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COMPARATIVE PHYTOCHEMICAL SCREENING OF MARCHANTIA POLYMORPHA AND MARCHANTIA GEMINATE

Maryanti Setyaningsih[1*] and Susilo Susilo[2]

[1, 2] Departement of Biology Education, Universitas Muhammadiyah Prof. DR. Hamka, Jl. Tanah Merdeka, Kp. Rambutan, Jakarta Timur, Indonesia.
**Correspondence Author Email: maryanti.setyaningsih@uhamka.ac.id*

Abstract

The presence of liverworts can provide clues to how to live in terrestrial environments. The Marchantia group has a variety of phytochemical components that can be a source of drugs in the pharmaceutical world. Although research on natural materials from plants has made significant developments, there is limited information on some Marchantia species. This study aimed to compare phytochemicals and total phenolic from Marchantia polymorpha and Marchantia geminate extracts. Phytochemical screening and total phenol content are carried out with standard protocols. Organoleptic tests are added to analyze differences in the content of phytochemical screening results. Color changes are observed for further qualitative analysis. The results of this study confirmed the presence of steroids, tannins, and flavonoids in the two types of Marchantia. Ethanol extracts from M. polymorpha contain phenol compounds not found in M. geminate. These results explain that M. polymorpha has the highest therapeutic value against various diseases.

Keywords: Phytochemicals; Marchantia Germinate; Marchantia Polymorpha

INTRODUCTION

Recently, the trend of medicinal plant research has focused on the search for new natural antioxidants. Much emphasis has been placed on finding new therapeutic agents from medicinal plants (1). It is a fact that society uses various herbs from different parts of the plant to treat various diseases (2). Antioxidants have an essential role in oxidative stress reduction power which is one of the causes that can damage biological molecules (3,4). The Bryophyta group in Indonesia is still primarily limited to diversity, and there is still little research on the usefulness of the compound content in it. Diversity of flora (biodiversity) means the diversity of chemical compounds (chemo diversity) in it is a source of the natural wealth of high value for humans (5). This spurs the research and tracing of chemical compounds, especially secondary metabolites contained in plants, and advances in science and technology, such as separation techniques, analytical methods, and pharmacological tests (6).

Many studies have examined the active components of plants reported to be medicinal (medicinal) and anti-microbial (7). Some Marchantia species are potent sources of many unsaturated fatty acids, and have a role in the sustainable environment (8), can be used in traditional Chinese medicine and have been used extensively to treat skin tumefaction, protect the liver and treat hepatitis and are also used as antipyretics in rural areas (9). Many degenerative diseases often suffered by the community can be caused by antioxidants in the body that cannot neutralize the increase in free radicals (10).

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The main problem in this research is that this moss plant is still considered useless in Indonesia and ignored. Bryophyta in Indonesia number around 648 species, consisting of 10 species in Madiun (11), 159 species in Bandung (12), 340 species in Sulawesi (13), 32 species in Bengkulu, and 85 species in Raja Ampat (14). As for Hepaticopsida (liverwort) in Indonesia, around 195 species, consisting of 1 species identified in Madiun, 134 species (included in 46 genera) in Sulawesi, and 60 species in Bandung (13). Of the number of liverworts that have not been studied, liverworts have the potential to have benefits ranging from alkaloid compounds, steroids, phenols, triterpenoids, tannins, and flavonoids (15,16).

Isolated or semi-synthetic compounds obtained from plants are essential for drug development (17). Some experts mentioned the presence of secondary metabolites as an attractor, repellant, and protectant (18). Secondary metabolites are typical products found in certain plants only (19). The role of biochemical compounds is usually to protect themselves from predators, such as young shoots and sprouts from predators, pollution, and toxic elements (20). In this study, Bryophyta is a plant with potential that can be considered in the study of beneficial metabolite compounds.

Many searches have been carried out for active components of plants that are reported to be medicinal (treatment) and anti-microbial (22). Marchantiophyta has quite a lot of chemical content; examples of species included are M. polymorpha and M. germinate, but the information is still not dug too deep. Therefore, the purpose of this study is for the initial screening of phytochemical compounds in M. polymorpha and M. germinate. This is the first study to study the phytochemical components of M. polymorpha, and M. germinate in Indonesia.

MATERIALS AND METHODS

Plant Material

M. polymorpha and M. germinate collected from Curug Cibeureum, Bogor, Indonesia (6°45'16.4"S 106°59'11.3"E). Some plant materials are identified in the Botanical Laboratory, National Research and Innovation Agency (BRIN). The B8-8765-086 specimen voucher has been stored at Herbarium Bogoriensis, Directorate of Scientific Collection Management, Indonesian Institute of Research Sciences (LIPI). Organoleptic experiments were conducted at the Genetic Physiology Laboratory, Bogor Agricultural University (ITB), Indonesia.

Chemicals

All chemicals, including 96% methanol solvent, 10% NaOH, chloroform, acetic acid anhydride, concentrated sulfuric acid, and 1% $FeCl_3$, are obtained from HiMedia. Magnesium powder, chlorhydrate alcohol (a mixture of 37% HCl and 95% ethanol of equal volumes), and amyl alcohol are obtained from Merck. The glassware used is from Borosilicate. Before use, glassware is washed with Sunlight detergent, rinsed with tap water, and then sterilized in an oven at 121°C for 3 hours.

Extraction

Each leaf is washed thoroughly with tap water and then dried in the sun until the water is gone. All ingredients are dried at room temperature in a dark, shaded place for 14 days. Each leaf sample

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is ground with an electric grinder of 40-60 mesh to easily mix with solvents following the previous research method (23). Next, the dry sample was immersed in 70% ethanol with a sample weight ratio and solvent volume of 1:10 for 24 hours. Marchantia polymorpha and Marchantia germinate powder was extracted in a ratio of 1:10 and then macerated for 6 hours while occasionally stirring with an orbital shaker, then the extract was allowed to stand for 24 hours. The obtained fiber is filtered and repeated three times with the same type and amount of solvent. All filtrate is collected and evaporated by a rotary evaporator at 40°C until a thick extract is obtained. The extract solution is filtered with filter paper and stored until further analysis (24).

Phytochemical Test

Phytochemical tests on the filtrate are carried out to determine the compound content in the sample. The phytochemical assays performed represent the main synthesis pathways of secondary metabolites. Filtrate testing included tests for phenols, steroids, triterpenoids, tannins, and flavonoids (24,25).

Total phenolic content

Phenolic compounds were analyzed by taking as much as 1 ml of extract, dripping it on a spot plate, and adding 10% NaOH. The formation of red indicates of phenol hydroquinone compounds. Analysis of flavonoid compounds was carried out by taking 1 ml of extract and put in a cup glass, and then boiling for 5 minutes in 100 ml hot water. and then filtering as much as 10 ml plus 0.5 grams of Mg powder and 2 ml of chlorhydrate alcohol. The development of red, orange, and yellow colours in the amylic alcohol layer indicates the flavonoids (26).

Analysis of total steroid and triterpenoid content

Steroid and triterpenoid compounds were analyzed by weighing as much as 1 mg of dry extract and dissolving it into 2 ml of chloroform. This is followed by 10 drops of anhydride acid and 3 drops of concentrated sulphuric acid. The solution is slightly agitated and left for a 10 minutes. A positive test is characterised by the green for steroid and red or violet fot triterpenoid (23).

Analysis of total tannin content

Tannin compounds were analyzed by weighing as much as 1 gram of extract that had been dried and put into a goblet glass, and then boiling for 5 minutes in 10 ml hot water. The filtrate was added to 10 ml of 1% $FeCl_3$. Dark blue or blackish green appearance is indicative of positive tannins (23,27).

DATA ANALYSIS

The data analysis technique was followed test result with the indicators in the determination of the presence of compounds in Marchantia polymorpha, and Marchantia germinate extracts (Table 1) (28).

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Table 1: Indicators of positive determination of the content of phytochemical

Compounds	Reagent	Positive Indicator
Phenol	NaOH 10%	Formed red color
Steroids	Anhydrous acetic acid, Chloroform, sulfuric acid	Formed green color
Triterpenoids	Anhydrous acetic acid, Chloroform, sulfuric acid	Formed red or purple color
Tannins	$FeCl_3$ 1%	Formed dark blue or blackish-green color
Flavonoids	Magnesium powder, chlorhydrate alcohol, amyl alcohol	Red, yellow, or orange color forms in the amyl alcohol layer

RESULT

The sampling location of Marchantia polymorpha and Marchantia germinate in Curug Cibeureum at the foot of Mount Gede Pangrango. This location has an altitude of 1675 meters above sea level, a humidity of 90%, a temperature of 23^0C, and an average rainfall of 4319 mm. The components contained in the methanol extracts of Marchantia polymorpha and Marchantia germinate were analyzed for compounds by a color test with several reagents for phenol group compounds, steroids, triterpenoids, tannins, and flavonoids (24). Various methods are used to identify an extract's secondary metabolites, including phenolic compounds, steroids, triterpenoids, tannins, and flavonoids (27,29). Phenolic compounds can be identified with a 10% NaOH reagent. As for identifying steroid compounds and triterpenoids using the Libermann-Burchard reagent (30). Identification of tannin compounds using a 1% FeCl3 reagent. As for flavonoids, use magnesium powder, chlorhydrate alcohol, and amyl alcohol. The content of each of these mosses can be summarized in Table 2.

Table 2: Content of Marchantia polymorpha and Marchantia germinate

Types of Moss	Phytochemicals				
	Phenol	Steroid	Triterpenoid	Tannins	Flavonoids
M. polymorpha	+	+	-	+	+
M. germinate	-	+	-	+	+

Table 1 shows that Marchantia polymorpha contains phenols, steroids, tannins, and flavonoids but does not contain triterpenoids. While in Marchantia germinate, there are steroids, tannins, and flavonoids, which do not contain phenols or triterpenoids. Test results on Marchantia polymorpha and Marchantia germinate shown in Figure 1.

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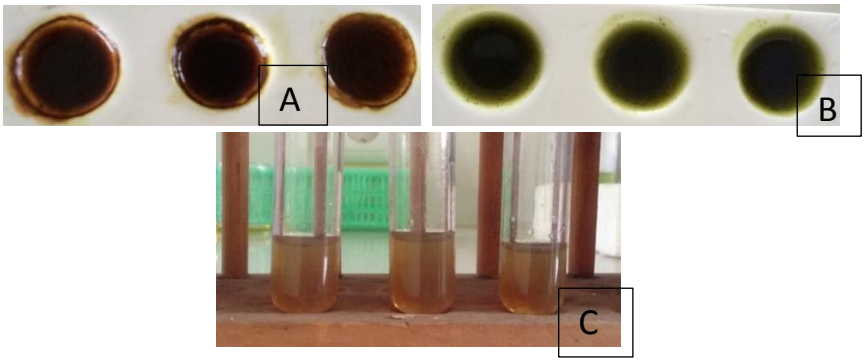

Figure 1: (A) Phenol test (+) Marchantia polymorpha; (B) Phenol test (+) on Marchantia germinate; and (C) Tannin test (+)

DISCUSSION

Based on the research conducted, Marchantia polymorpha contains phenols, steroids, tannins, and flavonoids, but no terpenoid compounds were found. Nevertheless, in research conducted by Wang et al., Marchantia polymorpha, there are ten compounds known as two steroids, six bibenzyls, flavonoids, and terpenoids(26). Likewise, previous studies show phenolic compounds, triterpenoids, and flavonoids in Marchantia paleacea (26).

Research conducted by Gupta et al., Marchantia convoluta extract can treat human liver tumors (31). The flavonoid extract of Marchantia convoluta also exhibits anti-microbial activity (7). The presence of phenolic compounds makes plants potential free radical scavengers, thus acting as natural antioxidants. Thallus contains 22,23-dihydro stigmasterol, stigmasterol, and n-hexadecanoic acid, octadecanoic acid as the main sterols and chemical compounds (7,18). Each class of bryophytes (hepaticopsides, bryopsides, and anthocerotopsides) was detected as positive for antibacterial activity, both gram-positive and gram-negative bacteria (32). This antibacterial activity may be due to flavonoids, steroids, terpenoids, and other polyphenolic compounds (7). The phytochemical screening method is carried out by looking at the color testing reaction using a color reagent. Essential things that play a role in phytochemical screening are solvent selection and extraction methods (33).

Phytochemical screening is a qualitative analysis of secondary metabolite compounds (22). An extract from natural materials consists of various secondary metabolites that play a role in biological activity (24). These compounds can be identified with reagents that characterize each secondary metabolite class. Chemical screening is a qualitative examination of chemical content to determine the class of secondary metabolite compounds with health benefits (27). The method used or chosen to conduct phytochemical screening must meet the requirements, including simple, fast, can be done with minimal equipment, selective to the class of compounds studied, semiquantitative (has a sensitivity limit for the compounds studied), and can provide additional information on the presence/absence of compound content (34).

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CONCLUSION

Marchantia polymorpha and Marchantia germinate contain bioactive compounds that can be antibacterial, antifungal, antioxidant, and anticancer. Marchantia polymorpha contains phenols, steroids, tannins, and flavonoids, while Marchantia germinates steroids, tannins, and flavonoids.

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