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To cite this article: E S Siregar *et al* 2021 *IOP Conf. Ser.: Earth Environ. Sci.* **713** 012061

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Antioxidant activity of liverworts *Marchantia paleacea* Bertol. from North Sumatra Indonesia

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Abstract. Research on antioxidant activity of *Marchantia* in Indonesia is still limited. The objective of the study is to investigate the antioxidant activity of *Marchantia paleacea* from North Sumatera, Indonesia. The method of extraction in this study using maceration with methanol. The antioxidant activity of extracts were evaluated by 1,1diphenyl-2-picrylhydrazyl (DPPH). Vitamin C was used as positive control. The results showed that the extract of *M. paleacea* has antioxidant content with LC50 value of 25.25 µg/mL. The antioxidant activity of *M. paleacea* is classified as a strong category.The results obtained in the recent study indicate that *M. paleacea* is a potential source of natural antioxidant.

1. Introduction

Marchantia is member of the genus of thalloid liverworts (Marchantiophyta). The genus is characterized by the presence of gemmae cup on dorsal thallus surface, with 4-10 rows of scales on ventral side of thallus [1]. Species of *Marchantia* are cosmopolitan, grow well on moist habitat in shady or open places. Substrate where it grows are soil, rocks, and river bank cliffs [2]. In some places it is considered as a weed, and often related to human activities.

The species of *Marchantia* play an important ecological role in the environment, and also used as traditional medicine [2]. Ecologically *Marchantia* able to compete well and colonize in deforested soils, so it greatly helps reduce erosion. The genus role as a pioneer plant in bare grounds, grows on substrates that cannot be covered by other plants, such as polluted soils. This is due to its ability to accumulate organic materials in polluted places, so as to provide life opportunities for other plants [3][4].

Besides having an important ecological role, *Marchantia* also has potential as a medicine. *Marchantia polymorpha* has been widely used as traditional herbal medicines for blister, poisonous snake bites, boils, pneumonia, and hepatic disorder. It is also has antifungal and antimicrobial properties [5–9]. However, so far the research which has been widely reported is *Marchantia polymorpha*, while the other species of *Marchantia* are still limited in their information. Research on the phytochemicals of *Marchantia* in Indonesia is still very limited. In North Sumatra, the study of *Marchantia* was limited in the species exploration and its morphology [10,11]. So far, the study on the chemical content and antioxidant activity of *Marchantia paleacea* were still less reported. Therefore, this study aims to investigate the antioxidants activity of a liverwort *Marchantia paleacea* of North Sumatra.

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2. Method

2.1. Preparation and extraction of sample

The thallus of *Marchantia paleacea* (figure 1) was collected from Panatapan Mount Sibayak, North Sumatera Indonesia (figure 2). The sample was washed by running water to remove the contaminants such as soils, gravels, and grass. The sample was spread out at room temperature for one week, then reduced to a fine powder with an electric blender. 100 g of finely powder sample was extracted with 500 mL of 70 % methanol for 24 hours, followed by an evaporation process to remove methanol. The extract was concentrated using a desiccator vacuum to obtain a thick extract that was ready for phytochemical screening tests.



Figure 1. *Marchantia paleacea*

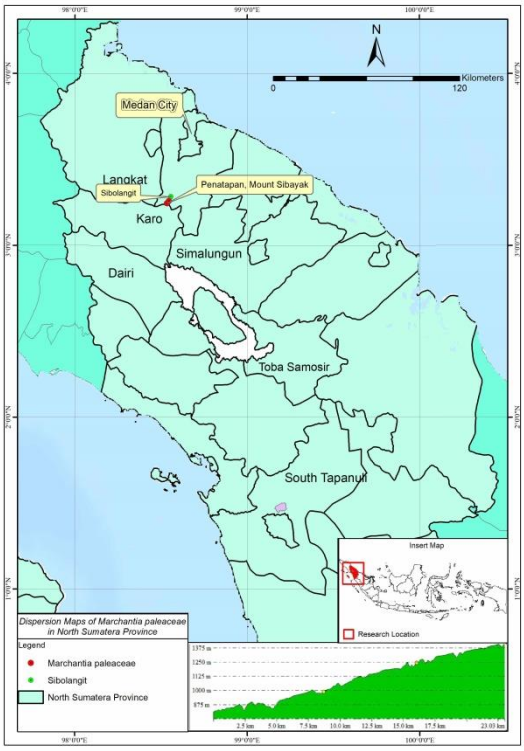


Figure 2. Map of the sample collection site in Panatapan, Mount Sibayak

2.2. Extract identification

Identification of secondary metabolites contained in extracts of *M. paleacea* using phytochemical screening, which consists of an examination of alkaloids, flavonoids, saponins, tannins, terpenoids, glycosides, and steroids. 1 g of extract was put into the test tube, added with 18 ml of distilled water and 2 ml of 2 N HCL then heated for 2 minutes. The test was carried out with Mayer/Dragendorff/Bouchardat reagent. Positive alkaloids resulted if turbid solutions were formed. 1 g of extract mixed with 20 ml of methanol, shocked and allowed for a moment. 0.1 gram of metal Mg and 5 drops of concentrated HCl. Orange-yellow to red solution indicating the existence of flavonoids. As much as 0.5 g of extract was mixed into 50 ml of distilled water and then filtered, adding 1 drop of 1% Fecl3 solution, bluish-green color indicates tannin compounds. 1 g of extract was mixed with 20 ml of hot water, shocked for 20 seconds. The saponins are positive if there is a stable foam formation for 10 minutes and not lost after adding 2 drops of 2N HCL. 3 g of extract was added to 30 ml of the ethanol-water mixture (7: 3), then added with concentrated sulfuric acid, refluxed in 10 minutes; 20 ml of filtrate was added with 10 ml of distilled water and 10 ml of lead (II) acetate 0.4 M, shaken and kept for 5 minutes; then extracted with 20 ml of the chloroform-isopropanol mixture of (3: 2); a green or blue color indicates the glycoside compounds.

2.3. Determination of antioxidant activity

Antioxidant activity is expressed by the Inhibitor Concentration 50 (IC50) value of each sample. The sample was reacted with DPPH (2,2-diphenyl-1 pycrylhydrazyl) solvent then measured using a UV-Vis spectrophotometer at a wavelength of 517 nm. Vitamin C was used as control or as a comparison. Approximately 9.8 mg of DPPH powder was weighed and dissolved with methanol up to 50 mL. A DPPH solvent of 200 ppm was obtained. Pipette standard solution DPHH as much as 5 mL, put into a 25 mL volumetric flask, then add methanol to the mark limit so as to obtain a solution with a concentration of 40 ppm. The maximum wavelength is measured using a UV-Vis spectrophotometer at wavelength of 517 nm.

Weigh the extract as much as 25 mg and was dissolved with methanol up to 25 mL, obtained a solution with a concentration of 1000 ppm. Taken 0.25 mL; 0.5 mL; 0.75 mL; 1 mL; 1.25 mL of the 1000 ppm extract solution, then 5 ml of DPPH solution (200 ppm concentration) was added to each concentration and added with methanol to the mark limit (25 mL volumetric flask), obtained concentrations of 10, 20, 30, 40, 50 ppm. Incubated for 30 minutes then absorbance was measured using a UV-Vis spectrophotometer at a maximum wavelength of 517 nm. Determination of the free radical trapping process by a test sample using the DPPH free radical scavenging method is calculated using the following formula:

$$\%\ Inhibition = \left[\frac{A0 - A1}{A0}\right] x100$$

Where, A0 = absorbance of blank, A1 = absorbance of the tested sample.

3. Results and Discussion

3.1. Phytochemical screening

This phytochemical test aims to identify the secondary metabolite compounds contained in the extract of sample. Phytochemical screening results of *Marchantia paleacea* can be seen in table 1.

Table 1. Phytochemical screening of methanol extract of *M. paleacea*

Secondary metabolite	Solvent	Detection
Steroids	Liebermann-Bouchart	-
Flavonoids	Mg(s) + HCl(p)	+
Saponins	Aquadest	+
	Aquadest+Alcohol 96%	+
Tannins	FeCl3 1%	+
Alkaloids	Bouchardart	+
	Dragendorff	+
	Mayer	+
Glycosides	H_2O+NaOH	+

Note: + = identified, - = unidentified

Phytochemical screening showed that methanol extract of *M. paleacea* contains flavonoids, saponins, tannins, alkaloids, and glycosides, but steroid was undetected. Flavonoids from *Marchantia convoluta* showed antimicrobial activity. It also has anti-hepatitis-B virus properties, effects of anti-inflammation, antibiosis, and diuresis in animals. The presence of flavonoids, tannins, and phenolic compounds cause the ability as a natural antioxidant that has free radical scavenging activity [2][12]. Tannin compounds can be applied as potential treatment agents as anti-carcinogenic, anti-inflammatory, and antiseptics [13]. Alkaloids have antimicrobial activity, used as anti-diabetes, and anti-tumors [14]. Based on the content of secondary metabolites possessed by *M. paleacea*, it is estimated that this plant has great potential as an herbal medicine for various types of diseases.

3.2. Antioxidant activity

The amount of antioxidants activity is characterized by IC_{50} (inhibitory concentration) values, that is the concentration of the sample solution needed to inhibit 50% of DPPH free radicals. The greater the IC_{50} value, the smaller the antioxidant activity and the smaller the IC_{50} value, the greater the antioxidant activity.

Table 2. Antioxidant activity of *Marchantia paleacea* extract

Concentration (ppm)	Inhibition (%)	LC50 (µg/ml)
0	0	
10	26.203	
20	44.449	25.25
30	60.782	
40	76.331	
50	89.519	

Based on the research conducted, IC50 values on *M. paleacea* methanolic extract were obtained at 25.25 µg/mL (table 2). The results of this study indicate that the species liverwort *M. paleacea* showed a strong activity by antioxidant activity methods with an IC50 value of 25.25 µg/mL. According to [15] there are five categories of IC50 values as antioxidants: very strong activity (<10 ppm), strong activity (10-50 ppm), moderate activity (50-100 ppm), low activity (100-250 ppm), and inactive (>250 ppm).

The results of IC50 obtained in this study are higher than [16] who study on antioxidant activity of *Marchantia paleacea* in Himalaya, IC50=18.18 µg/mL, but still in the same category, strong activity. However, the activity of *Marchantia paleacea* in the study is lower than [16], the higher the IC50 value, the lower antioxidant activity. The IC50 of *M. paleacea* in this study was higher than IC50 of

M. polymorpha in Turkish liverwort by [7] which showed moderate antioxidant activity. Other studies by [17] obtained the higher antioxidant activity *M. polymorpha* methanolic extracts from Yogyakarta, Indonesia with an IC50 value of 5.22, categorized as a very strong activity. The differences in the results obtained are likely due to the differences in species tested and the place of collection.

An antioxidant is one component that plays an important role to maintain cell function and cell integrity. Antioxidants can enhance the immune system, important in the prevention of carcinogenesis, contribute to health problems such as cardiovascular and inflammatory diseases [18,19]. Therefore, *M. paleacea* has great potential as a natural antioxidant due to its antioxidant activity. This plant can be processed into alternative herbal medicinal ingredients that play an important role in the treatment of various types of diseases.

4. Conclusion

The study provided the presence of secondary metabolite of *M. paleacea* consist of flavonoids, saponins, tannins, alkaloids, and glycosides. The results showed that the extract of *M. paleacea* has antioxidant content with LC50 value of 25.25 μg/m. The antioxidant activity of *M. paleacea* is classified as a strong category. Based on the content of secondary metabolites and antioxidant activity of *M. paleacea*, it is estimated that this plant has a great potential as herbal medicine for various types of diseases.

Acknowledgment

The authors thank the Directorate Research and Community Service, Ministry of Research Technology and Higher Education, Republic Indonesia for the 2019 Fundamental Research, through the "DRPM with contract number: 11/E1/KP. PTNBH/2019.

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