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Article
Volume 11, Issue 6, 2021, 13878 - 13884
https://doi.org/10.33263/BRIAC116.1387813884

Antioxidant Activity of Secondary Metabolite Compounds from Lichen *Teloschistes flavicans*

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Scopus Author ID 56648049800
Received: 16.01.2021; Revised: 15.02.2021; Accepted: 18.02.2021; Published: 1.03.2021

Abstract: In the last decade, lichen has become a research interest related to exploring secondary metabolites compounds. Lichen is a unique organism and contains specific chemical compounds rarely found in other natural sources; one species is the lichen *Teloschistes flavicans*. The objective study explores the toxicity effects and antioxidants activity properties from lichen *Teloschistes flavicans* isolate. The isolation of secondary metabolites was carried out by extraction, separation, and purification by means of gravity column chromatography, thin-layer chromatography, and purity tests. Toxicity activity test used the Mayer method by looking at the LC_{50} value, while the antioxidant activity test used the DPPH method by looking at the IC_{50} value. The results of the isolation obtained a needle-shaped crystal with the name of the compound is 3-[1'-(2",3"-dihydroxy-phenyl)-propyl]-7-hydroxy-chroman-4-one and the molecular formula $C_{18}H_{18}O_5$. The toxicity test showed that extract and isolate compounds have LC_{50} values of 9.38 and 162.18 µg/mL, indicating that the isolated compound was toxic. The antioxidant test showed that the extract and isolated compounds have antioxidant activity with IC_{50} values of 54.05 and 127.38. The results of these studies provide scientific development of natural compounds that have the potential to act as antioxidants for diseases in the human body.

Keywords: lichen; isolation; DPPH; toxicology, antioxidant.

1. Introduction

The unique lichen organism is very interesting to study because it has many benefits, such as medicinal ingredients, textile materials, perfume ingredients, and an indicator of a polluted environment. Lichen is a unique organism formed from two different organisms: fungi and algae [1–3]. Lichen produces secondary metabolites of the depsides, depsidone, dibenzofuran, xanthone, and terpenes, which are generally only found in lichen organisms [4–6]. Secondary metabolite compounds are currently a concern for researchers to study more seriously. The use is widely used in the health sector to seek remedies from natural ingredients. The secondary metabolite compounds of lichen have active substances with biological activity that can be developed as medicinal substances [7–10]. Several biological activities have been reported, namely antioxidant [11,12], antimalarial [13], antimicrobial [14,15], anticancer [16,17], antidiabetic [18], and anti-inflammatory [19].

One of the lichens that have the potential to be developed is the lichen *Teloschistes*. Lichen *Teloschistes* have been used by the general public as traditional medicine [20]. The pharmacological activity provided by lichen depends on the secondary metabolites contained therein. Lichen *Teloschistes flavicans* is a lichen species known as 'Golden Hair Lichen' [21–23]. Its bright color is one of the identifying identities of this lichen. The metabolite compounds produced by lichen *T. flavicans* need further investigation.

This research studies the active compounds of lichen *T. flavicans* by looking at their biological activity against antioxidants. Oxidative stress in the body is the beginning of degenerative diseases, such as hypertension, heart disease, diabetes, stroke, and cancer [24,25]. Antioxidant testing becomes necessary to report, given the lifestyle of people who are less concerned with health. The human body can provide defense by producing its own antioxidant compounds [26–28]. Defenses that are not optimal cause these healthy cells to be attacked or sick if the number of free radicals is greater than the body's supply of antioxidants. Therefore, antioxidants are needed from outside the human body. Some of the secondary metabolites of lichen that have antioxidant bioactivity are protolichesterinic acid [29], cryptostictinolide [30], resorcinol [31], and gallic acid [32].

This study is a continuation of the exploration of secondary metabolites from lichen of the *T. flavicans* type. Previous studies have reported the results of isolation from *T. flavicans* moss, namely compounds vicanicin [33] and 3-[1'-(2",3"-dihydroxy-phenyl) -propyl] -7hydroxy-chroman-4-one have antidiabetic and antifungal activity [34]. This isolate was obtained from the extraction using acetone and purification using gravity column chromatography, eluent n-hexane and ethyl acetate. It is necessary to explore other biological activities of 3-[1'-(2",3"-dihydroxy-phenyl)-propyl]-7hydroxy-chroman-4-one, namely antioxidants, and to test their toxicity effects.

2. Materials and Methods

2.1. Materials.

Lichen *T. flavicans*, acetone, hydrochloric acid, Bouchardic Reagent, Mayer Reagent, Dragendorf Reagent, magnesium powder, and $FeCl_3$ were obtained from Merck-Germany, distilled water (IPHA, Indonesia), Whatman No. 42 filter paper (Merck-Germany), 2,2-difenil-1-pikrilhidrazil (DPPH, Merck-Germany), *Artemia Salina* Leach larvae, and 3-[1'-(2",3"-dihydroxy-phenyl) -propyl] -7hydroxy-chroman-4-one compound [34].

2.2. Toxicity activity test.

The toxicity test used the Meyer method and *A. salina* Leach eggs as the test material [35]. *A. salina* Leach eggs were hatched in a container filled with seawater with the help of a 14-watt lamp. The extracts and isolates tested were made into various solutions with concentrations of 1000, 500, 100, and 10 µg/mL. The test was carried out for 24 hours and counted the number of dead and living shrimp larvae. The mortality rate is calculated by comparing the number of dead larvae divided by the number of dead and living shrimp larvae.

2.3. Antioxidant activity test.

The antioxidant activity test was carried out based on the Blois method using DPPH as the free radical [36]. The IC_{50} value was calculated based on the percentage of inhibition of

each concentration, namely 2.5, 10, 50, and 100 µg/mL. The percentage of inhibition (y) from each concentration (x), the points (x and y) are plotted on the coordinate plane then the line equation y = ax + b is determined by calculation using linear regression where a and b are constants, x is the concentration sample (ppm), and y is the percentage of inhibition (%). Antioxidant activity is expressed by IC_{50}, namely the sample's concentration that can reduce 50% of DPPH radicals [37].

3. Results and Discussion

3.1. Isolation of lichen T. Flavicans.

In previous studies, we have reported that the compound 3-[1'-(2",3"-dihydroxy-phenyl)-propyl]-7hydroxy-chroman-4-one has been isolated from lichen *T. flavicans*. The structural formula can be seen in Figure 1.

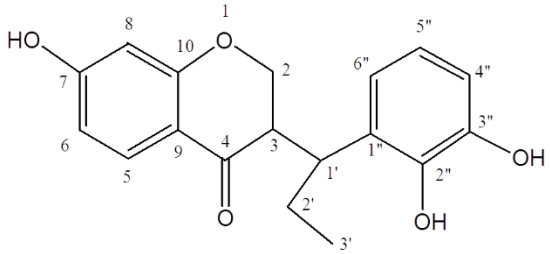

Figure 1. Structure of the compound 3-[1'-(2",3"-dihydroxy-phenyl)-propyl]-7hydroxy-chroman-4-one.

Table 1. Elucidation data of 3-[1'-(2",3"-dihydroxy-phenyl)-propyl]-7hydroxy-chroman-4-one [34].

Spectra Data	
Molecular weight ([M^+] (m/z))	315
UV (λ_{maks}, (nm))	270
IR ($\tilde{\upsilon}$, (cm^{-1}); functional groups)	978, C-O; 1037, C-O; 1625, C=C; 1740, C=O; 2932, Csp^3-H; 3559, OH and 3619, OH
^{1}H-NMR (δ_H (ppm) (∑H, mult, J (Hz))	7.612 (1H, d, 1.7); 7.296 (1H, d 1.6); 7.196 (1H, d, 1.69); 6.892 (1H, d, 1.6); 4.353 (2H, s); 3.784 (3H, s); 2.966 (2H, t, 7.18); 1.59 (1H, q); 1.337 (2H, m); 0.876 (3H, t, 7.12)

3.2. Toxicity test.

Based on the toxicity testing results, the extract and isolated compounds have LC_{50} values of 9.38 and 162.18 µg/mL, which indicated that the isolated compound was toxic, where a compound was said to be toxic if the LC_{50} value was ≤ 1000 µg/mL (Table 1). The mechanism of larval death is related to the content of flavonoid class compounds in an extract. The compound acts as stomach poisoning, so it irritates the larvae' digestive organs [38]. In addition, the taste receptors in the mouth area of the larvae are also inhibited, causing the failure of the taste stimuli in the larvae so that they are unable to recognize their food. Based on its toxic properties, it can be developed as an anticancer drug.

Table 1. Toxicity data of acetone extract and isolate compounds of lichen *T. Flavicans.*

Sample	**K (µg/mL)**	**Log K**	**Death**	**Life**	**Accumulation**		**Mortality**	**IC_{50} (µg/mL)**
					D	**L**		
Control	0	0	0	10	0	40	0	
	0	0	0	10	0	30	0	-
	0	0	0	10	0	20	0	
	0	0	0	10	0	10	0	
Aseton extract	10	1	9	21	9	30	23.08	9.38
of *T. flavicans*	100	2	21	9	30	9	76.92	
	500	2.7	30	0	60	0	100	

Sample	K (µg/mL)	Log K	Death	Life	Accumulation		Mortality	IC_{50} (µg/mL)
					D	L		
	1000	3	30	0	90	0	100	
Isolate	10	1	5	25	5	63	7.35	162.18
	100	2	3	27	8	38	17.39	
	500	2.7	22	8	30	11	73.17	
	1000	3	27	3	57	3	95.00	

3.3. Antioxidant test.

Antioxidant activity testing provides information about the ability of the test compound to react directly with DPPH radicals. The measurement results were obtained by observing the change in purple color to colorless due to its reduction to non-radical forms by monitoring its absorbance at the optimum wavelength of DPPH using a spectrophotometer [39,40]. The parameter as an interpretation of the test results is determined by the IC_{50} value, which is referred to as the concentration of the test sample, causing a reduction of DPPH activity by 50%. Table 2 shows the IC_{50} value of antioxidant activity by *T. flavicans* isolates and acetone extracts. The extract has an antioxidant activity to ward off DPPH radicals. The resulting IC_{50} value is 54.05 indicating strong antioxidant activity. The isolated compound has an IC_{50} value of 127.38, which is in the medium category.

Table 2. Antioxidant data of acetone extract and isolate compounds of lichen *T. Flavicans.*

Sample	Concentration (ppm)	IC_{50} (ppm)
Ascorbic acid positive control	10 25 50 100	5.05
Aseton extract of *T. flavicans*	10 25 50 100	54.05
Isolate	10 25 50 100	127.38

4. Conclusion

Isolation of lichen *T. flavicans* obtained pure compounds 3-[1'-(2",3"-dihydroxy-phenyl)-propyl]-7hydroxy-chroman-4-one. The toxicity test showed that extract and isolate compounds have LC50 values of 9.38 and 162.18 µg/mL, indicating that the isolated compound was toxic. The antioxidant activity showed an IC_{50} value of 161.273 µg/mL. The antioxidant test showed that the extract and isolated compounds have antioxidant activity with IC_{50} values of 54.05 and 127.38. The results of these studies provide scientific development of natural compounds that have the potential to act as antioxidants for diseases in the human body.

Funding

This research was funded by DRPM Menristek/BRIN of the Republic of Indonesia, grant number 171/SP2H/AMD/LT/DRPM/2020.

Acknowledgment

We are grateful for the laboratory facilities from the Research Center for Chemistry, Indonesian Institute of Sciences in conducting this research.

Conflicts of Interest

We declare that this article has no conflict of interest.

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