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PHYTOCHEMICAL COMPOUNDS AND ANTIOXIDANT ACTIVITY OF WATER CHESTNUT (Eleocharis dulcis) AND GIANT MOLESTA (Salvinia molesta) EXTRACT

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ABSTRACT

The purpose of this research was to observe of phytochemical content and antioxidant activity of chinese water chesnut (Eleocharis dulcis) and giant molesta (Salvinia molesta) extracts with three solvents of different levels of polarity. This study used an experimental laboratory method and data analysis was carried out descriptively. Some of the steps carried out include the stages of sampling, sample extraction, calculation of extract extracts, quantitative phytochemical analysis (flavonoid content, phenol content, phenolic content, and tannin content), and test of antioxidant activity (DPPH, ABTS and Reducing ability. The results showed that extracts of extract using ethanol in water chesnut and giant molesta had the highest values of 2.01% and 2.26%. Quantitative phytochemical tests on water chesnut and giant molesta extract showed that ethanol solvents at flavonoid levels had the highest values of 288.75 ppm and 267 ppm. The results of antioxidant activity with the water chesnut and giant molesta extracts had very weak IC50 values in n-hexan solvents valued at 1977.22 ppm and 3211.2 ppm. Water chesnut of extract had the highest ABTS value in ethyl acetate solvents at a concentration of 1000 ppm at 85.253% and in giant molesta extract at a concentration of 1000 ppm using ethanol solvent had the highest value of 76.665%. Ethanol extract in water chesnut extract and giant molesta had the highest reducing ability power with a mean absorbance of 0.477 and 0.520. The best solvent for extracting water chesnut and giant molesta were ethanol.

Keywords: Antioxidant, water chesnut, giant molesta, phytochemical

1. INTRODUCTION:

Water chesnut (*Eleocharis dulcis*) is one of the wild plants that can live in swamp waters. Water chesnut can also grow throughout the year, especially on watery land. Water chesnut have stems that are upright and not branched. Giant molesta (*Salvinia molesta*) is one of the aquatic plants whose life floats on the surface of the water. Giant molesta is also a water plant that is widely found in swamps, lakes and ponds that have calm water flow. Giant molesta live on the surface of the water by covering the surface of the water. Giant molesta also floats freely on the surface of calm water and its growth and development is so fast that it often covers the surface of the water.

Water chesnut and giant molesta are one of the swamp plants which are abundant in South Sumatra swamp waters especially in the Indralaya region. The swamp plants do not have high economic value and are not utilized by the surrounding community because many people do not know the use of the swamp plants. This plant has been known to contain phytochemical compounds.

Research on tuber skin extracts and fractionation from water chesnut (Eleocharis dulcis) using methanol which was then extracted using maceration to increase polarity using ethyl acetate, n-butanol and water solvents (Zhan et al., 2016). The ethyl acetate fraction (EF) of water chesnut showed that the ethyl acetate fraction had the strongest antioxidant activity compared to the n-butanol fraction and the water fraction, where the IC50 value of the ethyl acetate fraction was 0.36 mg/mL for DPPH and 0.40 mg/mL for ABTS. The results of this study also showed that the tuber skin from water chesnut could be potential as a source of natural antioxidants in nutraceuticals, useful as additives, and in the food industry. In the study of Ernaini et al. (2012), phytochemical compounds from giant molesta extracts contain alkaloids, phenols and saponins. Phenol compounds can function as primary antioxidants because they are able to stop free radical chain reactions in lipid oxidation. However, research that has used all parts of the water chesnut (*Eleocharis dulcis*) has not been carried out including identification of phytochemical compounds and antioxidant analysis (Rorong and Suryanto, 2010). Giant molesta research on quantitative phytochemical tests, including identification of phytochemical compounds and antioxidant analysis, has also never been done..

2. MATERIALS AND METHODS:

2.1. Materials

Tools used include analytical balance, rotary evaporator, dropper pipette, spectrophotometers, test tubes (iwaki), analytical scales (ohaus), aluminum foil and vortex (inesco). The main ingredient in this study are water chesnut (*Eleocharis dulcis*) and giant molesta (*Salvinia molesta*). The chemicals used for extraction are n-hexane, ethyl acetate, and ethanol. The analytical material used is aquadest, DPPH (2,2-diphenyl-1-picrylhydrazyl), phosphate buffer, potassium ferrisianide, and trichloroacetic acid.

2.2 Collection and sample preparation

Sample preparation is as follows: Swamp plants are washed with running water to remove impurities such as mud, wood, twigs, other types of plants and other foreign matter. The clean sample is then dried in the sun. The dried sample is then tested for water content, where the water content in the sample must be below 10%. After that the sample is blended using a blender until the sample becomes powder (simplicia).

2.3 Sample extraction

The extraction method used in this study is a multilevel extraction method. Harborne (1987) states that multilevel extraction is done by soaking samples with different solvents sequentially using n-hexane (nonpolar) solvent, ethyl acetate (semipolar), and ethanol (polar) sequentially for 2x24 h, respectively. The sample extraction method is as follows: Swamp plant powder is weighed as much as 100 grams and put into an erlenmeyer, then soaked with n-hexane (nonpolar) solvent until the final volume reaches 1000 mL with a ratio of 1:10 for 2x24 h at room temperature then filtered with Whatman 42 and produce n-hexane filtrate and residue. The residue was then soaked again using a solvent of ethyl acetate (semipolar) in a ratio of 1:10 for 2x24 h, then filtered with Whatman 42 and produced ethyl acetate and residue filtrate. 10 for 2x24 h, then filtered with Whatman 42 and produce ethanol filtrate and residue. The filtrate obtained from the three solvents was then evaporated using a vacuum rotary evaporator at 45℃. After evaporation, the extract was weighed to determine the extract yield, then it was put into a light-proof bottle and stored in a cabinet (4℃) until the extract was used.

2.4 Yield extract

Extract extract is used to determine what percentage of extract obtained from the solvent. The extract yield percentage can be calculated using the following formula:

PR=Be/Bs x 100%

Be = weight of extract produced

Bs = weight of simplicia used

2.5 Flavonoid content

Analysis of flavonoid content was carried out according to the method of Martinus dan Verawati (2015), making a solution of each sample extract, for total extract and polar extract at a concentration of 5 mg/mL by dissolving 0.125 g of the sample thick extract in a mixture of methanol and distilled water (1: 1) in a 25 mL volumetric flask to the mark limit. As for the non-polar extract with a concentration of 25 mg / mL by dissolving 0.625 g of the thick extract of the sample in a methanol mixture of distilled water (1: 1) in a 25 mL measuring flask to the mark limit. Then each extract was piped 0.5 mL and put into vials then mixed with 1.5 mL methanol, then added 0.1 mL of 10% aluminum chloride solution, 0.1 mL of 1 M sodium acetate and 2.8 mL of aquadest. The solution was homogenized and allowed to stand for 30 min, then the absorbance was measured at the maximum absorption wavelength using the UV-Vis spectrophotometer.

2.6 Phenol content

Phenolic compounds are important plant constituents with redox properties responsible for antioxidant activity (Soobrattee et al., 2005). The hydroxyl groups in plant extracts are responsible for facilitating free radical scavenging. As a basis, phenolic content was measured using the Folin–Ciocalteu reagent in each extract. The results were derived from a calibration curve (y = 9.53x − 0.13, R2 = 0.996) of gallic acid (0–250 µg/mL) and expressed in gallic acid equivalents (GAE) per gram dry extract weight

2.7 Phenolic content

Analysis of phenolic levels carried out according to the method of Andarwulan and Shetty (1999) is as follows: A total of 50 mg samples plus 2.5 mL of 95% ethanol in a test tube, then centrifuged 3500 rpm for 10 min. 1 mL of the supernatant was put into a test tube containing 1 mL of ethanol and 5 mL aquadest, then add 0.5 mL of Folin-Ciocalteu reagent, let stand 5 min. 1 mL Na_2CO_3 5% was divortexed and left in a dark room for 60 min. The sample was homogenized again to measure its absorbance at a wavelength of 725 nm. Gallic acid standard curves are used for calculations with the following formula

$$\text{Phenolic content} = \frac{y-b}{a} : \frac{\text{sample weight (g)}}{1000}\ x\ Fp$$

2.8 Taninn content

Quantitative estimation of tannin was performed by titrating the extract with standard potassium permanganate solution following the method of AOAC (19800.

2.9 Antioxidant analysis with DPPH method

The antioxidant activity uses the DPPH method (Falah et al., 2008) as follows: Take 1 ml of sample that has been dissolved in methanol with concentrations of 100 ppm, 200 ppm, 300 ppm and 400 ppm respectively. Then add as much as 2 ml of DPPH (0.003 g / 50 ml of methanol). The mixture is then homogenized with vortex and then incubated at room temperature for 30 minutes. Then measured with a spectrophotometer at a wavelength of 517 nm. Tests were also carried out on blank solutions (DPPH solution with the solvent). The absorbance value obtained is then used to get the percent of free radical capture.

The absorption value of DPPH solution before and after the addition of the extract was calculated as percent inhibition (% inhibition) with the following formula:

% Inhibition = [(Ao – As)/ Ao] x 100

Then the calculation results are entered into the regression equation. The IC50 value of the current calculation of% inhibition is 50%.

Y = aX + b (Zuhra et al., 2008)

2.10 Antioxidant analysis with the ABTS method

For ABTS assay, the procedure followed the method of Arnao et al. (2001) with some modifications. The stock solutions included 7 mM ABTS solution and 2.4 mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 14 h at room temperature in the dark. The solution was then diluted by mixing 1 ml ABTS solution with 60 ml methanol to obtain an absorbance of 0.706 ± 0.01 units at 734 nm using a spectrophotometer. Fresh ABTS solution was prepared for each assay. Plant extracts (1 ml) were allowed to react with 1 ml of the ABTS solution and the absorbance was taken at 734 nm after 7 min using a spectrophotometer. The ABTS scavenging capacity of the extract was compared with that of BHT and ascorbic acid and percentage inhibition calculated as ABTS radical scavenging activity (%)= Abscontrol−AbssampleAbscontrol where

Abscontrol is the absorbance of ABTS radical in methanol;

Abssample is the absorbance of ABTS radical solution mixed with sample extract/standard. All determinations were performed in triplicate (n = 3).

2.11 Antioxidant analysis with the reduction method

The reduction power of the extract was determined by the method of Oyaizu (1986) and modified by Lai et al. (2011), as follows: The test solution was dissolved in distilled water with a concentration of 100 ppm, 200 ppm, 300 ppm and 400 ppm as much as 1.25 ml then mixed with 1.25 ml of phosphate buffer 0.2 M pH 6.6 and 1.25 ml of K3Fe (CN)6 1%. The mixture was incubated at 50ºC for 20 min. After incubation, the solution was cooled immediately and added with trichloroacetic acid 10% as much as 1.25 ml. Pour 1 ml of the mixture into a new tube and add 1 ml of distilled water and add 0.1 ml of FeCl3 0.1%. Absorbance was measured at a wavelength of 700 nm with a spectrophotometer. The higher the absorbance indicates the higher the reducing power.

3. RESULTS AND DISCUSSION:

3.1 Yield extrct

Extract yield was calculated based on the ratio between the final weight (weight of extract produced) and initial weight (weight of simplicia used) multiplied by 100%. The higher the yield of extract produced indicates that the opportunity for greater raw material to be utilized. This extract was extracted using multilevel extraction using three different types of solvents. The type of solvent used can affect the active compound that is extracted. N-hexane solvents will attract non-polar compounds, ethyl acetate solvents will attract semi-polar compounds and ethanol can attract polar compounds. The yield value of water chesnut and giant molesta extract can be seen in Figure 1.

In Figure 1A it can be seen that the yield of water chesnut extract in ethanol solvent has the highest yield than n-hexane and ethyl acetate. At the first level the extract using n-hexane solvent yields a yield of 0.50% at the second extraction level using ethyl acetate solvent produces an extract yield of 1.15% and at the third level the extract uses ethanol solvent yield extract extraction of 2.01%. Baehaki et al. (2018) stated that the yield of water chesnut extract with the first level extract using n-hexane solvent yields a yield of 0.076%, at the second extraction level using ethyl acetate solvent an extract yield of 0.589 g %, and at the third level the extract using ethanol solvent resulted in an extract yield of 4.778%.

It can be seen in Figure 1B that the extract of giant molesta also has the highest value in ethanol solvent, where in the first level extract using n-hexane solvent yields a yield of 0.51% at the second extraction level using ethyl acetate solvent produces extract yield of 1.59% and at the third level extract using ethanol solvent resulted in an extract yield of 2.26%. Anggraini (2018) stated that the yield of giant molesta extract with the first level of extract using n-hexane solvent yields a yield of 0.27%, at the second extraction level using ethyl acetate solvent produces an extract yield of 1.12%, and at the third level the extract using ethanol solvent resulted in an extract yield of 2.24%.

In this study the extract yield produced by using different solvents has different amounts of extracts. This was due to the ability of each solvent to dissolve compounds according to their polarity. The high yield of extracts produced by ethanol solvents shows that ethanol is a universal solvent capable of dissolving polar, semi-polar and non-polar compounds.

3.3 Phytochemical compounds

Phytochemical tests of water chesnut (*Eleocharis dulcis*) and giant molesta (*Salvinia molesta*) were carried out using three solvents namely n-hexane, ethyl acetate and ethanol with different polarity which will be tested quantitatively for phytochemical compounds. Phytochemical results of water chesnut and giant molesta extract is shown in Table 1 and 2.

Based on Table 1 and 2 quantitative phytochemical tests of water chesnut and giant molesta extracts showed that the phyochemical compounds (favonoid, phenol, phenolic and tannin) had the highest value in ethanol extract then ethyl acetate and n-hexane. In the water chesnut and giant molesta using n-hexane solvent has the lowest value indicating that n-hexane was not the right solvent to be able to extract phyochemical compounds. This was because n-hexane is a non-polar solvent. Sudirman et al. (2017) states that the flavonoids contained in ethanol extract contain polar flavonoids while the soluble flavonoids in ethyl acetate and n-hexane extracts are thought to be flavonoid aglycones which are less polar so that they can dissolve in the solvent.

3.4 Antioxidant activity with DPPH method

The DPPH method in testing antioxidant activity has an easy and fast procedure for evaluating the activity of capturing free radicals from non-enzymatic antioxidants. The testing principle was the transfer of electrons and the transfer of hydrogen atoms between antioxidants and DPPH radicals, so that DPPH (Diphenyl Pikril Hidrazil) will be reduced to DPPH-H (diphenyl picril hydrazine) and a color change from purple to yellow. Antioxidant activity of DPPH method of water chesnut and giant molesta extract is shown in Figure 2 and 3.

In Figure 2. the percentage results of water chesnut samples from n-hexane extract with a concentration of 1.000 ppm was 32.28% of free radical inhibition which is the largest percentage in testing the antioxidant activity of n-hexane extract then the results of the percentage of inhibition power from extraction with solvents ethyl acetate with a concentration of 1.000 ppm resulted in a higher percentage of free radical inhibition than n-hexane extract, which was 45.704%, while the percent inhibition of DPPH in ethanol extract showed the highest percentage of DPPH inhibitors among n-hexane and ethyl acetate solvents, because the solvent ethanol which is polar. Ethanol extract at a concentration of 1.000 ppm has an absorbance value of 56,644%.

In Figure 3 it can be seen that the results of the percentage of inhibition power from extract of giant molesta by using n-hexane, ethyl acetate and ethanol at a concentration of 1000 ppm has the highest absorbance value. The n-hexane solvent with a concentration of 1.000 ppm produces a percentage of free radical resistance of 20.134%. Percentage of DPPH inhibition with ethyl acetate solvent with a concentration of 1.000 ppm produces a higher percentage of free radical inhibition than n-hexane extract that is equal to 66.107% and the results of the percentage of inhibition power from the third stage extraction with 70% ethanol solvent shows the highest percentage of DPPH inhibitors with concentration of 1000 ppm which has an absorbance value of 71.8115%.

The ethanol extract at a concentration of 1.000 ppm from the kimbang extract had the highest absorbance value compared to n-hexane and ethyl acetate solvents, indicating that the greater the absorbance value, the higher the reducing power by antioxidant compounds found in the plant. 50% free radical activity inhibition concentration (IC_{50}) values can be calculated using regression obtained from the relationship of the sample concentration and the percentage of free radical activity inhibitors. The results of the antioxidant activity test are expressed by the value (IC_{50}).

In Figure 4 shows that the extract of water chesnut and giant molesta by using solvent n-hexane, ethyl acetate and ethanol has a very weak antioxidant activity that is on the water chesnut extract IC_{50} value of n-hexane solvent was 1977.22 ppm, ethyl acetate solvent worth 1083.61 ppm and 70% ethanol extract worth 854 ppm. Whereas the Giant molesta extract IC50 value obtained from n-hexane extract was 3211.28 ppm, ethyl acetate was 825.20 ppm and 70% ethanol was 726.80 ppm.

According to Mardawati et al. (2008), a compound said to be an antioxidant was very strong if the IC50 value <50 ppm, strong if the IC50 value was 50-100 ppm, while if IC50 was 100-150 ppm, it was weak if IC50 was 150-200 ppm and very weak if the IC50 value> 200 ppm. These results indicate that extracts from water chesnut and giant molesta using n-hexane, ethyl acetate and ethanol solvent has very weak antioxidant activity because it has an IC50 value> 200 ppm. In extracts of water chesnut and giant molesta which had very weak IC50 values were n-hexane extracts worth 1977.22 ppm and 3211.2 ppm compared with ethanol. Agustin (2017) states that the hyacinth n-hexane extract has a very weak antioxidant activity value because the IC50 value obtained> 200 ppm. This was thought to be caused by extracts from water chesnut and giant molesta with ethanol solvents containing more compounds classified as natural antioxidants such as flavonoids.

3.5 Antioxidant activity with the ABTS method

The ABTS method was a method of determining antioxidant activity obtained from the oxidation of potassium persulfate with ABTS diammonium salt. The presence of antioxidant activity from the sample is marked by the loss of blue in ABTS reagents (Molyneux, 2004). The principle of ABTS test is the removal of ABTS cation color to measure the antioxidant capacity that reacts directly with ABTS cation radicals, the presence of ABTS activity can be inhibited by antioxidants in the reaction medium with the activity affected by the reaction time and the amount of antioxidants (Yu, 2008). Antioxidant activity of the ABTS method of water chesnut and giant molesta extract is shown in Figure 5 and 6.

In Figure 5 the results of the ABTS method analysis using water chesnut extract showed that the n-hexane extract had the highest reducing power at a concentration of 1000 ppm with an absorbance value of 58,343%. Then followed by ethyl acetate extract at a concentration of 1000 ppm with an absorbance value of 85.253% and ethanol extract at a concentration of 1000 ppm with an absorbance value of 65.041%. The absorbance value of ethyl acetate extract showed that this ethyl acetate extract had the highest reducing ability which was 85.253%.

In Figure 6 ABTS analysis of giant molesta extract has the highest value at a concentration of 1000 ppm, where n-hexane extract has an absorbance value of 63.6315. Then the absorbance value of ethyl acetate extract at a concentration of 1000 ppm is 75.147% and ethanol extract 70% with an absorbance value of 76.675%. This value also shows that the extract from ethanol has the highest reducing ability by 0.520% compared to ethyl acetate and n-hexane extracts. This shows that the greater the absorbance value, the higher the reducing power by antioxidant compounds found in these plants.

3.6 Antioxidant activity with the reducing power method

Reduction power test is a method to strengthen the results of the antioxidant activity test using the DPPH method. According to Oyaizu (1986), the reducing power is related to the ability to release H atoms to react with free radicals to form antioxidant radicals. The basic principle in this reduction power method is that antioxidant compounds will reduce Fe3+ ions to Fe2+ ions to make it more stable.

In Figure 7 it can be seen that the difference in each concentration used, where the higher concentrations made indicate that the absorption value in the sample was increasing as well. Based on the results of the analysis of the reduction power method by using water chesnut extract in Figure 4.8. showed that ethanol extract had the highest reducing power at a concentration of 1000 ppm with an absorbance value of 0.477. Then followed by ethyl acetate extract at a concentration of 1.000 ppm with an absorbance value of 0.373 and n-hexane extract had the highest reducing power at a concentration of 1.000 ppm with an absorbance value of 0.347. The absorbance value of ethanol extract shows that it has the highest reducing ability that is equal to 0.477.

In Figure 8 the results of the analysis of the reduction power of giant molesta extract has the same reducing power as the highest extract of water chesnut at a concentration of 1000 ppm using 70% ethanol solvent with an absorbance value of 0.520. Then the absorbance value of ethyl acetate extract at a concentration of 1000 ppm was 0.495 and n-hexane extract has the highest reducing power at a concentration of 1000 ppm with an absorbance value of 0.373. This value also shows that the extract from ethanol has the highest reducing ability of 0.520 compared to ethyl acetate and n-hexane extracts. Agustin (2017) states that the analysis of reducing power using water hyacinth extract (*Eichhornia crassipes*) shows that methanol extract has the highest reducing power at a concentration of 2000 ppm with an absorbance value of 0.2420, ethyl acetate at a concentration of 2000 ppm of 0.1815 and n-hexane at a concentration of 2000 ppm of 0.0830. This shows that the greater the absorbance value, the higher the reducing power contained in the plant as an antioxidant compound.

4. CONCLUSION:

The highest yield of extracts from the extract of water chesnut and giant molesta extracts were found in ethanol solvent. The fraction producing the most bioactive components was found in the ethanol fraction. The highest content of bioactive components found in extracts of water chesnut and giant molesta were found in flavonoid levels of 288.75 mg/mL and 267 mg/ml. Ethanol fraction has a very weak antioxidant activity with IC_{50} values> 200 ppm of 854 ppm in water chesnut extract and 726.80 ppm in giant molesta extract. Antioxidant ABTS antioxidant test results had the highest absorbance value in ethyl acetate extract at a concentration of 1.000 ppm at 85.235 ppm, while giant molesta in ethanol extract at 76.675 ppm.

5. ACKNOWLEDGMENTS:

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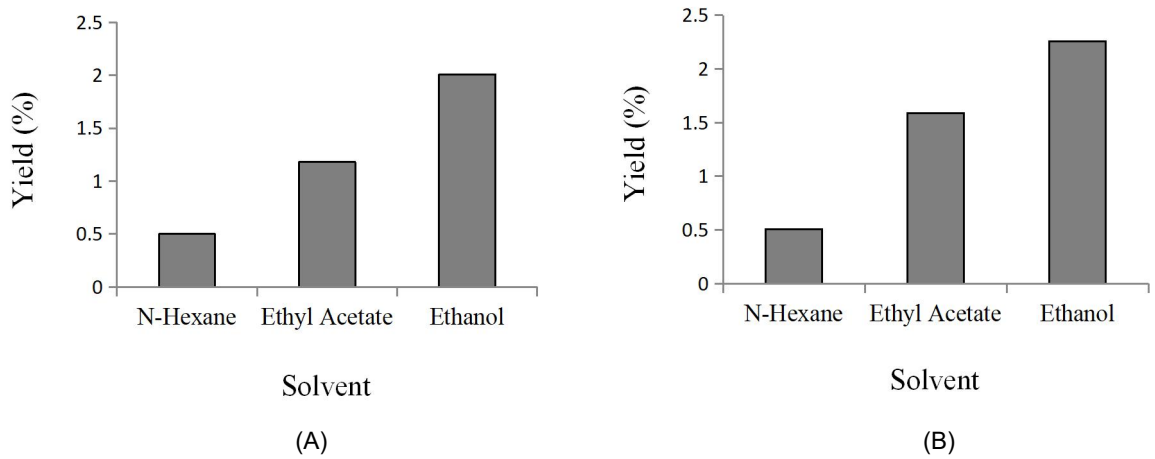

Figure 1. Yield extraction (A=water chesnut extract, B= giant molesta extract)

Table 1. Phytochemical results of water chesnut extract

Phytochemical Compound	Solvent		
	N-Hexane	Etyl acetate	Ethanol
Flavonoid	87.50	171.25	288.75
Phenol	24.21	45.43	53.33
Phenolic	30.00	74.00	86.00
Tannin	20.49	37.07	47.42

Table 2. Phytochemical results of giant molesta extract

Phytochemical Compound	Solvent		
	N-Hexane	Etyl acetate	Ethanol
Flavonoid	55.00	192.00	267.00
Phenol	32.60	64.20	100.20
Phenolic	115.33	138.00	175.66
Tannin	51.30	60.57	67.62

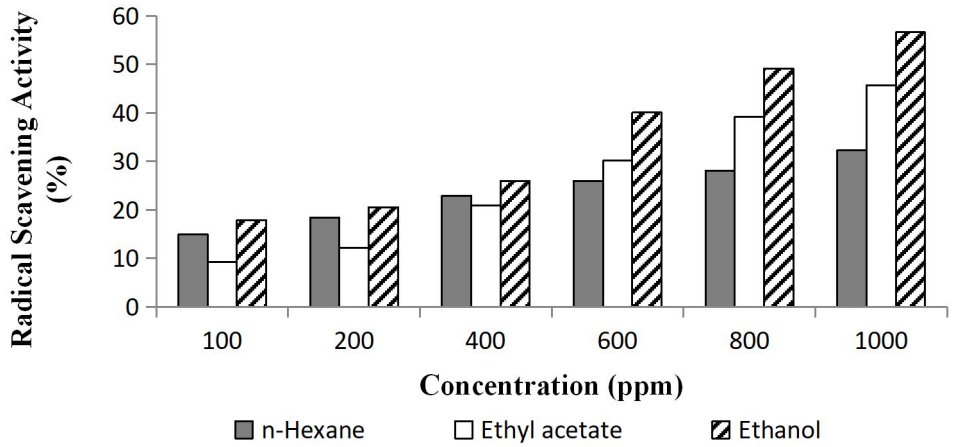

Figure 2. Antioxidant activity of DPPH method of water chesnut (*Eleocharis dulcis*) extract

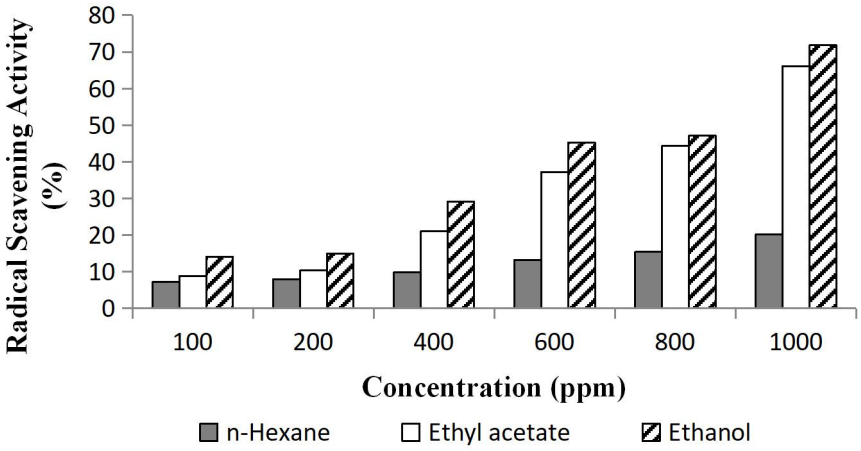

Figure 3. Antioxidant activity method of DPPH of giant molesta extract

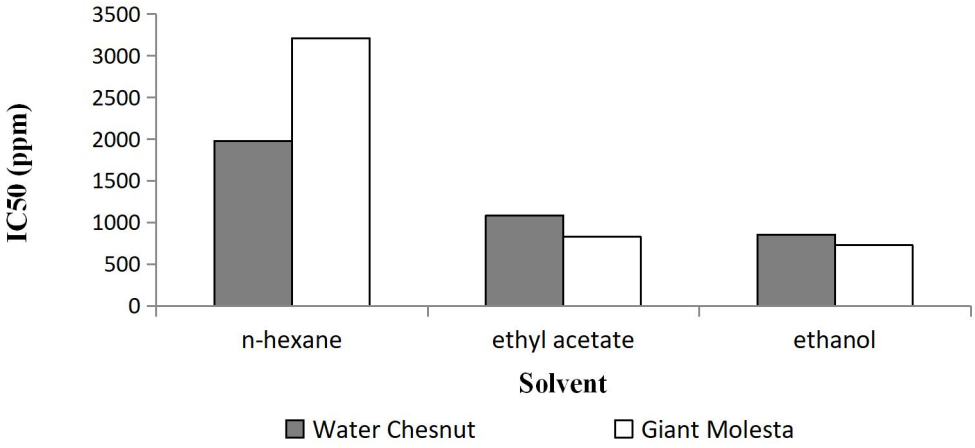

Figure 4. IC_{50} value of water chesnut and giant molesta extracts

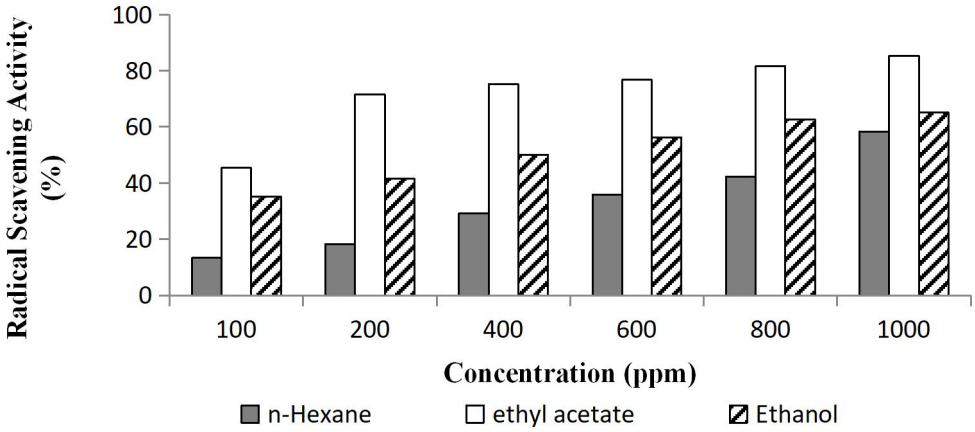

Figure 5. Antioxidant activity of the ABTS method of water chesnut extract

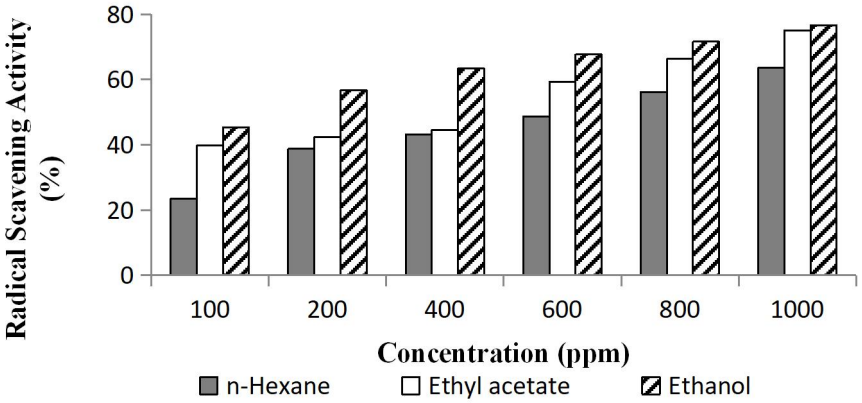

Figure 6. Antioxidant activity of the ABTS method of giant molesta extract

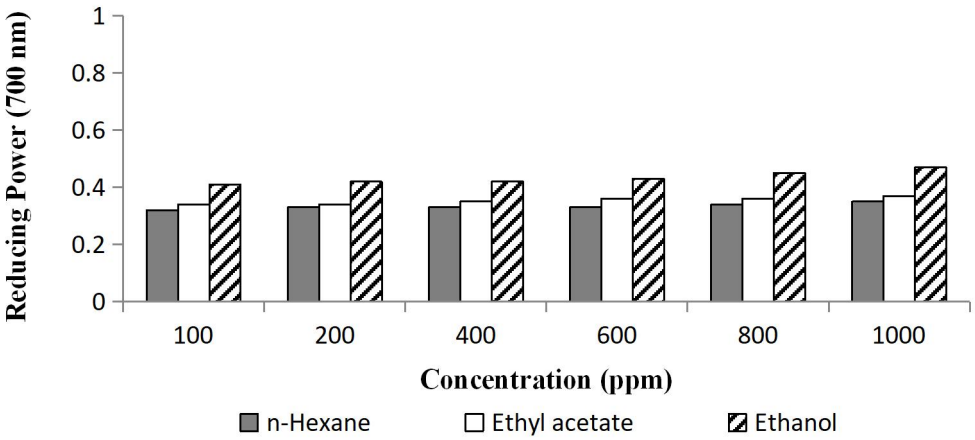

Figure 7. Antioxidant activity of the reduction method of water chesnut extract

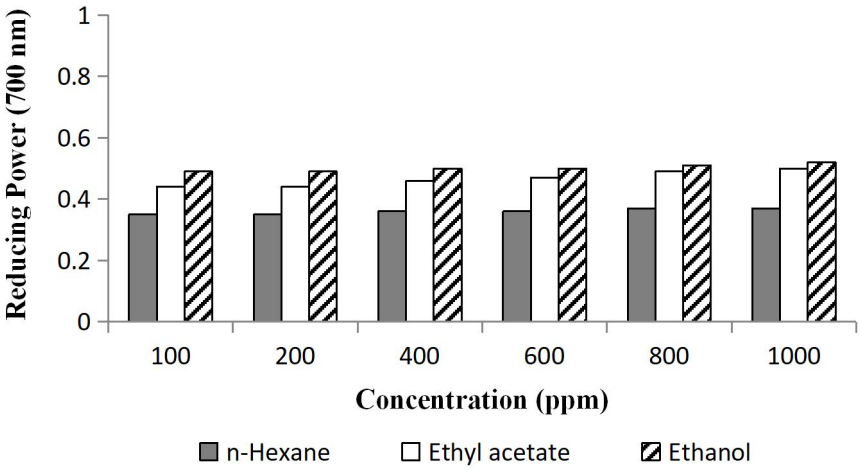

Figure 8. Antioxidant activity of the reduction method of giant molesta extract