Evaluation of the Phytochemical, Total Phenolic Content, and Antioxidant Activity of Banto Grass (*Leersia hexandra*) Using an Ecological and Ethnobotanical Approach

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Abstract. Banto (*Leersia hexandra*) is an essential plant for the Minangkabau people. Banto grass is a weed plant that easily grows anywhere. This plant can live in dry, watery, or damp areas. This study aimed to evaluate the phytochemical and total phenolic content of banto grass using an ecological and ethnobotanical approach. Banto grass samples originate from Lintau Buo and Pariaman. The phytochemical content of banto grass was determined using a qualitative screening method, and the total phenolic content was determined using gallic acid (GAE) standards. The ecological and ethnobotanical aspects of banto are studied through literature studies including information on botany, ecology, distribution, local names and traditional medicine. The results showed that both banto grass from the high and lowlands extracted with methanol had positive results for flavonoids, alkaloids, tannins, saponins and steroids. However, quantitatively, the total phenolic content of highland banto extract has a higher value than lowland, namely 842.593 mg/L and 649.471 mg/L respectively. Based on ethnobotanical aspects, the leaves of Banto have long been used to make a drink which functions to eliminate toxins in the body, asthma, and shortness of breath. Apart from that, our ancestors also used banto leaves as a toothbrush. Banto grows scattered around areas with lots of water, therefore, the Minang people also use banto as an indicator for digging wells.

1 Introduction

The balance of free radicals and antioxidants is closely related to disease incidence. Many diseases, one of which is a degenerative disease, are initiated by oxidation reactions in the body. According to WHO, degenerative diseases are the deadliest diseases in Southeast Asia, with 8.5 million deaths annually [1]. The negative effects caused by free radicals can be prevented by using natural materials rich in antioxidant compounds.

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Antioxidants are compounds that can overcome the negative effects of oxidation on living cells, such as damage to vital elements of cells, by donating one electron to oxidative compounds so that their activity can be inhibited [2, 3]. Based on the source, antioxidants can be divided into two, namely endogenous antioxidants and exogenous antioxidants [4, 5]. Exogenous antioxidants are antioxidants that come from outside the body. These antioxidants are found in natural materials as secondary metabolites classified as phytochemical compounds [6–8].

Phytochemicals are active chemical compounds produced by plants. The content of phytochemical compounds contained in a plant will affect the benefits of the plant. Phytochemical compounds are classified into five main groups, namely polyphenols, terpenoids, alkaloids, phytosterols, and organosulfur compounds [9]. Phytochemicals are widely used as dyes, food aromas, and medicines [8, 9].

One of the plants used among the people of West Sumatra as a traditional drink is banto grass. This grass is traditionally processed into a drink called "aia banto". People believe in the efficacy of "aia banto" to eliminate toxins in the body, asthma, and shortness of breath [10]. Banto grass is a weed that can easily grow anywhere. This plant can live in dry, watery, and humid areas. Sometimes, these plants form dense populations that cover the water surface.

Plant growth and development can be influenced by internal and external factors. Internal factors such as genes and external factors such as light, temperature, humidity, pH, nutrient content in the soil and altitude. Based on the definition of ecological zones, humidity and altitude are the main environmental factors in plant growth [11]. Differences in altitude will affect the growth and development of plants. As a result, a series of metabolic processes in these plants will also be disturbed so that the compounds produced from these processes will be different at each altitude [11, 12].

2 Materials and methods

2.1 Sample preparation

Banto grass was obtained from different ecological zones in West Sumatra province based on the altitude at which it grows, Lintau Buo and Pariaman. Clean, fresh banto grass was cut into small pieces and then air-dried for two days. Then, the sample was made into powder using a blender and sieved with a 20-mesh sieve.

2.2 Extraction

The powder was taken as much as 50 g for maceration extraction using three solvents that have different levels of polarity, namely methanol p.a, ethanol p.a, and distilled water. The 50g extract was soaked using 500 mL of each solvent for three days. After that, it was filtered to separate the filtrate and the pulp. The extract obtained was then evaporated using a rotary evaporator at 60°C to obtain concentrated extracts in each solvent.

2.3 Phytochemical screening

2.3.1 Identification of saponins

The concentrated extract of banto grass was put into a test tube. Then 10 mL of distilled water was added. Next, the sample was heated in a water bath with a temperature of 60 for 10

minutes. After that, cool the sample and shake it vigorously vertically to cause foam. Foam formation indicates a positive test result for saponins [13].

2.3.2 Alkaloid identification

The concentrated extract of banto grass was added with 1 mL of chloroform and 0.5 mL of ammonia. The sample was shaken, and a few drops of 2 N sulfuric acid were added. Then, the sample is shaken again until two layers are formed, namely the top layer, which is the acid layer and the bottom layer, which is the chloroform layer. The top layer is taken and then dripped on a drip plate. After that, the drip plate is dripped with Mayer reagent, and the formation of an orange or brown precipitate indicates a positive test result for alkaloids [13].

2.3.3 Flavonoid identification

The thick extract of banto grass is taken and then dissolved with 3 mL of ethanol. Then stir and heat; after that, stir again and then filter. Then, the sample is dropped on a drip plate, and 0.2 mg of magnesium and ten drops of concentrated hydrochloric acid are added. The formation of orange to red indicates positive flavonoid test results [13].

2.3.4 Identification of steroids and triterpenoids

A total of 50 mg of thick extract of the sample was given chloroform. Then, the sample is allowed to stand until the chloroform evaporates. Then, the Lieberman-Burchard reagent is added to the sample. The formation of red to orange colour indicates the presence of tertripenoid compounds in the sample. If the colour formed is green to blue, it indicates the presence of steroid compounds in the sample [13].

2.3.5 Identification of tannins

The thick extract of banto grass was added to 20 mL of distilled water and then heated and filtered. The filtrate is then added with a few drops of 1% ferric chloride. The formation of green to brown or blue to black color indicates the presence of tannin compounds in the sample [13].

2.4 Determination of total phenolic content

2.4.1 Preparation of standard solution

The mother liquor was prepared by dissolving 10 mg of gallic acid in a 10 mL volumetric flask using methanol solvent to obtain a 1000 mg/L concentration. Concentration variations were made by pipetting 10.1, 0.2, 0.4, 0.6, and 0.8 mL of 9 parent solutions and then putting them into a 10 mL volumetric flask. 0.5 mL of Folin-Ciocalteu reagent was added to each flask and allowed to stand for 5 minutes. After that, 1 mL of 20% sodium carbonate solution was added and diluted with distilled water until the limit was reached. The mixture was allowed to stand for 120 minutes. The absorbance value was measured at a wavelength of 760 nm. Based on the absorbance value obtained, a calibration curve was made and the regression equation of the standard solution was obtained.

2.4.2 Preparation of test solution

A total of 10 mg was weighed from each extract and dissolved in 10 mL of methanol to obtain a concentration of 1000mg/L. 0.5 mL of the solution was put in a 10 mL volumetric flask. 0.5 mL of Folin-Ciocalteu reagent was added to the volumetric flask and allowed to stand for five minutes. Then, 1 mL of 20% sodium carbonate solution was added and diluted with distilled water until the limit was reached. The mixture was allowed to stand for 120 minutes. Then, the absorbance was measured at a wavelength of 765 nm. The total phenolic concentration of the test solution was determined from the regression equation of the standard solution curve. The total phenolic in the extract was expressed as Gallic Acid Equivalent (GAE).

2.5 Antioxidant activity testing

2.5.1 Preparation of DPPH (1,1-Diphenyl-2-Pricrylhydrazyl) solution

A total of 4 mg DPPH was dissolved in 100 ml methanol p.a. Then the solution was stored in a dark place.

2.5.2 Preparation of test solution

Each thick extract sample was weighed at 10 mg and then dissolved with methanol p.a 10 ml. A concentration series of 10 ml was made from the mother liquor, 20 ml, 30 ml, 40 ml, and 50 ml.

2.5.3 Testing

A total of 1.5 ml of the test solution was added with 2.5 ml of DPPH, after which the solution was incubated in a dark room for 30 minutes. Then the absorbance was measured at a wavelength of 517 nm. For control, 2.5 ml of DPPH solution was used, which was then added to 1.5 ml of methanol solution p.a. based on absorbance obtained, calculate the % inhibition or per cent attenuation value of each concentration using the formula: Antioxidant activity was described by percentage inhibition as % inhibition = (A 0 - A 1) / A 0 × 100 where A0 is the absorbance of the control and A1 is the absorbance of the sample.

Analysis of antioxidant test results is carried out quantitatively by looking at the values IC50. To obtain the IC50 data value, a linear equation curve was created, connecting the concentration of ingredients with the percentage of inhibition. From the linear curve, the linear equation y = ax + b is obtained. The IC50 value is the effective concentration of extract required to reduce 50% of total DPPH so that the value 50 is substantiated into the y-value. After substantiating the value 50 on the y-value, the x-value will be obtained as the IC50 value [3,13,14].

3 Result and discussion

3.1 Ecological approach of *L. hexandra*

Based on data from the Central Statistics Agency (BPS), ecological condition data was obtained from both highland and lowland areas where banto grass was taken.

Table 1. Ecological Aspect of *L. hexandra* habitat

No.	Condition	BE Highland	BE Lowland
1.	Height of the living area	200 - 750 m	0 - 25 m
2.	Rainfall	147 mm^3/month	384.88 mm^3/month
3.	Wind speed	3 km/h	8 km/h
4.	Soil types	Andosol	Regosol and Alluvial
5.	Slope	8-25%	0–2% (flat)
6.	Temperature (min-max)	22-33°C	28-33°C

3.2 Phytochemical screening of banto grass extracts

Phytochemical screening was conducted to identify secondary metabolites contained in banto grass extract. Based on the results of the phytochemical screening of banto grass extract using methanol solvent, saponins, flavonoids, alkaloids, steroids, triterpenoids, and tannins were identified.

Table 2. Phytochemical screening of banto grass extracts

Phytochemical	BE Highland			BE Lowland		
	EtOH	MetOH	Aq	EtOH	MetOH	Aq
Saponins	+	+	-	+	+	-
Flavonoid	+	+	+	+	+	+
Alkaloid	+	+	+	+	+	+
Steroids	+	+	-	-	-	-
Triterpenoids	-	-	+	-	-	+
Tannins	+	+	+	+	+	+
Yield (%)	1.58	1.34	1.62	1.82	2.48	1.72

BE = Banto Extract (+) = Present, (-) = Absent

In determining the levels of total phenolic compounds, gallic acid was used as a standard solution. The maximum absorption of gallic acid was obtained at a wavelength of 765nm. Before measuring the total phenolic content, a standard curve of gallic acid with concentrations of 100, 125, 150, 175, and 200 mg/L was first made. From the examination of the standard curve, a calibration curve was obtained with the equation Y = 0.00075x + 0.1098, and the value of the correlation coefficient (R) is 0.96843.

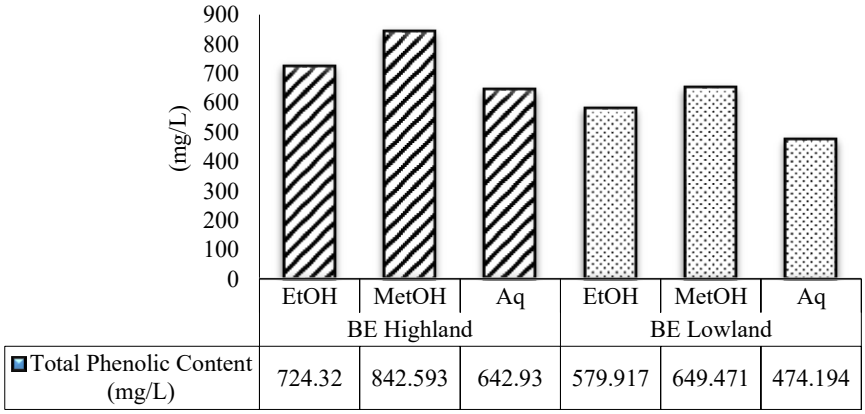

Fig. 1. Total phenolic content of banto grass extracts

The result of the phytochemicals screening and the value of total phenolic content from the highland sample was higher rather than the lowland sample. Accordingly, only samples from the highland were used for the antioxidant activity assay using the DPPH method, and the results are in Table 3.

Table 3. Antioxidant activity of banto grass extract from the highlands

Sample	Solvent	% Inhibition	IC50	Value
Highland Banto Grass	Methanol	24.94	39.35	Strongest [15]
		32.42		
		46.08		
		48.69		
		58.69		
	Ethanol	6.1	123.46	Moderate [15]
		7.04		
		8.95		
		17.48		
		21.11		
	Aquades	6.32	148.76	Moderate [15]
		9.48		
		12.93		
		14.8		
		19.4		

A few consider that height is one of the variables that impact the development of a plant [12]. The phytochemical substance of auxiliary metabolites, such as flavonoids from a plant, will vary in each locale since it is impacted by a few variables. The environment joins light, temperature, pH and the stature of the creating put which can affect the phytochemical substance of a plant [16]. Separated from this, preparing crude materials can impact the chemical substance extricated. The sort of dissolvable utilized can influence the compound extricated from a plant [17].

Based on detailed investigations on therapeutic plants, numerous restorative plants contain expansive amounts of cancer prevention agents. Phytochemical screening was conducted to determine the course of compounds in banto grass extrication. Positive outcomes results appeared within the saponin, flavonoid, alkaloid, steroid and tannin tests. According to Widyasari [18] and Endarini et al. [19], the foam formed identifies the presence of saponins in the sample. This is because saponins contain compounds partially soluble in water (hydrophilic) and compounds insoluble in water (hydrophobic), which can reduce surface tension. The property of saponins that can reduce surface tension can be useful as an emulsifying agent for two liquids that do not mix with each other, such as oil and water. In addition, saponins can also maintain a suspension of glycosides that are insoluble in water [19]. Flavonoids are one of the biggest classes of normal phenols. For the most part, flavonoids are found to tie to sugars to make glycosides, which cause these compounds to break down effectively in polar solvents [20]. Alkaloids are cyclic compounds containing nitrogen molecules whose dispersion is constrained in living beings. Alkaloids can be characterized as characteristic items determined from plants, creatures, microbes, and parasites. Be that as it may, the biggest dispersion of alkaloids is found in plants. Steroids are a lesson of triterpenoid compounds with a perhydrophenanthrene cyclopentane center, comprising three cyclohexane rings and one cyclopentane ring. These compounds are often displayed in free shape and as straightforward glycosides [21]. Tannins are a course of polyhydroxy phenols (polyphenols) that can be recognized from other phenols because of their capacity to accelerate proteins [19]. Based on the inquiries that have been done, the most noteworthy add-up to phenolic substance is found in banto grass tests that live within

the swamps. In this study to decide the full phenolic substance in banto grass extract, gallic corrosive (GAE) was utilized as a standard arrangement. Typically, gallic corrosive is one of the normal and steady phenols. Gallic corrosive is included in phenolic compounds inferred from hydroxybenzoic corrosive which is classified as a basic phenol. Gallic corrosive response with Folin-Ciocalteu reagent produces a yellow colour, demonstrating that it contains phenol, after which it is included with Na_2CO_3 arrangement to create a blue colour. Phenolic compounds respond with Folin-Ciocalteu reagent in a soluble climate to separate protons in phenolic compounds into phenolic particles, so the Na_2CO_3 arrangement is included [22]. Phenolic compounds are compounds of characteristic materials that are very broadly utilized. Phenolic compounds, as organically dynamic compounds, play a major part in the human interface. One of them is an antioxidant for avoiding and treating degenerative maladies, cancer, untimely maturing, and resistant framework clutters [22]. Another factor that can affect plant content is the environment. This is evidenced in research by Katuuk et al., who concluded that the altitude of the place affects the saponin content in babadotan weeds [11]. Another study was conducted by Akhmad [23] on the effect of plant location altitude on the antioxidant power of kirinyuh leaves. In his research, it is known that antioxidant power will increase as the height of the place grows.

4 Conclusion

According to the findings of this study, altitude is one of the factors that influence the growth of banto grass. Banto from both of altitudes showed that extracted with methanol had positive results for flavonoids, alkaloids, tannins, saponins and steroids. The total phenolic content of highland banto extract has a higher value than lowland respectively.

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