, barbituric acid, salazinic acid, diffractaic acid, and con salazinic acid, demonstrate antibacterial activity[9,17]. A secondary metabolite compound from genus *Pseudevernia furfuracea* that is chloroatranorin demonstrates antibacterial activity [18]. A research by Vivek et al. reported that lichen extract from genus *Parmotrema* exhibits gram-positive antibacterial activity [19]. In addition to its function as antibacterial agents, lichen has many bioactivities such as anticancer, anti-fungi, antioxidant, and anti-malaria [20]. Lichen contains secondary metabolite compounds that can prevent and cure illness, such as infection [21]. Infection is an illness caused by bacterium i.e. *Escherichia coli, Staphylococcus aureus*, and *Salmonella typhii* are the examples of bacteria that set off infection [22-24]. In Indonesia, infection ranks is the first in term of its dissemination. The main remedy for infectious diseases caused by bacterium is antibacterial. Antibacterial is particular compound that can prevent the growth process of bacterium. Based on those elaborations, several studies on bioactivity of lichen had been conducted. However, the objective of this study is to identify of secondary metabolite from lichen *Usnea* sp. originating from Sulawesi. Therefore, it is required to conduct isolation and identification of the structure of the secondary metabolite content of lichen *Usnea* sp. and its bioactivity testing as antibacterial agent towards *Escherichia coli* (ATCC 35218), *Staphylococcus aureus* (ATCC 25923), and *Salmonella typhii* (YCTC).

MATERIALS AND METHODS

Materials:Materials used in this research were: Lichen (*Usnea* sp.), methanol, acetone, ethyl acetate, n-hexane, chloroform p.a. (Merck), ethanol 95%, Whatman 42 filter paper, GF254 p.a silica gel (E. Merck), G.60 silica gel, sea sand, $CeSO_4$, media of Nutrient Agar (NA), antibiotics (chloramphenicol), and bacterial culture of *E. coli* (ATCC 35218), *S. aureus* (ATCC 25923), and *S. typhii* (YCTC).Research Methods:The stage of the research which was conducted includes isolating secondary metabolite compound from lichen. The isolation itself consists of several stages, including sample preparation, extraction, fractionation, and purification. It was explained as follows, the powder of lichen *Usnea* sp. has been mashed then weighed of 550 g and extracted using methanol for 3 x 24 hours; furthermore, the separated (solvent and extract) using a rotary evaporator. The results of crude extract in a partition using n-hexane solvent (n-hexane fraction) and tested the antibacterial activity.TLC test followed by the comparison eluent to obtain a suitable solvent for the separation process in the GCC (diameter 13 cm). Then, GCC process and uniting of the extract from the TLC results. Further, the extract was recrystallized and tested purity by TLC using three eluent systems. The identification of the structure was performed by using spectroscopy of NMR 1D (^{13}C-NMR, ^{1}H-NMR) and NMR 2D (HMQC, HMBC, and COSY) and bioactivity testing as an antibacterial agent.

RESULTS AND DISCUSSION

1. Isolation of Secondary Metabolites Compounds

Extracted methanol was partitioned using n-hexane as the solvent as depicted by chromatography; that is illustrated by several compounds with low polarity that have been distributed into n-hexane solvent.The separation of secondary metabolite compound was performed using GCC which produced fractions distributed based on each compound's polarity difference. The compound separation process causes transfer based on the solubility of compounds at solvent or eluent of two combined solvents and the polarity of compound which experiences stationary phase (silica) and solvent as mobile phase. The solvent used is 100 mL of n-hexane 100%; this solvent was used to separate oil content from the extract. Then, solvent or n-hexane eluent and ethyl acetate with the ratios of 9:1, 8:2, 7:3 were mixed up until the eluent became more polar. The polarity was increased in order to make the compound of lichen easily distributed that the compound possesses greater degree of polarity. The gradient system of eluent used can be seen in Table 1.

Table 1
Gradient System of Eluent used in the fraction of n-hexane

Solutions System (mL)	Solution Volume	Fraction Stage	Colors of Fractions
n-hexane 100%	100 mL	1-3	Clear
90 : 10 mL *n*- hexane – ethyl acetate	200 mL	4-14	Dark yellow
80 : 20 mL *n*- hexane – ethyl acetate	300 mL	15-17	Yellow-black
70 : 30 mL *n*- hexane – ethyl acetate	300 mL	18-20	Black
60 : 40 mL *n*- hexane – ethyl acetate	100 mL	21-24	Greenish yellow
50 : 50 mL *n*- hexane – ethyl acetate	100 mL	25-27	Light yellow
40 : 60 mL *n*- hexane – ethyl acetate	100 mL	28-29	Light yellow
30 : 70 mL *n*- hexane – ethyl acetate	100 mL	30-31	Tawny
20 : 80 mL *n*- hexane – ethyl acetate	100 mL	32-33	Light yellow
10 : 90 mL *n*- hexane – ethyl acetate	100 mL	34-35	Light brown
100% Methanol	100 mL	36	Clear

Two grams of extract of lichen from the fraction of n-hexane were separated using GCC (the diameter is 3 cm and the length is 1 m) with 20 gram G60 silica and a mixture of n-hexane as stationary phase and ethyl acetate as mobile phase. GCC process produced 35 fractions with various fraction colors (eluate) generated based on the order of separation; i.e. orange fraction, light yellow fraction, black fraction, and light brown fraction. Those 35 fractions were then tested using TLC to identify compound patterns of the fractions using the ratio (8:2) of n-hexane and ethyl acetate (Figure 1).

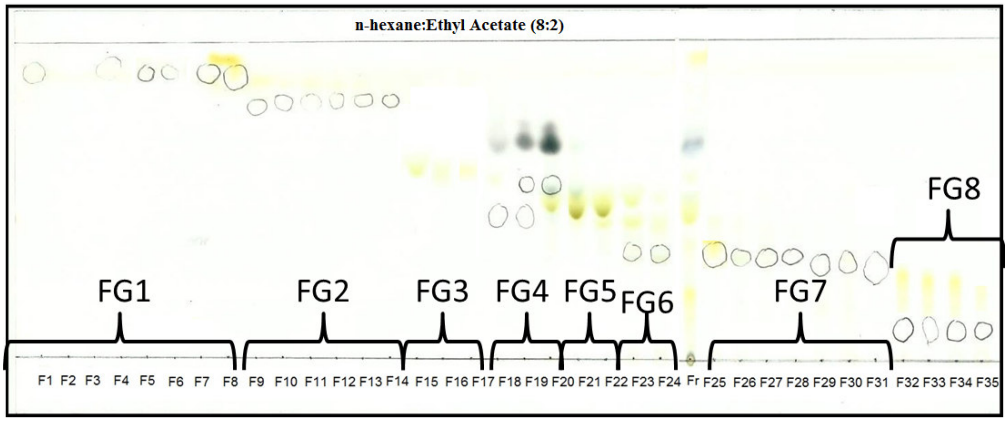

Figure 1
Chromatogram from the Results of GCC

Fractions from the combination were then tested using TLC with the ratio of n-hexane and ethyl acetate of 8:2 to identify their compound patterns. Fraction 3 shows stain spot which is practically pure as exhibited in Figure 2.

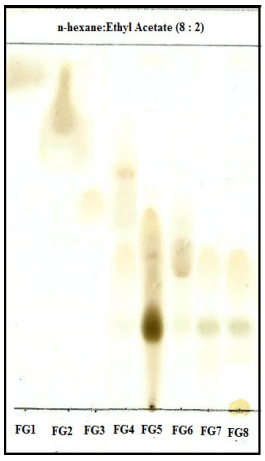

Figure 2
Chromatogram from the Combination

The result of TLC from combined fractions as displayed in Figure 2 depicts various stain spots which have different Rf values; however, the chromatograph shows that compound spot which is segregated well is observed in fraction 3 thus it becomes the target for the next separation and purification. Fraction 3 in the form of crystal which is further purified produced white crystals. There are also sediments that attach to crystals; hence, purification was conducted by rinsing the crystals with n-hexane solvent which cannot dissolve crystals but the

debris attached to them. The crystals were then further purified by implementing recrystallization process using heated methanol as the solvent used to facilitate the process of solvent evaporation and the formation of crystals. Fraction 3 was recrystallized during TLC to observe the purity pattern of the compound (Figure 3A). Chromatogram of TLC can be seen in Figure 3B.

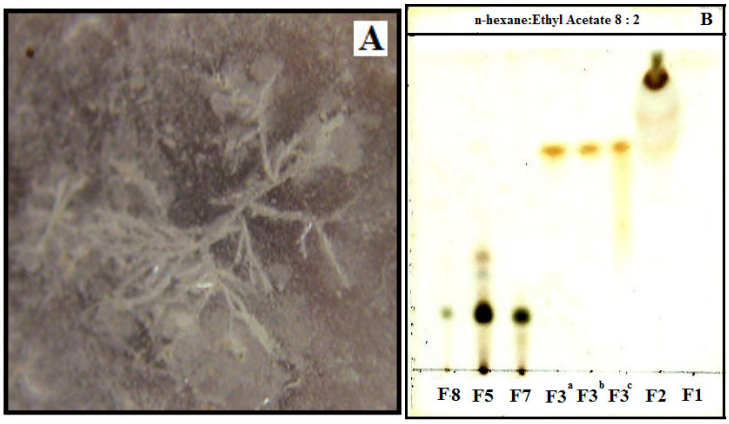

Figure 3
A). White Crystals from Fraction 3, B) Chromatogram of TLC.

1.1 Chromatogram from the results of recrystallization of F1, F2, F3, F5, F7, and F8

Chromatogram from the results of recrystallization in Figure 3 represents compound spot that had been pure which is F3; F3 was then further examined to identify its degree of purity. To see the degree of purity of particular compound, purity testing must be undertaken using 3 kinds of solvent with 3 different polarity qualities in one eluent, i.e. polar, semi-polar, and non-polar. Those solvents are acetone, methanol, ethyl acetate, chloroform, and n-hexane. The results of purity testing using 3 eluent systems are displayed in Figure 4.

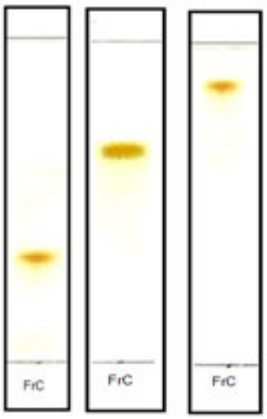

Figure 4
Isolates Chromatogram with 3 eluent systems
(a) chloroform : aceton (8:2) (b) n-hexane : Ethyl acetate (8:2) (c) n-hexane : chloroform : methanol (8 : 1.5 : 0.5)

Chromatogram of purity testing using 3-solvent system exhibited in Figure 4 represents stain spot which cannot be separated again (single) thus it can be stated that this compound is pure. If the compound stain spot experienced separation during this test due to the different polarities in one solvent system, it can be stated that the compounds are not pure yet. Pure compound depicted in chromatogram has only 1 spot which is then called as isolates. The structure of isolates is identified by using spectroscopy technique.

1.2 dentification of the Structure of Isolates

Isolates which is obtained from lichen *Usnea* sp. is solidified white crystal shaped like nail. The identification of the structure of isolates was carried out using ^{1}H-NMR and ^{13}C-NMR spectrophotometry methods with DEPT homonuclear (1D) and heteronuclear (2D) techniques including HMBC, MHQC, and COSY.

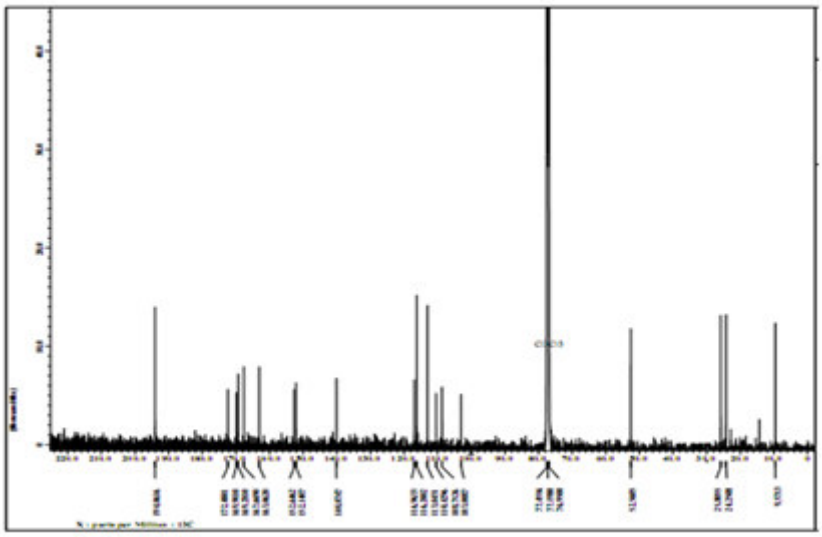

Figure 5
The Spectrum of ^{13}C-NMR isolates

Figure 5 represents the data of isolates which contains 19 carbon signals indicating that the isolates has 19 carbon atoms which consist of 3 carbons from carbonyl group during the shift of δ_C = 169.9010; 194.0616; and 172.4001 ppm. The value of normal carbonyl chemical shift is 205 ppm and yet the result of measuring the spectrum of ^{13}C-NMR decreased; this was because carbonyl group was conjugated with double group that triggered resonance inside benzene ring. Three carbon methyl atoms were in δ_C = 24.2508; 25.8055 and 9.5713; the chemical value of one carbon methyl which was bonded in ester group is δ_C = 52.5606 ppm. Four quaternary C atoms which bond O atom were represented by chemical shift of δ_C = 167.6690; 167.6690; 152.1407 and 163.0620 ppm

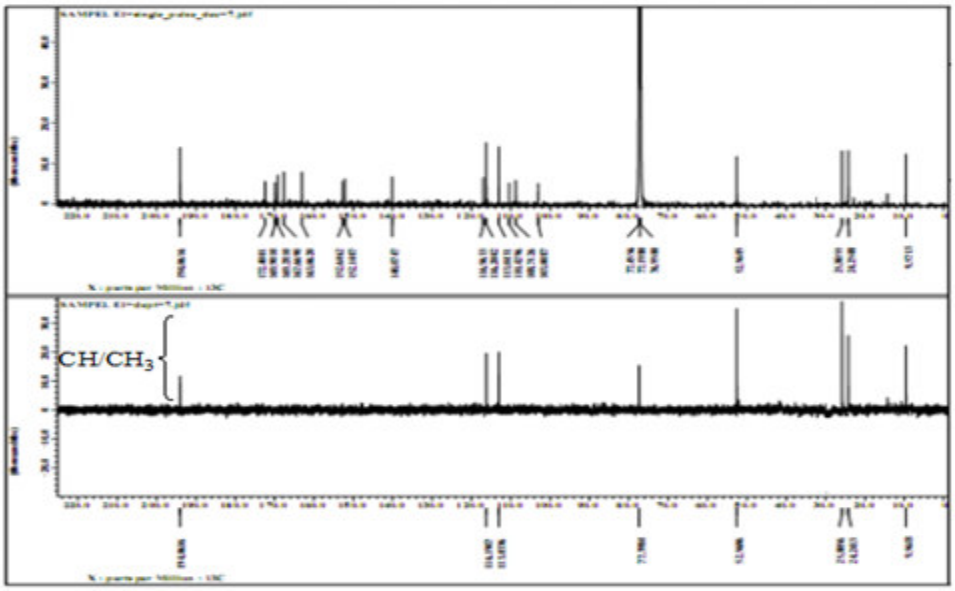

Figure 6
^{13}C-NMR with DEPT technique

^{13}C-NMR analysis using DEPT technique depicted in Figure 6 was implemented to identify three kinds of carbon from the separation of spectrums of carbon detected. The kinds of carbon are methylene (CH_2), methyne (CH) and methyl (CH_3). The spectrums of carbon methyne (CH) and methyl (CH_3) will emerge at the top based on each own value of chemical shift, while carbon methylene (CH_2) will be at the bottom. The results of analysis on ^{13}C-NMR spectrum 500 Hz using DEPT technique show that isolates has three carbon methyl, 1 methyl from carboxylic acid that is ester ($COOCH_3$) and 2 carbon methyne (CH) from aromatic. While the ^{1}H-NMR spectrum of isolates can be seen in Figure 7. The data of chemical shift ^{1}H-NMR and ^{13}C-NMR of the isolates can be illustrated on Table 2.

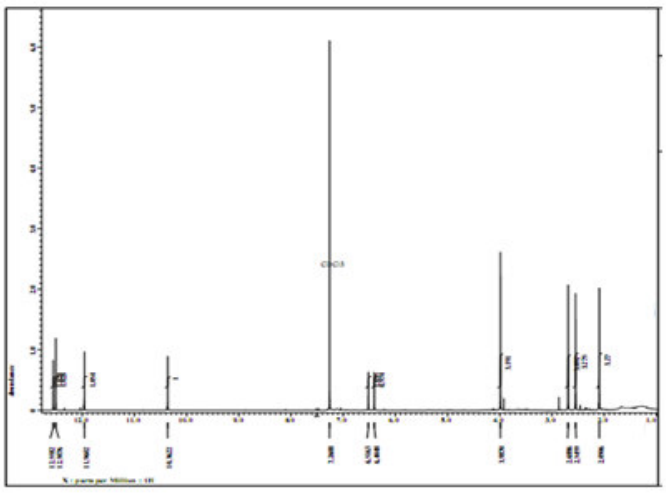

Figure 7
^{1}H-NMR Spectrum of Isolates

Table 2
The Data of Chemical Shift ^{1}H-NMR and ^{13}C-NMR of Isolates

C Position	^{1}H-NMR	^{13}C-NMR	Carbon Type
1		103.0087	**C**q
2	12.5076 (1H,s, HO-2)	169.2810	**C**-OH
3		108.7126	**C**q
4	12.5582 (1H,s,HO-4)	167.6690	**C**-OH
5	6.4048 (1H,s,H-5)	113.0431	**C**-H
6		152.6462	**C**q
7		169.9010	**C**O
8	10.3622 (1H,s,CHO-8)	194.0616	**C**HO
9	2.5459 (3H, s, Me-9)	24.2508	**C**H_3
1’		116.2002	**C**q
2’	11.9602 (1H,s,HO-2’)	163.0620	**C**-OH
3’		110.4296	**C**q
4’		152.1407	**C**q
5’	6.5163 (1H,s,H-5’)	116.9633	**C**-H
6’		140.0747	**C**q
7’	3.9870 (3H,s,COOMe-7’)	172.4001	**C**OOMe
8’	2.0906 (3H, s, Me-8’)	25.8055	**C**H_3
9’	2.6886 (3H,s, Me-9’)	9.5713	**C**H_3
		52.5606	O-**C**H_3

NB: Cq is quartenary carbon
Based on the calculation, the value of the total double bonds of isolates is:
F = X – 0,5Y + 0,5Z + 1, maka F = 19 - 0,5 (18) + 1 = 11. Where :
F = the total of rings or double bonds
X = the total of tetravalent atoms (C)
Y = the total of monovalent atom (H, F, B, Cl)
Z = the total of trivalent atom (N, P)

From the result of calculation, it can be stated that isolates has 11 double bonds with the molecular formula of $C_{19}H_{18}O_8$ which is represented by the predited structure of isolates in Figure 8.

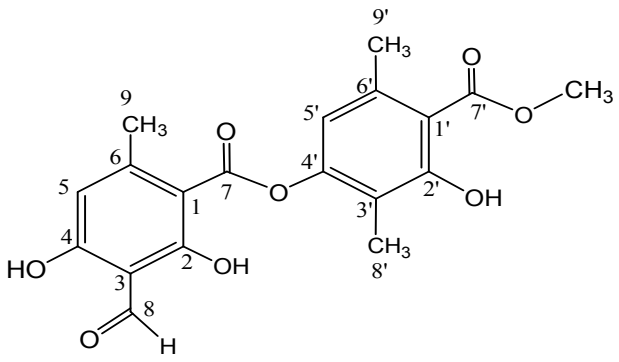

Figure 8
Predicted Structure of Isolates

Measurement was then conducted using NMR-2D spectroscopy with HMQC and HMBC techniques to validate the predicted structure of isolates. HMQC spectrum of isolates is exhibited in Figure 9.

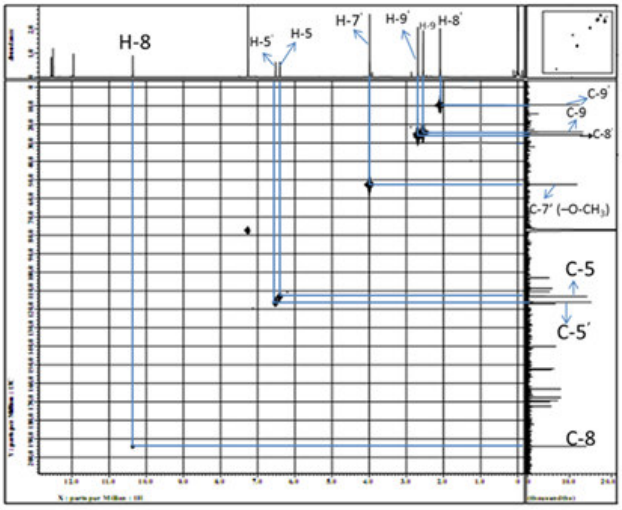

Figure 9
HMQC Spectrum of Isolates

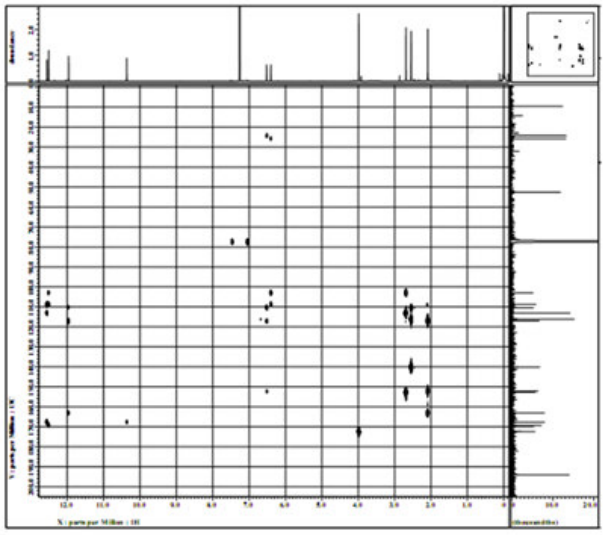

Figure 10
HMBC Spectrum of Isolates

The spectrum and data of HMBC Spectrum of isolates are listed in Figure 10 and Table 3, while HMBC structure of isolates is displayed in Figure 11.

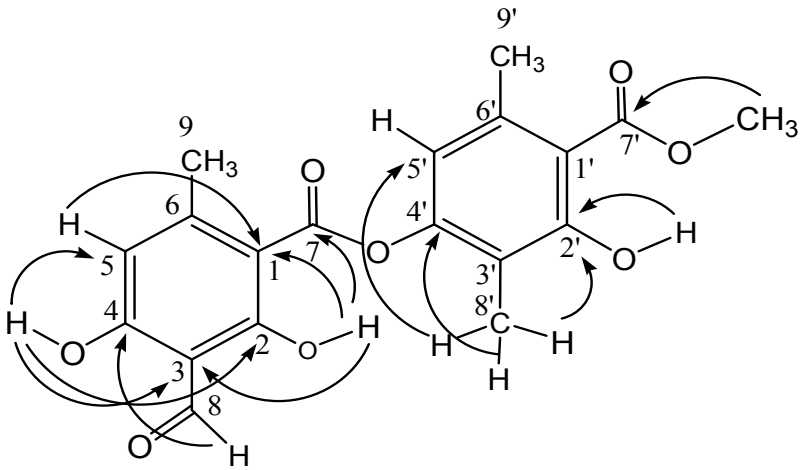

Figure 11
HMBC Structure of Isolates

Table 3
Data of HMBC Spectrum of Isolates

C Position	δ ^{13}C (ppm)	δ ^{1}H (ppm) (J in Hz)	HMBC
1	103.0087		
2	169.2810	12.5076 (1H,s, HO-2)	C-1, C-3, C-7
3	108.7126		
4	167.6690	12.5582 (1H,s, HO-4)	C-3, C-5, C-2
5	113.0431	6.4048 (1H,s, H-5)	C-8', C-1, C-3
6	152.6462		
7	169.9010		
8	194.0616	10.3622 (1H,s, CHO-3)	C-4
9	24.2508	2.5459 (3H,s, CH_3-6)	C-3', C-1', C-6'
1'	116.2002		
2'	163.0620	11.9602 (1H,s, HO-2')	C-3', C-5' C-2'
3'	110.4296		
4'	152.1407		
5'	116.9633	6.5163 (1H,s, Ar-H)	C-9, C-3, C-5, C-4
6'	140.0747		
7'	172.4001	3.9870 (3H,s, O-CH_3)	C-7'
8'	25.8055	2.0906 (3H,s, CH_3-3')	C-3, C-5', C-4', C-2'
9'	9.5713	2.6886 (3H,s, CH_3-6')	C-1, C-5, C-6
7'	52.5606		

The next measurement was carried out using H-^{1}H COSY spectroscopy. COSY spectrum shows stereochemical relationship between protons which have the same pattern of space. Based on the result of COSY measurement of isolates, there was no pairs of proton's spin which have inter-proton correlation. The spectrum of ^{1}H-^{1}H COSY can be seen in Figure 12.

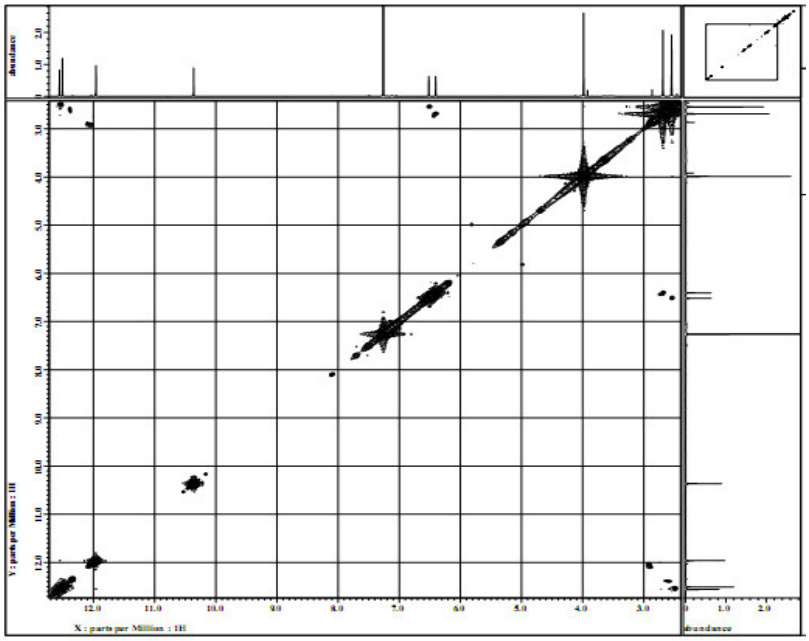

Figure 12
COSY Spectrum of Isolates

The 3D structure of isolates can be seen in Figure 13; the structure was created based on the data of measurements using ^{1}H-NMR, ^{13}C-NMR and NMR 2D spectroscopy as well as the results of literature reviews related to compounds with similar data of ^{1}H-NMR and ^{13}C-NMR that the isolates shares similarity with atranorin [9] ; it is depicted in Table 4. After the spectrums were compared, those data happened to be accordant that it can be concluded that isolates was Atranorin. This compound had been isolated from *Usnea* sp., a species of lichen. Atranorin has also found in *Usnea hirta, U. subcabrosa, U. cornuta, U. hesperina, U. ceratina, U. rubicunda, U. glabrata, and U. fragilescens* [17]. Besides from the genus of *Usnea*, this compound had once been isolated from genus *Parmotrema tinctorum, U. cenariensis* [9], *Hopea sangal* [25] and *Everniastrum cirrhatum* [26].

Table 4
Comparative Data of Chemical Shift of ^{1}H-NMR and ^{13}C-NMR of Isolates with Atranorin obtained from literatures

No C	Atranorin (*Parmotrema tinctorum*) [9]		Atranorin (*Hopea sangal*) [25]	Isolates Compound (*Usnea* sp.)	
	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{1}H-NMR	^{13}C-NMR
1		103.0			103.0087
2	12.51 (1H, s, HO-2)	169.0	12.52 (1H, s, Ar-OH)	12.5076	169.2810
3		108.7			108.7126
4	12.56 (1H, s,HO-4)	167.5	12.57 (1H, s, Ar-OH)	12.5582	167.6690
5	6.40 (1H, s,H-5)	112.8	6.40 (1H, s)	6.4048	113.0431
6		152.3			152.6462
7		169.6			169.9010
8	10.36 (1H, s,CHO-8)	193.6	10.37 (1H, -CHO)	10.3622	194.0616
9	2.69 (3H, s, Me-9)	24.4	2.70 (3H, s, Ar-Me)	2.5459	24.2508
1’		116.8			116.2002
2’	11.96 (1H, s,HO-2’)	162.8	12.05 (1H, s, Ar-OH)	11.9602	163.0620
3’		110.4			110.4296
4’		152.1			152.1407
5’	6.51 (1H, s,H-5’)	116.0	6.53 (1H, Ar-H)	6.5163	116.9633
6’		139.8			140.0747
7’	3.99 (3H,s,COOMe-7’)	172.1	4.00 (3H, s, -CO_2Me)	3.9870	172.4001
8’	2.09 (3H, s, Ar-Me)	23.8	2.10 (3H, s, Ar-Me)	2.0906	25.8055
9’	2.69 (3H, s, Ar-Me)	9.4	2.55 (3H, s, Ar-Me)	2.6886	9.5713
7’		52.2 (COOMe)			52.5606

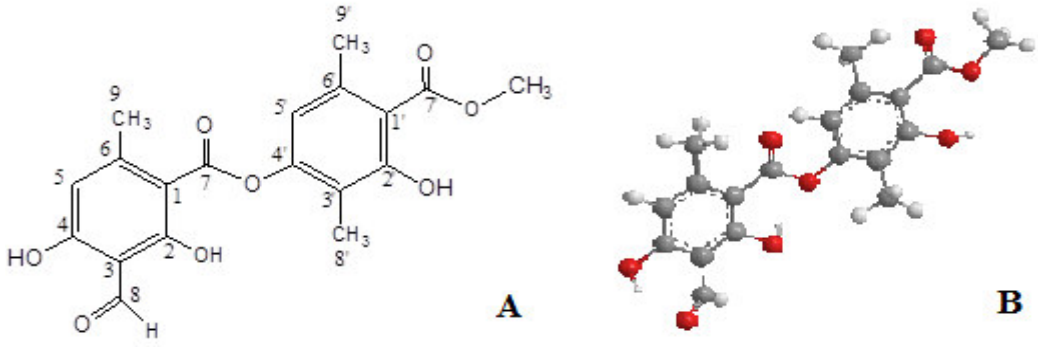

Figure 13
A) Structure of isolates compound; B) The 3D structure of isolates

2. n-hexane Fraction and Isolates Antibacterial Activity Testing

This antibacterial activity made use of triplo, the antibacterial activity testing used positive control and negative control. Negative control which was used is solvent that can dissolve fraction and isolates, while positive control is benchmarking antibiotics, chloramphenicol in this instance. Positive control was utilized to identify the effectiveness of the tested isolates; if the diameter of inhibition zone of isolates is bigger than the clear zone of positive control, it can be stated that the isolates is highly effective as antibacterial. If the inhibitor zone of isolates is smaller or even none, it can be said that the isolates is less effective or inapt to be used as antibacterial [27]. Negative control was used as benchmark between isolates and the used solvent thus the effectiveness of active isolates is not influenced by the solvent.

2.1 n-Hexane Fraction Antibacterial Activity

The n-hexane fraction activity testing towards bacteria made use of triplo with agar diffusion method using paper disc with 6 mm of diameter. The test was conducted towards 3 types of bacteria, i.e. gram-positive bacterium of *S. aureus* and gram-negative bacterium of *E.coli*, and *S. typhi*. The results are listed in Table 5.

Table 5
Data of n-hexane antibacterial activity

Bacteria Species	Diameter of inhibition zone (mm)																				
	n-hexane fractions (mg/mL)																C -			C+	
	100			Average	250			Average	500			Average	1000			Average					
E.coli	7	7	6	6.66	8	8	7	7.66	12	8	8	9.33	14	11	14	13	0	7	8	6	7
S.auerus	6	8	9	7.66	7	8	10	8.33	12	9	11	10.6	14	11	12	12.3	0	7	8	9	8
S. typhi	6	7	9	7.33	8	8	10	8.66	12	9	11	10.6	15	12	12	13	0	9	7	6	7.33

NB:The diameter of zone is excluded from the diameter of paper disc (6 m)
C+ = benchmarking antibiotic (Chloramphenicol 250 mg/L)
C - = $CHCl_3$ as solvent

Based on the results of the test, n-hexane fraction turned active in response to *S. auerus, S. typhi*, and *E.coli*. The diameters of inhibition zones which were formed within each concentration exhibit antibacterial activity. The n-hexane fraction offers considerable antibacterial activity because it contains usnic acid compound [9,16]. The inhibition zones formed are not too much different to the benchmarking antibiotic compound, that is cholaramphenicol. The inhibition zones from the test of n-hexane towards *E. coli, S. auerus*, and *S. typhi* within the concentration of 250 mg/mL are 7.66 mm, 8.33 mm, and 8.66 mm, respectively.

2.2 Isolates Antibacterial Activity

Isolates antibacterial activity test made use of triplo and used three kinds of bacterium, i.e. *E.coli, S. typhi* and *S.auerus*. The results of this test can be seen in Table 6

Table 6
Data of Isolates Antibacterial Compound

Bacteria Species	Diameter of inhibition zone (mm)																			
	n-hexane fractions (mg/mL)																C -	C+			Average
	50			Average	100			Average	250			Average	500			Average					
	a	b	c		a	b	c		a	b	c		a	b	c			a	b	c	
E.coli	1	1	0	0.66	2	2	1	1.66	3	2	1	2	4	3	2	3	0	7	6	5	6
S.auerus	1	1	1	1	2	2	2	2	3	3	3	3	5	4	3	4	0	8	7	4	6.33
S. typhi	1	1	0	0.66	4	2	1	2.33	5	3	3	3.66	9	3	4	5.33	0	10	7	7	8

The diameter of zone is excluded from the diameter of paper disc (6 m)
a = First test, b = Second test, c = Third test, Isolates = Atranorin

The results of isolates antibacterial activity can prevent the growth of bacteria; however, it shares significant difference to n-hexane fraction where isolates has less antibacterial activity than n-hexane fraction. In term of structure, isolates has 3 hydroxyl groups which are hydrophilic that make it difficult to penetrate hydrophobic cell walls of bacterial; this makes it have low activation. On the other hand, isolates has two benzene rings and carbonyl group, but it has no oxygen bridge bond, cyclical ether for this instance, which connects two units of benzene. The compound is in the form of cyclical ring and it has carbonyl group as well as active oxygen bridge bond (R-O-R) that it can act as DNA intercalators which can be attached between two unit of alkali pairs of DNA and that it can interact with DNA via Van der Walls bond which can destroy double helix and prevent DNA cloning [28-29]. Based on the data displayed in Table 6, it can be seen that there are significant differences between n-hexane fraction isolates where n-hexane reveals strong antibacterial activity towards *S. auerus, S. typhi* and *E.coli*, while isolates has low antibacterial activity. Activity testing towards *E.coli* within each concentration of isolates – 500, 250, 100, and 50 mg/mL – results in average inhibition potencies by 3, 2, 1.66 and 0.66 mm, respectively. Activity testing towards *S. auerus* within each concentration of isolates – 500, 250, 100, and 50 mg/mL – results in average inhibition potencies by 4, 3, 2, and 1 mm, respectively. Activity testing towards *S. typhi* within each concentration of isolates – 500, 250, 100, and 50 mg/mL – results in average inhibition potencies by 5.33, 3.66, 2.33 and 0.66 mm, respectively.

CONCLUSION

This research has successfully isolated particular secondary metabolite compound in the form of white crystal from *Usnea* sp., a species of lichen by using GCC and TLC techniques. After being identified using ^{1}H-NMR and ^{13}C-NMR spectroscopy with homonuclear (1D) and heteronuclear (2D) HMBC, HMQC, and COSY DEPT techniques as well as literature reviews, it was found that the isolates is atranorin with molecular formula of $C_{19}H_{18}O_8$. The results of n-hexane fraction and isolates antibacterial activity testing exhibit antibacterial activity towards *E. Coli* (ATCC35218), S. *Aureus* (ATCC25923), and *S. typhi* (YCTC).

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CONFLICT OF INTEREST

None of the authors having any conflict of interest

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