IDENTIFICATION OF AQUATIC PLANT SPECIES FROM BANTIMURUNG WATERFALL AND THEIR PHYTOCHEMICAL COMPOUNDS ANALYSIS

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Abstract

A total of 21 aquatic plant species were collected from Bantimurung waterfall areas, South Sulawesi, Indonesia, in October 2017. These plant materials were subjected to both species and phytochemicals identification. The aims of this study were to determine the species or taxonomic rank of Indonesian aquatic plants collected from Bantimurung waterfall, South Sulawesi, Indonesia and to identify their chemical compounds (phytochemicals) as a candidate for new herbal medicine. Plant genetic materials used in this study were collected from Bantimurung Bulusaraung waterfall and were then identified based on standard botanical techniques for species identification in the Herbarium Bogoriense, Research center for Biology Indonesian Institute of Science (LIPI), Cibinong, West Java. The samples were subjected to the phytochemistry screening such as alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids and anthraquinone followed the procedures of Indonesian Materia Medika and Harborne. Results showed that all collected aquatic plant samples were able to be identified, including their species names. Phytochemical screening of each sample revealed the presence of glycoside in all of the tested species. However, no alkaloids, anthraquinones, and terpenoids were observed in those tested plant samples. Of the total 21 aquatic plants, 14 species contained flavonoids, 8 species contained phenol compound, and 10 species contained saponins. Among these species *Donnax canniformis* possessed good antioxidant activity, which correlated to its total phenolic and flavonoid contents. Our results would be beneficial for any future effort in the development of new herbal drugs derived from aquatic plants.

Key words: Aquatic plants, Antioxidant activity, Flavonoid, Phenolic,

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Introduction

Aquatic biodiversity is a sensitive thematic issue because it serves as a component of global biodiversity and a model for basic research on water ecological studies in evolutionary aquatic ecosystems (Xu *et al.*, 2007). Aquatic plants act as “engineering and pioneer species” (Ansari *et al.*, 2017), and their disappearance may have drastic effects on trophic and functional status of the habitats within water bodies (Bouma *et al.*, 2000; Scheffer *et al.*, 2003). Nevertheless, aquatic plants often remain unrecognized in broad-scale investigations, a condition that can lead to wrong or to conflicting evaluations in analysing their current spatial patterns and rarity (Alahuhta *et al.*, 2017).

Drought in the riverine areas can be examined by identifying the original source from which the water flows. Bantimurung is one of the regions that has a vast and long riverine area. It is believed that water plants can be an indicator to

DOI: http://dx.doi.org/10.14203/ann.bogor.2019.v22.n2.41-48

determine whether or not a particular aquatic ecosystem is healthy. The availability of water which falls into extremely low levels in a long period is required in order to keep the aquatic ecosystems stable. According to Parenti, (1998) the aquatic plants which grew along the vast riverine area affected water health and provided several benefits in fulfilling future human needs.

The existence of aquatic species reflects the condition of waters and ecosystems. Aquatic plant species from various families serve as reliable indicators for biological monitoring of aquatic ecosystems (Ansari *et al.,* 2017). The loss of vulnerable species leads to changes in species composition and in ecosystems. Therefore, maintaining aquatic plant biodiversity and their adaptability to environmental changes, including climate change, is very important. Aquatic plant biodiversity plays a vital role in people's livelihood, but it is being threatened by many factors such as climate change. It is generally acknowledged that aquatic plants, despite representing only a small fraction of the total vascular plant diversity (~1%), are one of the most critical groups of threatened species worldwide (Saunders *et al.,* 2002; Chambers *et al*., 2008).

Based on the Ramsar Convention of 10 June 2011, the total area of important Indonesian wetlands was 964,600 ha (Gopal, 2013). Bantimurung waterfall is located in Maros District, South Sulawesi Province, Indonesia, and the areas surrounding the waterfall and river have various plant species. Bantimurung area (43,750 ha) is located in Wallace line region with highly endemic and specific mix of flora and fauna between Asia and Australia (Wakka *et al*., 2012). Bantimurung is located in the early tertiary isolated small land area in Sulawesi, and this is probably the factor which caused the endemism of its flora and fauna. Based on the Wallace theory, many endemic and special flora and fauna live in this region because their range of diversity is often strongly related to suitable habitats and climate, or because certain floral elements were present on both sides of the Wallace line before the formation of Makassar straits (Parenti, 1998). In addition, Thomaz & Cunha (2010) reported that waterfalls in mountains and rivers also support a rich assortment of aquatic biological diversity that contributes to the economy, culture, health (as the source of nutrition and medicine), and social recreation of human beings. Experiments with several aquatic plants reported by Gesberg *et al.,* (1986) could act as a medium for waste water treatment and removed ammonia, nitrogen TSS (Total Suspended Solids), and BOD (Biochemical Oxygen Demand). Although, studies on numerous phytochemicals derived from different plants have been published, but only few studies reported such as phytochemicals investigated from aquatic plants. A broad body of knowledge about chemical compounds resulting from aquatic plants serves as a valuable material for developing traditional medicine in future. Therefore, we have to give more attention to the biodiversity of aquatic plant species and the prospect for developing them into herbal drugs for fish and humans.

Aquatic plant biodiversity has a great potential for bringing about new pharmaceutical inventions derived from fresh water aquatic flora. It is expected that those species can contribute to food security and meet future challenges in the development of new herbal drugs for controlling fish or human diseases. This paper provides the first comprehensive inventory and phytochemical analysis of endemic aquatic plants found in Bantimurung, South Sulawesi Province, Indonesia. The aims of this study were to identify the diversity of aquatic plant resources collected from Bantimurung waterfall zones and to analyse their potential phytochemicals as a candidate for new herbal medicines

Materials and Methods

Study area

A total of 21 aquatic plants samples were collected from the areas surrounding Bantimurung Bulusaraung waterfall, Maros District, South Sulawesi Province, Indonesia, from October 2017. Sampling site was determined according to the remote sensing data from the preliminary location surveys and geobiophysical information provided by WorldView-2 satellite imageries (Indonesian National Institute of Aeronautics and Space–LAPAN)

The aquatic plants were identified based on standard botanical techniques for species identification in the Herbarium Bogoriense, Research center for Biology Indonesian Institute of Science (LIPI), Cibinong, West Java. The fresh samples were washed with tap water to remove impurities on the sample surface and were dried in the greenhouse of the Indonesian Centre for Agricultural Biotechnology and Genetic Resources Research and Development

DOI: http://dx.doi.org/10.14203/ann.bogor.2019.v22.n2.41-48

(ICABIOGRAAD), Bogor. Those dried samples were powdered using a blender.

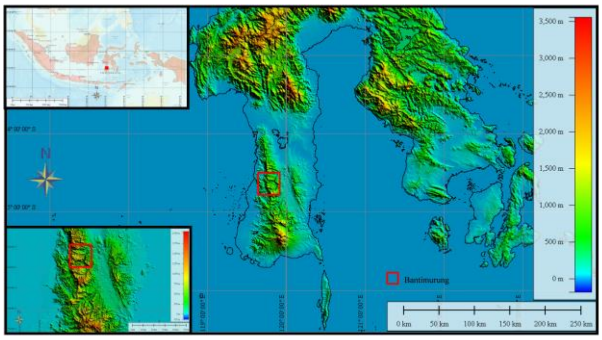

Figure 1. Bantimurung Bulusaraung National Park, South Sulawesi (4^{0}58' 08.68' S dan 119^0 40' 56.94' E); and the detected sites.

Procedures and Data analysis

Chemical and Reagents

The chemicals used in this study including gallic acid standard, quercetin standard, 1,1-Diphenyl-2-picrylhydrazine (DPPH) were purchased from Sigma-Aldrich, Folin Ciocalteu's phenol, methanol for analysis, ethanol for analysis were purchased from Merck Indonesia.

Extraction Procedure

Aquatic plants samples were extracted according to the procedures described by Indonesian Materia Medika Departemen Kesehatan Republik Indonesia (1995) and Harborne, (1998). The dried powder sample (1 g) was diluted in 10 mL ethanol 70% and extracted using a sonicator for an hour. Then, the filtrate and the residue were separated using a funnel

Phytochemistry screening

The aquatic plants extracts were diluted using ethanol. Identification of the presence of Total phenolic content, flavonoid content, antioxidant activity, accordance with procedures of Indonesian Materia Medika Departemen Kesehatan Republik Indonesia. (1995) and Harborne, (1998).

Determination of Total Phenolic Content

The determination of total polyphenol content was performed using the 96-well microplate reader method 20 µL of sample or standard was added to 100 µL reagent of 25% Folin-Ciocalteu solution, which was homogenized for 1 minute and then allowed to stand for 4 minutes. Then, 75 µL of 10% sodium carbonate solution was added and homogenized for 1 minute. Mixed solutions were incubated for 2 hours at room temperature with dark condition. Gallic acid was used as the standard (Ahmad *et al.*, 2017). The calibration curve of gallic acid which measured at a wavelength of 750 nm by using a microplate reader (VersaMax^{TM} ELISA Microplate reader, USA) with equation y = 0.047x + 0.087 R^2 = 0.992. The total phenolic contents of aquatic plants were measured using Folin Ciocalteu's method and were reported as gallic acid equivalents method by Bobo-Garcia *et al*., (2014) with some modifications. The yield of GAE in extracts was determined by comparing its absorbance with the standard.

Determination of Total Flavonoid Content

The total flavonoid content in aquatic plants was determined using the aluminium chloride colorimetric assay. 50 µl of extracts or standard solution of quercitin (6.25, 12.5, 25, 50, 100 µg/ml) in 70% ethanol was added to 10 µl of 10% aluminium chloride solution, followed by 150 µl of 95% ethanol. 80% ethanol was used as reagent blank. 10 µl of 1 M sodium acetate was added to the mixture in a 96-well plate. All reagents were mixed and incubated for 40 minutes at room temperature, protected from light. The absorbance was measured at 415 nm using a Microplate Reader (VersaMax^{TM} ELISA Microplate reader, USA).[19]

Determination of antioxidant activity

Antioxidant activity was determined using the 96-well plate assay as proposed in several previous studies (Bobo-García et al., 2014), with some modifications. A total of 20µL of sample solution was added to 180µL of DPPH solution (300 µM) in methanol, and then was homogenized for 60s in a microplate reader. The reagents were incubated for 40 minutes in the dark at room temperature. The absorbance was measured at 517 nm in a microplate reader. Quercetin was used as the standard.

$$\%\ DPPH\ inhibition = \frac{A\ sample - A\ blanko}{A\ blanko}\ x\ 100\%$$

Results

Determination of Plants

The species of the collected plants were identified. They consisted of 14 families and 21 species of plants, as shown in Table 1.

DOI: http://dx.doi.org/10.14203/ann.bogor.2019.v22.n2.41-48

Table 1. Name of aquatic plant list from waterfall Bantimurung Bulusaraung South Sulawesi

No	Species Name	Famili	Part of Plant
1	*Anadendrum microstachyum* (de Vriese & Miq.) Backer & Alderw	Araceae	Herbs
2	*Donax canniformis* (G.Forst.) K. Schum.	Maranthaceae	Stem & root
3	*Donax canniformis* (G.Forst.) K. Schum.	Maranthaceae	Leaf
4	*Diplazium*	Athyriaceae	Herbs
5	*Elatostema* sp	Urticaceae	Leaf
6	*Epipremnum* sp	Araceae	Herbs
7	*Hemigraphis* sp	Acanthaceae	Herbs
8	*Lindsaea scandens* Hook.	Lindsaeaceae	Herbs
9	*Murdannia* sp	Commelinaceae	Herbs
10	*Oldelandia* sp	Rubiaceae	Herbs
11	*Stenosemia aurita* (Sw.) C. Presl	Tectariaceae	Leaf
12	*Staurogyne elongata* Kuntze	Acanthaceae	Stem & root
13	*Staurogyne elongata* Kuntze	Acanthaceae	Leaf
14	*Staurogyne* sp	Acanthaceae	Herbs
15	*Schismatoglottis calyptrata* (Roxb.) Zoll. & Moritzi	Araceae	Herbs
16	*Schismatoglottis* sp	Araceae	Herbs
17	*Selaginella plana* (Desv. Ex Poir.) Hieron.	Selaginellaceae	Herbs
18	*Senna alata* (L.) Roxb	Leguminosae	Herbs
19	*Persicaria barbata* (L.) H.Hara	Polygonaceae	Herbs
20	*Pellionia* sp	Urticaceae	Herbs
21	*Pothos tener* Wall	Araceae	Herbs

Phytochemical Screening

The result of phytochemical compounds obtained from 21 aquatic plant samples were presented in Table 2. Of these, flavonoid and glycoside were found in all the samples, phenol and saponin were detected only in several samples. While, there where no alkaloid, terpenoid and antraquinon were found in the samples.

Total Flavonoid Contents

The total flavonoid contents extracted from dried samples were shown in Table 3. The equation for calibration curve of the quercetin standard was y = 0.037 x + 0.205, with R^2 = 0.994.

Among the samples, *Donax canniformis* (G. Forst.) K. Schum (root and stem) showed the highest (11.95 mgQE/g) amount of total flavonoid content, , while the lowest (0.01mgQE/g) ones was in *Persicaria barbata* (L.) H. Hara (herb) (Table 3).

Total Phenolic Contents

The total phenolic contents of the 21 aquatic plants samples were shown in Table 4. Based on total phenolic content analysis, we found that amount of total phenolic content ranged from 0,558 mgGAE/g to 8,864 mgGAE/g. The highest amount of total phenolic contents was detected in *Donax canniformis* (G. Forst.) K. Schumin stem and rootpart of the plant . While the lowest amount of total phenolic content was detected in *Murdannia* sp.

Antioxidant Activity

Eight out of 21 aquatic plants samples were identified to have the ability to scavenge DPPH radicals. Of these, three samples, i.e Donax canniformis, Persicaria barbata, and Diplazium have high ability to scavenge free radicals compared to other samples. . The ability to scavenge free radicals is defined in terms of inhibition concentration (IC_{50}) as shown in Figure 2.

DOI: http://dx.doi.org/10.14203/ann.bogor.2019.v22.n2.41-48

Table 2. List of Phytochemical each aquatic plant from Bantimurung Bulusaraung South Sulawesi.

No	Species name	Part of plant	Phytochemical screening result						
			Alkaloid	Flavonoid	Terpenoid	Phenol	Antraquinon	Saponin	Glycoside
1	*Anadendrum microstachyum* (de Vriese & Miq.) Backer & Alderw	Herbs	-	+	-	-	-	-	+
2	*Donax canniformis* (G.Forst)K.Schum	Stem & root	-	+	-	+	-	+	+
3	*Donax canniformis* (G.Forst.) K.Schum	Leaf	-	+	-	+	-	+	+
4	*Diplazium*	Herbs	-	+	-	+	-	+	+
5	*Elatostema* sp	Leaf	-	+	-	-	-	-	+
6	*Epipremnum* sp	Herbs	-	+	-	-	-	-	+
7	*Hemigraphis* sp	Herbs	-	+	-	-	-	+	+
8	*Lindsaea scandens* Hook	Herbs	-	+	-	-	-	-	+
9	*Murdannia* sp	Herbs	-	+	-	-	-	-	+
10	*Oldelandia* sp	Herbs	-	+	-	-	-	-	+
11	*Stenosemia aurita* (Sw.)C. Presl	Leaf	-	+	-	-	-	-	+
12	*Staurogyne elongata* Kuntze	Stem & root	-	+	-	-	-	+	+
13	*Staurogyne elongata* Kuntze	Leaf	-	+	-	-	-	-	+
14	*Staurogyne* sp	Herbs	-	+	-	+	-	-	+
15	*Schismatoglottis calyptrate* (Roxb.) Zoll.& Moritzi	Herbs	-	+	-	-	-	-	+
16	*Schismatoglottis* sp	Herbs	-	+	-	-	-	+	+
17	*Selaginella plana* (Desv. Ex Poir) Hieron	Herbs	-	+	-	+	-	+	+
18	*Senna alata* (L) Roxb.	Herbs	-	+	-	-	-	-	+
19	*Persicaria barbata* (L) H. Hara	Herbs	-	+	-	+	-	+	+
20	*Pellionia* sp	Herbs	-	+	-	+	-	-	+
21	*Pothos tener* Wall	Herbs	-	+	-	+	-	-	+

Based on antioxidant activity extracted from the leaf of *Donnax canniformis* (G. Forst.) K. Schum, an observed inhibition concentration of IC_{50} value was 67.053 µg/mL and that of While, extracted from from the herbs part of *Persicaria barbata* resulted in 73.745 µg/mL of the IC_{50} concentration value. While, antioxidant activity obtained from the *Diplazium* spp. extracted from herbs part was 90.292 µg/mL of the IC_{50} concentration value. These three aquatic plant samples would be potentially used for future purposes to prevent free radicals.

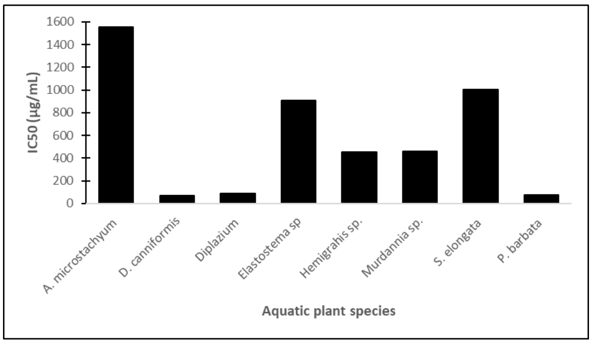

Figure 2. Histogram of antioxidant activity obtained from 8 aquatic plant species collected in Bantimurung Bulusaraung.

Discussion

Based on botanical identification, all aquatic plant samples collected in Bantimurung waterfalls were successfully identified. To our best knowledge, identification study of aquatic plants live at the Bantimurung waterfalls was not reported and this finding was the first report in relation to their botanical names. Prior to the phytochemical compound analysis, present study indicated that two major compounds, including flavonoid and glycoside were detected and all the samples. Two phytochemical compounds such as phenols, and saponin were mostly detected in several aquatic plant samples and no alkaloid, terpenoid and antraquinon were detected.. In current study, for example, *Donnax canniformis* which showed the highest amount of total flavonoid and phenolic contents with high antioxidant activity could be potentially used as source of herbal medicine.

DOI: http://dx.doi.org/10.14203/ann.bogor.2019.v22.n2.41-48

Table 3. Total flavonoid content each species aquatic plant.

No	Species Name	Part of Plant	Total Flavonoid Contents (mgQE/g)
1	*Anadendrum microstachyum* (de Vriese & Miq.) Backer & Alderw	Herbs	5,03
2	*Donax canniformis* (G.Forst.) K. Schum.	Stem & root	11,95
3	*Donax canniformis* (G.Forst.) K. Schum.	Leaf	3,95
4	*Diplazium*	Herbs	4,26
5	*Elatostema* sp	leaf	10,01
6	*Epipremnum* sp	Herbs	0,07
7	*Hemigraphis* sp	Herbs	2,42
8	*Lindsaea scandens* Hook.	Herbs	4,53
9	*Murdannia* sp	Herbs	0,84
10	*Oldelandia* sp	Herbs	1,29
11	*Stenosemia aurita* (Sw.) C. Presl	Leaf	2,18
12	*Staurogyne elongata* Kuntze	Stem & root	0,18
13	*Staurogyne elongata* Kuntze	Leaf	7,70
14	*Staurogyne* sp	Herbs	11,4
15	*Schismatoglottis calyptrata* (Roxb.) Zoll. & Moritzi	Herbs	1,49
16	*Schismatoglottis* sp	Herbs	4,01
17	*Selaginella plana* (Desv. Ex Poir.) Hieron.	Herbs	11,84
18	*Senna alata* (L.) Roxb	Herbs	2,27
19	*Persicaria barbata* (L.) H.Hara	Herbs	0,01
20	*Pellionia* sp	Herbs	5,57
21	*Pothos tener* Wall	Herbs	7,16

However, to meet this purpose, the detailed further study is still needed. Similarly, the two aquatic plants such as *Persicaria barbata* and *Diplazium esculentum* were also promisingly sources of new herbal medicine plants. They showed a good ability to possess antioxidant activity with the IC_{50} values of less than 100µg/mL. The phenolic and flavonoid compounds available in *Diplazium esculentum* species could potentially play a key role in inhibiting DPPH free radicals. Maisuthisakul (2012) reported that flavonoid compounds contained a number of phenolic hydroxyl groups attached to aromatic ring structures, which confer the antioxidant activity. Flavonoids inhibit antioxidant in four ways, consisting of (1) quenching free radical elements, (2) chelating metal, (3) suppressing the enzymes associated with free radical generation, and (4) stimulating internal antioxidant enzymes (Banjarnahor & Artanti., 2014).

Overall, our present study implied that identification of the phytochemical compounds in aquatic plants, especially in Indonesia should be considered as an important aspect to study. Aquatic plants could also potentially be used as source of herbal medicine and to take advantage of their future purposes the detailed further study is still needed.

CONCLUSION

A total of 21 aquatic plants species names were succesfully identified in present study. Phytochemical compound analysis of the aquatic plants resulted in 4 major components including flavonoid, phenol, saponin and glycoside. Three aquatic plant species *Donax canniformis* (G.Forst.) K. Schum., *Diplazium*, *Persicaria barbata* were found to have a high antioxidant activity, which beneficial to attack free radicals.

DOI: http://dx.doi.org/10.14203/ann.bogor.2019.v22.n2.41-48

Table 4. Total Phenolic Contents from each species of aquatic plants.

No	Species Name	Part of Plant	Total Phenolic Contents (mgGAE/g)
1	*Anadendrum microstachyum* (de Vriese & Miq.) Backer & Alderw	Herbs	3,933
2	*Donax canniformis* (G.Forst.) K. Schum.	Stem & root	8,864
3	*Donax canniformis* (G.Forst.) K. Schum.	Leaf	7,071
4	*Diplazium*	Herbs	6,185
5	*Elatostema* sp	Leaf	3,252
6	*Epipremnum* sp	Herbs	0,775
7	*Hemigraphis* sp	Herbs	0,862
8	*Lindsaea scandens* Hook.	Herbs	1,348
9	*Murdannia* sp	Herbs	0,558
10	*Oldelandia* sp	Herbs	0,621
11	*Stenosemia aurita* (Sw.) C. Presl	Leaf	0,924
12	*Staurogyne elongata* Kuntze	Stem & root	3,471
13	*Staurogyne elongata* Kuntze	Leaf	0,629
14	*Staurogyne* sp	Herbs	0,791
15	*Schismatoglottis calyptrata* (Roxb.) Zoll. & Moritzi	Herbs	0,733
16	*Schismatoglottis* sp	Herbs	2,106
17	*Selaginella plana* (Desv. Ex Poir.) Hieron.	Herbs	2,857
18	*Senna alata* (L.) Roxb	Herbs	2,026
19	*Persicaria barbata* (L.) H.Hara	Herbs	6,52
20	*Pellionia* sp	Herbs	5,903
21	*Pothos tener* Wall	Herbs	8,519

Acknowledgements

The Authors are grateful thank to Insinas KEMENRISTEK DIKTI for financial support during the various expedition and research aquatic plant in Sulawesi for 2017-2018. We also greatly thanks to Prof Andi Akhmad Mustafa Chairman Balai Riset Perikanan Budidaya Air Payau dan Penyuluhan – BRSDM KKP Maros, Dr. Darmawan, Director of Politeknik Negeri Pangkep South Sulawesi, Ir. Mastur, Ph.D Chairman of Indonesian Center for Agricultural for Biotechnology and Genetic Resources Research and Development , Dr. Idil Ardi Chairman of Indonesian Riset center for ornamental fish. Dr. Andi Parenrengi, Debora Ayu Christyandari, AMD, and Daswar. We also thanks to the people of South Sulawesi who have helped us and all of heads o regions in the area of South Sulawesi.

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DOI: http://dx.doi.org/10.14203/ann.bogor.2019.v22.n2.41-48