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Bioactive Compounds and Antioxidant Activity of Lindur Stem Bark (*Bruguiera gymnorrhiza*)

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Abstract

Mangrove has a lot of benefits that intersect directly with human life ranging from ecology to benefit as a source of food, shelter and medicine. One of them is often found in Indonesia is lindur (*Bruguiera gymnorrhiza*). This study aimed to identify the content of bioactive compounds and antioxidant activities of lindur stem bark extract in various solvents. Antioxidant assay used *Free Radical Scavenging Assay* was estimated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and total penolic content in the extracts was calculated as gallic acid equivalent (GAE). The results showed that phytochemical screening that n-hexane extract showed positive results on the parameters of steroids, the ethyl acetate extract on the parameters of steroids, flavonoids, tannins, saponins and phenols hydroquinone, methanolic extract on the parameters of steroids and flavonoids. Based on the antioxidant activity assays and total phenolic content, ethyl acetate extract showed the highest values of IC_{50} and phenolic content (37.23 ppm and 73.24 mgGAE/g). The results indicate mangrove species potential for the utilization as significant source of natural antioxidant.

Keyword

Antioxidant, *Bruguiera gymnorrhiza*, Mangrove, Phytochemical, Total Phenolic

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1. Introduction

Mangrove is a tropical forest that is easy to grow and has not been widely utilized. This is supported by the fact that in this world there are approximately 250.000 types of high plants, but just more than 0.4% the chemical contents that has been investigated (Darminto etal. 2009). Mangroves in Indonesia is the largest in the world, both in quantity of area (± 42.550km^2) and the number of species (± 45spesies) (Spalding etal. 2001). Mangrove species which often found in Indonesia are api-api (Avicenniasp.), bakau (Rhizophorasp.), lindur (Bruguierasp.), and pedada (Sonneratiasp.) (Bengen2001).

Mangrove has a lot of benefits that intersect directly with human life on land, ranging from ecology to benefit as a source of food, shelter and medicine. Mangrove commonly used as drugs and side dishes, but not much information about the bioactive compounds. Several studies in the past reported the presence of the anti-inflammatory, antioxidant, anti-bacterial and antiviral ability of extracts from various species of mangrove (Wibowo 2009). The description indicates the potential of mangrove to be directed to the utilization of marine resources that have potential in pharmacologic.

One of the potential of mangrove extracts is a source of natural antioxidants. According Winarsi (2007) antioxidants are known to inhibit the action of free radicals. The human body does not have much reserve of antioxidants, so if there is exposure of radicals that too excessive, the body requires exogenous antioxidants. Antioxidant compounds are of two types, natural antioxidant and synthetic antioxidants. Antioxidants from natural materials received great attention from the public because it is safer than the use of synthetic antioxidants. The possibility of unknown side-effects of

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synthetic antioxidants makes natural antioxidants into an alternative that potential to developed. Natural antioxidants can protect the body against damage caused by reactive oxygen species, can inhibit the occurrence of degenerative disease and inhibit the forming of lipid peroxide in food. Based on the research of Wichi (1988), synthetic antioxidant compounds include BHA (butylatedhydroxyanisole) and BHT (butylatedhydroxytoluena) potentially carcinogenic.

Exploration of the bioactive compounds of mangrove plants is necessary to find new therapeutic agents and this information is very important for the community. According to Purnobasuki (2004) there are two important reasons to study the chemical constituents of mangrove plants. First, mangrove rain forest is one that is easy to grow and not widely utilized. Second, the chemical aspects of mangroves is very important because of it's potential to develop agrochemical and valuable medical compounds. One of the expectation sources of natural antioxidants that can replace the role of synthetic antioxidants is lindur (*Bruguiera gymnorrhiza*) stem bark. This study aimed to identify the content of bioactive compounds and antioxidants activities of lindur stem bark extract in various solvents.

2. Method

2.1. Materials and Device

The materials needed for testing proximate include lindur stem bark distilled water, selenium, H_2SO_4, boric acid (H_3BO_3) containing 2% indicator of bromcerosole green - methyl red (1:2), 0.10 N HCl, $AgNO_3$, n-hexane, ethyl acetate, and methanol (pa), Wagner reagent, Meyer reagent, Dragendroff reagent, chloroform, acetic anhydride, sulfuric acid, magnesium powder, amyl alcohol, ethanol, $FeCl_3$, DPPH, ascorbic acid, Folin-Ciocelteau reagent andNa_2CO_3 5%.

The tools used in this study include digital scales, homogenizer, volumetric pipette, bulb, test tube, rotary vacuum evaporator (Heidolph VV 2000), orbital shaker, tweezers, microplate, spectrophotometers (UV Vis RS 2500), desiccator, oven, electric stove, Soxhlet tube, water bath, Kjeldahl flask, distillation apparatus, Erlenmeyer flask, burette, volumetric pipette, mortars, centrifuges, funnels, vortex(Pasolina type NS-8).

2.2. Research Method

This research was conducted in six stages, first is sample preparation, the measurement of yield, proximate test of lindur stem bark which refers to the SNI 01-2891-1992 (BSN 1992) (moisture, fat, protein, ash) and crude fiber (AOAC 2005), extraction of plant material (Quinn 1988 referred to Darusman *et al.* 1995), qualitative phytochemical screening refers to Harborne (1987) (alkaloids, steroids, flavonoids, saponins, phenols hydroquinone), free radical scavenging assays (Salazar *et al.*2009) as well as determination of total phenolic content (AOAC 2005).

3. Result and Discussion

3.1. Rendement of Lindur Stem Bark

The percentage rendement of the stem bark is calculated based on the weight ratio of the stem bark and the branches lindur that taken. Rendement of stem bark obtained to 35.15%. Proportion value is used to determine the effectiveness of a material. Lindur plant part that used is the stem bark. Tissue was found in the stem bark are epidermis, parenchyma, cortex and pith. The outer part of the dicotyledonous is bark consisting of epidermal tissue, cork cambium, cortex, and phloem (Duke and James 2006).

3.2. Proximate Result of Lindur Stem Bark

The chemical composition of bark lindur tested include moisture content, ash, protein, fat and crude fiber. Lindur bark chemical composition of fresh and after drying are presented in Table 1. Proximate test generally conducted to determine the key elements in the form of water, ash, carbohydrates, proteins and fats. The chemical composition of a substance contained in foods are various because of differences in nutrition, species and age of the material (Kusumo 1997). Proximate of stem bark can be done to predict the chemical composition in connection with a secondary metabolite produced which usually act as bioactive components in plants.

Table 1. Chemical composition of *B. gymnorrhiza*stem bark.

Chemical Composition	fresh	dried	stem bark of
	B. gymnorrhiza	***B. gymnorrhiza***	***Avicennia marina****
Water content	65.18%	9,.18%	55%
Ash content	1.99%	7.28%	9.6%
Fat content	0.66%	1.98%	1.55%
Protein content	1.89%	4.31%	6.4%
Crude fiber	6.48%	21.70%	

Information: *Handayani (2013)

Testing the water content can be used to determine interval stored time of the material. Water content in a material is a medium for the microorganisms to grow. The water content in the material also determines the received value, freshness and durability of the material. Water content in foodstuffs can be affected by habitat or environment. Mangrove habitat located generally grows on the coast. The measurement result showed that the water content of the fresh bark has a moisture content of 65.18% and bark that has been dried decreased to 9.18%. This result is a bit different from the result of research from Handayani (2013) that has moisture content of fresh A. marina by 55%.

Ash content of fresh stem bark was 1.99 % while after drying was 7.28%. Increased levels of ash in the material caused by the decreased percentage of water content after drying process, so that the proportion of other chemical content is increased. The different ash content may be caused by differences in habitat or environment. In addition, each organism also has a different ability to regulate and absorb minerals, so this will also affect the values of ash content in each material (Winarno 2008).

The result of measurements of fat content fresh stem bark was 0.66%, while dried bark was 1.98%. Increased fat content maybe caused by reduced water content after drying, so the fat will be proportionally increased. This is appropriate with the opinion of Yunizalet al. (1998) which states that water levels are generally inversely related to fat content. This is caused increasingly levels of fat, if the water content contained in the material decreased.

The results of protein measurements content of the fresh stem bark was 1.89 %, while the protein content of dried stem bark was 4.31%. The results of this study are lower when compared with studies of Handayani (2013) which gives 6.4% in the protein assay. Some differences may be due to influence of several factors; habitat, age and metabolic rate. The presence of proteins in the stem more as part of the existing enzymes as well as proteins that are bound in chlorophyll which serves as a regulator of the body's defense and controlling of growth (Marlina 2011).

The results of crude fiber determination of the fresh stem bark amounted to 6.48%, whereas after drying was 21.70%. Crude fiber contains cellulose, hemicellulose and lignin. Crude fiber is the part of food that can't be hydrolyzed by acid or alkali (Muchtadi 2001). Carbohydrates included crude fiber which is the highest chemical composition of mangroves plants.

4. Lindur Stem Bark Extraction Results

The end results of the process extraction are crude extracts in the form of a thick paste with different polarity and colors. The yield of the extract is the ratio between the amounts of extract produced by the number of samples. The yield of extract is expressed in percent.

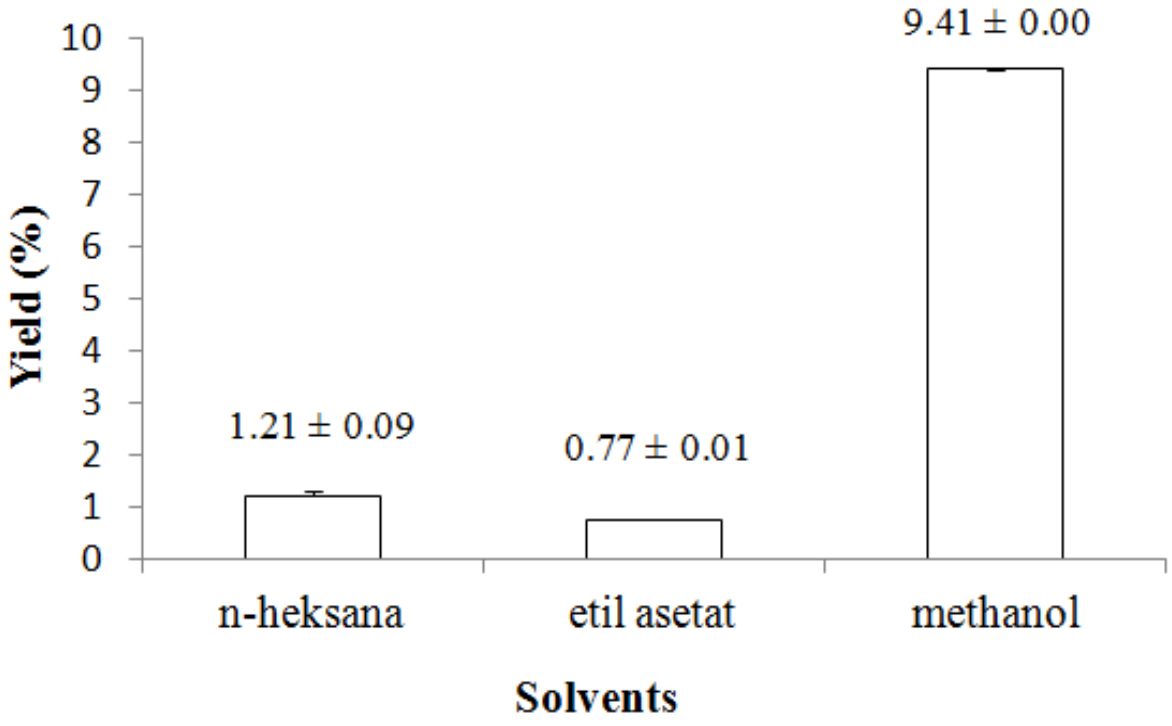

Figure 1. Diagram of yield of stem bark crude extract.

Figure 1 showed that the extraction with methanol has the largest yield, 9.41%. The data showed that the most abundant component in the stem bark is a component that has polar characteristic. It is also proved that methanol is able to separate the compounds better than other solvents.

Extraction results obtained will depend on several factors, including the condition of the natural compounds, the extraction method used, sample particle size, condition, storage time and ratio of solvent extraction by samples (Darusman*et al.* 1995). According to Salamah *et al.*(2008) yield results maceration extracts with different solvents produce different percentage yield. Each differences polarity of solvents dissolving different bioactive components. According to Hougton and Raman (1998), extracts of n-hexane (nonpolar) contains components that are non-polar including wax, fats and essential oils, while the ethyl acetate extract (semipolar) mostly contain alkaloid compounds, aglycones and glycosides, whereas for polar solvents such as methanol and ethanol it can extract phenolic compounds, such as steroids, terpenoids, alkaloids and glycosides.

5. Bioactive Compounds and Antioxidant Activity of Lindur Stem Bark

Phytochemical screening of three stem bark extracts with different solvents showed different results. The results of identified bioactive compounds of stem bark are presented in Table 2.

Table 2. Result of phytochemical test, antioxidant activity and total phenolic content stem bark extract.

Parameters	Crude Extract		
	N-Hexane	Ethyl Acetate	Methanol
Phytochemical	Saponins	Steroids Flavonoids Phenol Hydroquinon Saponins Tanins	Steroids Flavonoids
IC_{50}	1858.36 ppm	37.23 ppm	56,93 ppm
Phenolic content	6.58 mgGAE/g	73.25 mgGAE/g	43.12 mgGAE/g

According to Kannan *et al.*(2009) bioactive compounds are not limited to the results of secondary metabolism, but also include primary metabolites that provide functional biological activities, such as proteins and peptides. Generally lindur stem bark extract has bioactive compound of phenols and terpenoids group. Bioactive compound of phenols detected in the extract include flavonoids, phenols hydroquinone and tannins, whereas the group of terpenoids include steroids and saponins. Most of these bioactive compounds that although there is free form but there is as glycosides, esters of organic acids and in some ways bound to proteins (Harborne 1987).

Bioactive components found in ethyl acetate and methanol extracts one of which is a steroid. Steroids / Triterpenoids are compounds with carbon of 6 units of isoprene biosynthesis and is made from squalene, a C30 acyclic hydrocarbon. Triterpenoids have a cyclic structure and is relatively complex, consisting of alcohols, aldehydes or carboxylic acids (Harborne 1987). Steroid group-containing ethylene avenasterol, proved to have oxidative activity as peroxyl radical can separate atom H from the group (Belitz*et al.* 2009).

Other bioactive components contained in the ethyl acetate and methanol extracts are flavonoids. Flavonoids are found in all parts of the plant, including the fruit, pollen and roots in the form of glycosides. Flavonoids are classified into flavones, flavonols, flavanones, flavanonol, isoflavones, calkon, dihidrokalkon, auron, anthocyanidins, catechins and flavan-3,4-diol (Harborne 1987). Antioxidant properties of flavonoids are derived from the ability to transfer an electron to the free radical compounds and also form complexes with metal. Research Redha (2010) showed that the antioxidant activity of flavonoids rooted in the ability to donate hydrogen atom or through its ability chelating metal.

Phenolic components of an aromatic structure that binds to one or more hydroxyl groups, some may be replaced with a methyl group or a glycosyl. Free phenolic compounds usually found in woody tissue, while the phenolic compounds are usually somewhere else in the form of glycosides. Phenol hydroquinone functions as inhibitors of oxidative free radicals to bind and react with compounds Reactive Oxygen Species (ROS) to form a more stable compound (Eastman 2009). Agati*et al.* (2007) stated that phenolic compounds can protect mangroves from damage caused by ultraviolet radiation.

Saponins including terpenoid classes are available in the form of glycosides and not as alcohol-free (Harborne 1987). Xiong*et al.* (2010) stated that the saponin compounds are antioxidants and radical scavenger by forming hydrogen

peroxide as an intermediate and can donate hydrogen to DPPH radical compounds that terminate radical chain reactions.

Tannins are secondary metabolites that are widespread in plants, especially vascular plant. Tannins are derived polyphenolic compounds with characteristics that can form complexes with other macromolecules. Hagerman (1998) stated that the tannins have the ability to capture free radicals. Tannins are very effective as an electron donor and a hydrogen atom and chelating metals, because these compounds have a hydroxyl group and conjugated double bonds that allow the delocalization of electron.

The results showed that stem bark extract has antioxidant activity. Indication of the power of reducing power lindur bark extract and vitamin C depends on the percent inhibition and IC_{50} values were obtained. Based on the research that has been conducted, IC_{50}values from free scavenging assay of crude extract with n-hexane at1858.36ppm which is much different from the ethyl acetate extract and methanol at37.23ppm and 56.93ppm. Statistical analysis showed that different solvent treatment gave evident effect($p<0.05$) on the antioxidant activity. Duncan test showed that the antioxidant activity of the n-hexane extract contained significantly different from the activity of antioxidants found in extracts of ethyl acetate and methanol.

Lindur stem bark extract which has highest antioxidant activity is ethyl acetate extract as compared with other. Inhibitory activity by ethyl acetate extract estimated because this solvent can extract antioxidant compounds that are polar or non-polar. Tensiska *et al*. (2007) stated that the ethyl acetate solvent may contain more non-polar compounds isoflavones (aglycone) and polar compounds (glikon). Usually component that have antioxidant activity are phenolic compounds that have a hydroxyl group substituted in the ortho position to the -OH and -OR (Andayani *et al*. 2008). Natural antioxidants generally are phenolic compounds or group of polyphenolic like flavonoids, coumarins and tocopherols (Trilaksani 2003).

Lindur stem bark extract using methanol solvent has IC_{50} values of 56.93 ppm. This resulted likely to be lower when compared with the results by Herawati *et al.* (2011) who tested the antioxidant activity of *Sonneratia alba* stem bark with the same solvent and obtained IC_{50}values of 12.20 ppm. Factors that influence these results could be different due to different plant species as well as the length of time the extraction is done. This is in line with research from Hardiningtyas (2012) who showed a decrease in antioxidant activity due to the length time of extraction. Chew *et al.* (2011) and Chirinos *et al.* (2007) also adds that too long time extraction time will cause more oxygen exposure and thus improve the chances of oxidation of the phenolic compounds. N-hexane extract requires very high concentrations to achieve IC_{50}values caused by the property of the non-polar solvent containing only non-polar compounds only such as essential oils, fat, and wax that does not potential as antioxidant (Suratmo 2009).

According to Molyneux (2004), a compound is classified as a very powerful antioxidant when IC_{50} value is less than 50ppm, strong when IC_{50}value is between 50-100ppm, medium if the IC_{50} values ranged between 100-150ppm and weak when IC_{50}values ranging from 150-200ppm. Based on this value, the ethyl acetate extract is a very powerful antioxidant, methanol extract is included into powerful antioxidants, while then-hexane extracts included very weak antioxidant.

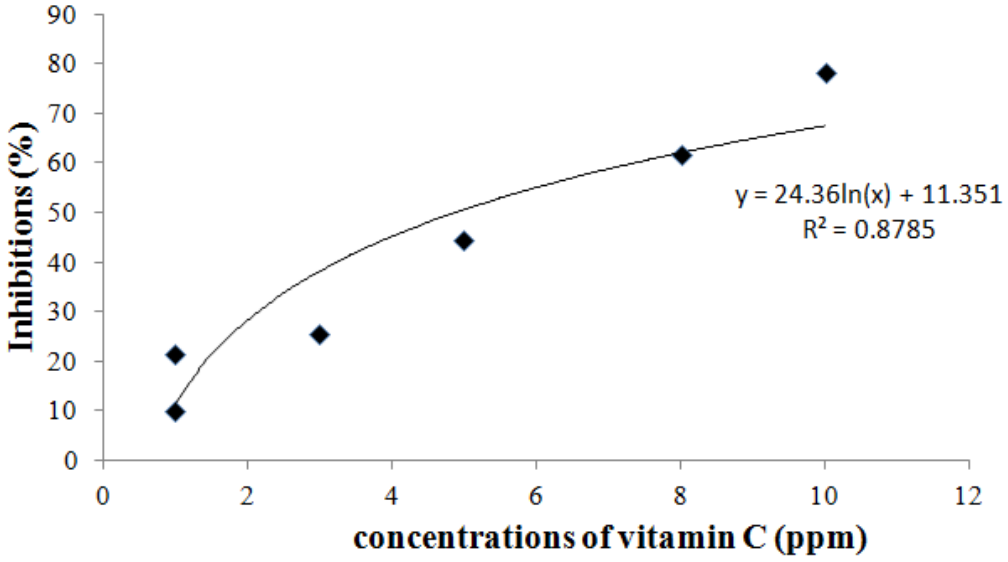

Figure 2. Graph of relation between concentrations of vitamin C and inhibitions percentage by DPPH.

Antioxidant used as a positive control in this study was vitamin C. Purwaningsih (2012) stated that vitamin C is used as a comparison because usually people take vitamin C as a scavenger of free radicals, in this case is done to obtain a description of the antioxidant activity of the stem bark extract when compared with conventional antioxidants such as vitamin C. The relation between the concentration of vitamin C (ascorbic acid), concentration of stem bark extract and percent inhibition of DPPH is presented in Figure 2and 3.

Graph showing the relation between the percent inhibition at concentrations of vitamins C and stem bark extracts if a straight upright line drawn on the chart will show that vitamin C is required to achieve IC_{50} values is much smaller when compared to the concentration stem bark extract, which is in the range of numbers 4 - 6 ppm. Vitamin C is an antioxidant synthetic that has been purified, while the stem bark extract is still a crude extract that has the possibility of having non-antioxidant compounds that do not have antioxidant activity or either can be the inhibitor.

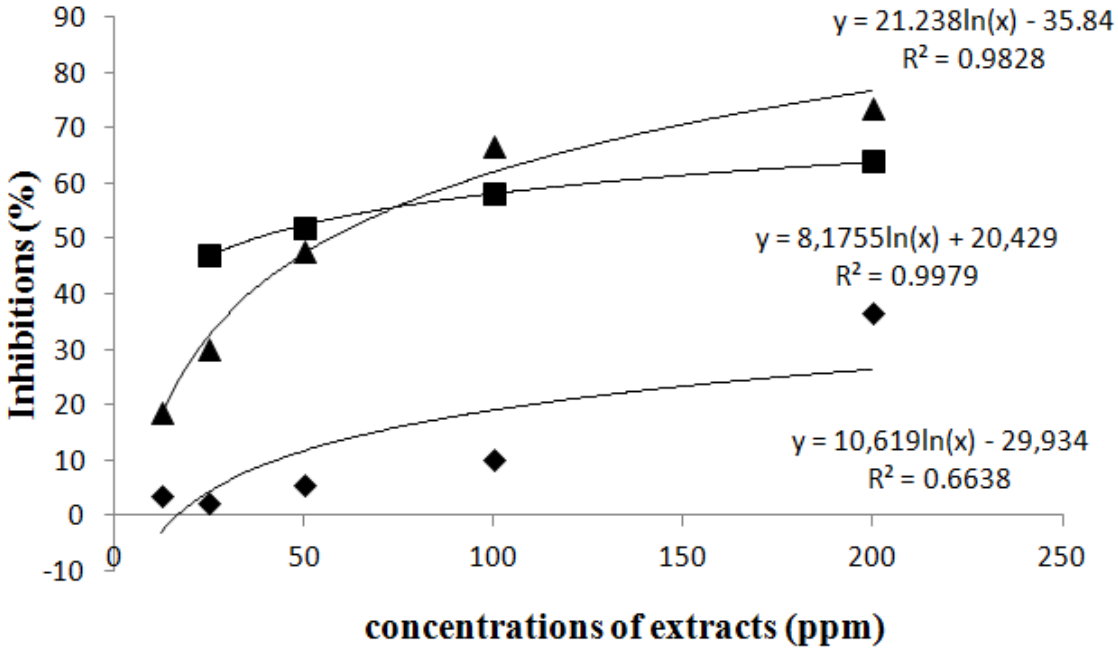

Figure 3. Graph ofrelationbetween concentrations of stem bark extractsand inhibitions percentage by DPPH. (◊) n-hexane, (□) ethyl acetate, (Δ) methanol.

The results of phytochemical screening showed stem bark extract has alleged phenol components affect the antioxidant content in the stem bark extract. Determination of total phenolic content by Folin-Ciocalteu method performed by the Folin-Ciocalteu reagent ability to oxidize the hydroxyl group (-OH) of phenols group. Determination levels of total phenols showed the highest total phenolic stem bark extract produced by ethyl acetate extract is equal to 73.25mgGAE/g. Content of total phenols in a material is usually calculated as gallic acid equivalents (GAE) in milligrams per gram of dry sample. The relation between total phenols and flavonoids with antioxidant activity in plants is the increasing concentration of total phenolic or flavonoid compounds, the higher level of antioxidant activity of the plant (Erukainure 2011). Banerjee *et al*. (2008) explained an inclined of production phenolic compounds will increase if mangroves grow and survive in the depressed condition.

The highest result of total phenolic compound present in the crude extract with ethyl acetate is equal to 73.25mgGAE/g. These results are consistent with the results of phytochemicals screening and antioxidant activity of extracts stem bark with ethyl acetate solvent. According Hardiana *et al.* (2012) generally flavonoid compounds are polar and soluble in polar solvents, such as methanol, but there are several flavonoid compounds that are semi-polar as aglycone flavonoids. It is proved that phenol is very important because of its ability to inhibit free radicals depends on the hydroxyl group or by directly acting as an antioxidant. Meenakshi *et al*. (2009) and Lim *et al.* (2002) stated that the relation between total phenolic compound and antioxidant activity if the material has a high concentration of phenolic compounds, the antioxidant activity of the material is also high. This is carried as by Hardiana *et al.* (2012) stated that the phenolic compounds known to contribute significantly to the antioxidant activities, the greater the content of phenolic compounds either with the antioxidant activity.

6. Conclusion

Generally lindur stem bark extract has bioactive compound of phenols and terpenoids group. Phytochemical screening in n-hexane extract showed positive results on steroids; the ethyl acetate extract on steroids, flavonoids, tannins, saponins and phenols hydroquinone, while the methanol extract on steroids and flavonoids. Based on the antioxidant activity assays, lindur stem bark extract which has best antioxidant activity is ethyl acetate extract with IC_{50} values of 37.23 ppm, followed by extraction with methanol at 56.93 ppm and n-hexane has a very weak antioxidant activity with IC_{50} values at 1858.36 ppm. The highest total phenol present in the ethyl acetate extract is 73.25mgGAE/g followed by methanol extract of 43.12mgGAE/g and n-hexane extract of 6.58mgGAE/g. All experiments that have been done show the best results found in the crude extract of the stem bark extract with ethyl acetate solvent. Tests have been done proved that the phenolic content also relates to the ability of a compound to inhibit free radicals.

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