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Proximate compositions, bioactive compounds and antioxidant activity from large-leafed mangrove (*Bruguiera gymnorrhiza*) fruit

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Abstract

Antioxidants are compounds that can inhibit or prevent the oxidation of the easily oxidized substrate. One of the plants as a potential source of bioactive compounds and antioxidant activity is large-leafed mangrove (*Bruguiera gymnorrhiza*). This plant was commonly found in the Pacific region of Southeast Asia, Ryukyu Islands, Micronesia and Polynesia (Samoa) to subtropical regions of Australia and has been used by the society. This study aimed to determine the proximate compositions, bioactive compounds and antioxidant activity from large-leafed mangrove fruit which extracted by methanol. It have high carbohydrate content is 29.28%, 66.39% moisture, 2.11% protein, 1.07% fat and 1.15% ash. Old fruit yield greater than young fruit with value 9.94% and 6.83%, respectively. Old fruit has a more effective antioxidant activity (13.47 ppm) compared to young fruit (81.60 ppm) and classified as a very strong antioxidant (IC_{50} < 50 ppm). Bioactive compounds that act as antioxidants are phenol group. By TLC chromatography technique produces the best eluent, namely methanol and water (4:1) and result 3 fractions. Fraction III had the most effective antioxidant activity with IC_{50} value of 26.69 ppm.

Introduction

Antioxidants are compounds that can inhibit or prevent the occurrence of oxidation of the substrate is easily oxidized and has been used by society. Limited reserves of antioxidants in the body so that in the event of exposure excess free radicals, the body requires sources of antioxidants that come from outside. Antioxidants are grouped into two categories based on the source, which are natural antioxidant and synthetic antioxidants. Natural antioxidants are antioxidants obtained natural and be a potential alternative to be developed as a replacement synthetic antioxidants (Winarsi, 2007). Natural antioxidants contain bioactive compounds.

Bioactive compounds can be determined by phytochemical tests. These compounds act as a stamina strengthen, immune system and prevent several diseases, such as cancer, heart disease, stroke, high blood pressure, cataract, osteoporosis and infections digestive tract. Phytochemical compounds found in plants, namely alkaloids, flavonoids, quinones, tannins, polyphenols, saponins, steroids and triterpenoids (Juniarti *et al*., 2009). One of the plants as a potential source of bioactive compounds is large-leafed mangrove (*Bruguiera gymnorrhiza*).

Large-leafed mangrove live in mangrove forest ecosystems. Mangrove forest is widespread areas in the tropics. Indonesian mangrove forest area reached 50% of the total mangrove Asia and nearly 25% of the world's mangrove forests, which is about 3.7 million Ha (Onrizal, 2010). Mangrove forests as one of the wetlands in the tropics with easy access and usability components of high biodiversity and land has made these resources as resources tropical are threatened sustainability (Onrizal, 2005). This is due to the conversion of mangrove forests continue increased so it is necessary to do research on the utilization of mangrove plants, such as the development of functional foods that can serve as antioxidant, antihypertensive and hepatoprotective.

Large-leafed mangrove plants are found in tropical regions of the Pacific from Southeast Asia, Islands Ryukyu, Micronesia and Polynesia (Samoa) to subtropical regions of Australia and has been used by the society. Fruit of this plants have antiviral activity and can against Sarcoma I80 tumor and Lewis lung carcinoma cancerous and high carbohydrate that has the potential to become a new food source. The bark is used to treat burns in the Solomon Islands, diarrhea and malaria in Indonesia and Cambodia (Allen and Duke, 2006). Compounds that play a role as anticancer, anti-diarrhea and malaria has not been studied scientifically. Compound antioxidants thought to play a role in it.

Research on antioxidants and bioactive

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compounds of plants and plant water mangrove has been done, e.g. research velvetleaf plants (Jacoeb *et al*., 2010), water clover (Nurjanah *et al*., 2012), water spinach (Nurjanah *et al*., 2014) and *Sonneratia caseolaris* fruit (Santoso *et al*., 2011), but research on *B. gymnorrhiza* plant still very little. Research of Jacoeb *et al.* (2013) produced the largest yield of crude extract of large-leafed mangrove (*B. gymnorrhiza*) fruit were extracted with methanol, which is 7.85%. The study also result highest antioxidant activity in methanol extracts with IC_{50} 9.42 ppm, but compounds that act as antioxidants are not done isolation and separation (purification) so that the study of this needs to be done.

This study was aims to determine the proximate compositions, antioxidant activity and bioactive compounds as antioxidants function as free radical scavengers from large-leafed mangrove (*Bruguiera gymnorrhiza*) fruit extracted by methanol. The study began with the collection and preparation of samples, proximate analysis, extraction, and separation (purification) using thin layer chromatography (TLC) and column chromatography. Antioxidant activity assay performed before and after separation (purification).

Materials and Methods

Raw materials

The materials used in the study is the large-leafed mangrove (*B. gymnorrhiza*) fruit, methanol pa. (extraction). and materials for separation of bioactive compounds and antioxidant activity assay (free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) Alorich Chem. and Ascorbic acid HmBG Chem.) and phytochemical test. Tools used, i.e. orbital shaker WiseShake, rotary vacuum evaporator Rot Buchi R-205, UV-Vis spectrophotometer Hitachi U-2800, separation by Thin Layer Chromatography (TLC) and Column Chromatography.

Sample preparation and extraction

The study consisted of two stages. The first stage are the collection and preparation of sample, proximate analysis, extraction, phytochemical test and antioxidant activity assay of crude extracts. The second stage are the separation or purification and antioxidant activity test of fractions (results purification). Its fruit used, the old fruits (fruit green-brown, reddish brown petals) and young (green fruit, green tanned petals). Preparation process includes cleaning and drying fruit. Fruits what had clean to reduced size and dried in the sun for 3 days. Fruits that has been dried to obtain a powder of crushed dried and stored in plastic at chilling temperatures for process next research.

Powder of fruits dried were extracted using methanol with the method maceration at room temperature with orbital shaker at 150 rpm. Comparison of sample and solvent is 1:4 (w/v). Extraction carried out for 48 hours and then performed using paper filtration Whatman 42 (Hardiningtyas, 2012). The extraction process is not done until the filtrate colored (clear). The filtrate is collected and then evaporated using a rotary vacuum evaporator at 40°C and then dried using a freeze dryer thus obtained crude extracts of fruit in solid form.

Proximate analysis

Proximate compositions of large-leafed mangrove determined by proximate analysis. Proximate test performed based on AOAC (2005). Analysis was conducted on the test moisture content, fat, protein and ash. Carbohydrate content obtained by difference.

Antioxidant activity assay

Antioxidant activity determined DPPH method by Hanani *et al*. (2005). DPPH test method is one of the methods most widely used to determine the efficiency of the performance of a substance that acts as a antioxidants (Molyneux, 2004). The early stages of testing antioxidant activity is prepare the test solution. Crude extract of samples were dissolved in methanol with concentrations of 10, 20, 30 and 40 ppm. Ascorbic acid was used as positive control and comparison with the concentration of 2, 4, 6 and 8 ppm.

DPPH solution concentration used was 1 mM. The solution used in fresh condition and protected from light. A total of 4.5 ml of test solution included in a test tube is then reacted with 0.5 ml of DPPH solution. Test tube is covered with aluminum foil and incubated at 37°C for 30 minutes then the absorbance was measured using a UV-Vis spectrophotometer at length wave 517 nm.

The antioxidant activity of each sample was expressed in percentage inhibition of free radicals which is calculated by the formula:

$$\%\text{inhibition} = \frac{\text{blanko absorbance} - \text{Sample absorbance}}{\text{blanko absorbance}} \text{ x } 100\%$$

Concentration and barriers to extract the value of each plotted on the x axis and y. Obtained equation in the form [y = b(x) + a] is used to find the value of IC (Inhibitory concentration) with a stated value of y is 50 and the value of x as IC_{50}. IC_{50} values is the concentration of the sample solution is required to reduce DPPH by 50%. This test is performed

three replications. The data obtained analyzed descriptively.

Phytochemical test

Phytochemical testing conducted to determine the bioactive components contained in the crude extract of the fruit elected. Phytochemicals test based on Harborne (1987) include alkaloids, steroids, flavonoids, phenols hydroquinone and tannin.

Separation of the bioactive compounds

Separation of the bioactive compounds selected fruit extracts done in two stages separation based on Sarker *et al.* (2006). The first stage is the separation by thin layer chromatography (TLC) which aims to determine the appropriate eluent to separate the active compounds in the crude extract. The second stage is the separation by column chromatography to collecting fractions of active compounds using the appropriate eluent later each fraction was tested antioxidant activity.

Results and Discussion

Proximate compositions

Large-leafed mangrove fruit used by people as a food substitute for rice in when a bad season. The fruit is usually treated with boiled, dried and can be stored for a long time. Proximate compositions of large-leafed mangrove fruit are presented in Table 1. It's that used in the study have the proximate composition is not much different from it fruit used by other researchers, namely Jacoeb *et al.* (2013) and Fortuna (2005). Carbohydrate content is higher than that obtained with fruit Avicennia marina fruit (Table 1). Differences in the chemical composition in a material can be influenced by the environment or habitat and species (Megayana *et al*., 2012).

Crude extract

Fruit powder were extracted using methanol solvent. Solvent methanol selected based on research conducted by Jacoeb *et al.* (2013). The study resulted in the largest yield and antioxidant activity effective on large-leafed mangrove fruit extracted using methanol solvent. Prior *et al.* (2005) which states that methanol is a polar compound that is easy to position the atoms hydrogen from a compound or hydroxyl groups to form hydrogen bonds so as to facilitate movement of protons (hydrogen atoms antioxidants) to free radicals. Extract the results obtained dark brown. Old fruit yield (9.94%) has a value greater than young fruit (6.83%). It can caused by different harvesting. Harvesting is one of the factors that affect the chemical composition or content of bioactive

Table 1. Proximate composition of fruit large-leafed mangrove (*B. gymnorrhiza*) and *Avicennia marina*

Proximate composition (*wet basis*)	*Bruguiera gymnorrhiza*			*Avicennia marina*
	Current result (%)	Jacoeb *et al.* (2013) (%)	Fortuna (2005) (%)	Hardiningtyas (2012) (%)
Moisture	66.39	62.92	73.76	68.16
Ash	1.15	1.29	0.34	4.45
Crude protein	2.11	2.11	1.13	3.67
Crude lipid	1.07	0.79	1.25	0.72
Carbohydrate	29.28	32.91	23.53	23.00

Table 2. Antioxidant activity of crude extract and ascorbic acid

Sample	Linear line	IC_{50} value (ppm)	Antioxidant[1]
Crude extract			
Old	y = 0.86 + 38.41	13.46	Very strong
Young	y = 0.22x + 32.13	81.60	Strong
Ascorbic acid	y = 7.01x + 22.77	3.88	Very strong

[1]Molyneux (2004)

Table 3. TLC result of crude extract by methanol:water (4:1)

Spots	Solvent (cm)	Compound (cm)	*Rf* value
I	8.00	2.50	0.31
II	8.00	2.70	0.34
III	8.00	3.60	0.45

Table 4. Antioxidant activity of fraction

Sample	Linear line	IC_{50} value (ppm)	Antioxidant[1]
Fraction I	y = 1.48x + 0.58	33.50	very strong
Fraction II	y = 1.28x + 0.45	38.79	very strong
Fraction III	y = 2.17x – 7.99	26.69	very strong

[1]Molyneux (2004)

materials (Megayana *et al*., 2012). Yield results that are suspected to be high content of bioactive found in old fruits.

The results obtained are supported by research conducted Khaerana *et al*. (2008) which states that the impact of harvesting on the content xanthorrhizol on ginger rhizome. The content of these compounds generally increased in plants were harvested at 7 months compared to 5 months of age were harvested. This result is also in accordance with research conducted by Megayana *et al.* (2012) which produces content of Na-alginate seaweed *Sargassum* sp. harvest at the age of 2 weeks (5.54%) increasing in the harvest age 6 weeks (10.62%).

Antioxidant activity of crude extract

Antioxidant activity of the test method used in this study is the method DPPH. This method is the most widely used method for estimate the effectiveness of the performance of substance that acts as an antioxidant. This method chosen because it is a method that is simple, easy, fast, sensitive and only require the least amount of material. Antioxidant activity of large-leafed mangrove crude extract and ascorbic acid presented in Table 2. Old fruits has a higher antioxidant activity than young fruit. This results proves that the extract yield contains a bioactive component obtained high. Old fruit and ascorbic acid has a very strong antioxidant activity, while the young fruits including strong antioxidants (Table 2).

Bioactive compounds of crude extract

Phytochemical content of the methanol extract of the large-leafed mangrove fruit is determined by phytochemical test. Phytochemical compounds

contained in the crude extract of the large-leafed mangrove fruit are steroids, flavonoids, phenols hydroquinone and tannin.

Phenolic compounds are compounds that are soluble in polar compounds and slightly polar. Phenolic compounds can be flavonoids, phenols simple monocyclic, phenyl propanol and phenolic quinones (Harborne, 1987). Prasad *et al*. (2009) states that flavonoids are a group of natural compounds of the most diverse and dispersed wide. These compounds have chemical and biological activity spectrum broad including the scavenging of free radicals. Wu *et al.* (2004) states that the antioxidant a phenolic components work easily in donating a hydrogen atom the peroxyl radical (ROO^*). Valgimigli *et al*. (1995) adds that the effectiveness reduction of free radicals by polyphenols is affected number of hydroxyl groups on the compound. Prior *et al.* (2005) asserts that the effectiveness and strength of activity antioxidant is determined by the ability to move the hydrogen atom and transfer of a single electron.

Antioxidant compounds separation by chromatography

Chromatographic techniques are used for separation by thin layer chromatography (TLC) and column chromatography. TLC technique used to determine the solution developers (eluent). Obtained the best eluent for the separation of crude extract of large-leafed mangrove fruit parents of methanol:water (4:1). This results in the separation of compounds with 3 fractions point (spotting) are different. TLC results can be seen in Table 3. Eluent is then used in the chromatography column. The separation yield 3 fractions based on the same pattern of spots. Fraction is then freeze-dried (freeze dry) so that the fraction obtained in solid form. Fraction then tested the antioxidant activity.

Antioxidant activity of fractions

Solid fractions obtained from the separation tested antioxidant activity with concentrations of 10, 20, 30 and 40 ppm. The test results presented in Table 4 that produces fraction III as a fraction whose activity best antioxidants, which amounted to 26.69 ppm. The value of antioxidant activity differences between the crude extract and fractions. Difference might be due to the antioxidant activity the effect of synergism between the active compounds contained in fruit extracts so that when the compound is separated, then the activity decreased. This case supported by Moure *et al.* (2001), that the combined antioxidant components produce synergistic antioxidant effectiveness higher than the activity in a single component. Mu *et al*. (2007), also produce a combination components of flavonoids and triterpenoids which have neuroprotective effects, antioxidant activity and anti-inflammatory more effective than the single components. Research Santos *et al.* (2010) produce effects synergism between the components quinonemetida flavonoids and triterpenes in providing antioxidant effects.

Conclusions

Large-leafed mangrove (*B. gymnorrhiza*) fruits have high carbohydrate content that is 29.28%, moisture content 66.39%, 2.11% protein, 1.07% fat and 1.15% ash. Old fruit yield more than young fruits with values 9.94% and 6.83%, respectively. Old fruit has a more effective antioxidant activity (13.47 ppm) than younger fruit (81.60 ppm) and classified as a very strong antioxidant (IC_{50} <50 ppm). Classes of active compounds that act as antioxidants are phenol group. By TLC chromatography technique produces the best eluent, namely methanol:water (4:1) and yielded three fractions. Best fraction (fraction III) have antioxidant activity is 26.69 ppm.

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