

Free Radical Scavenging Activity of Ethanol Extract Fraction of Water Hyacinth Stem (*Eichhornia Crassipes* (Mart.) Solms) Using DPPH Method

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Abstract: **Background:** Water hyacinth has been shown to have free radical scavenging activity as an antioxidant. Several secondary metabolites contained in water hyacinth have antioxidant activity. **Objective:** This study aims to identify the antioxidant activity of ethanol extracts, fractions, and isolates of water hyacinth stems. **Materials and Methods:** Water hyacinth stem extraction was carried out by maceration using 96% ethanol solvent. The antioxidant activity test of the extract, fraction, and isolate was carried out using the DPPH (1,1-Diphenyl-2-picrylhydrazyl) method to determine the IC₅₀ value. Phytochemical screening to determine the compounds contained in the isolation. The active fraction was then isolated using FTIR and GC-MS to determine the compounds. **Results:** Phytochemical screening tests on isolate B8 were suspected to be positive for containing terpenoid and monoterpenoid compounds. The functional groups of the active ethanol extract isolates of water hyacinth stems (*Eichhornia hyacinth*) *crassipes* (Mart.) Solms consist of functional groups CH, CH aliphatic, CH₃, CO, C=C aromatic, carboxylic acid stretch, methylene CH bend, tert-butyl.

Keywords: Water hyacinth, antioxidant, 1,1-Diphenyl-2-picrylhydrazyl, ethanol extract

INTRODUCTION

The development of science in free radicals has recently brought developments in the health sector. Free radicals are chemical compounds with unpaired electrons. The presence of free radicals carries certain properties that are very reactive. Free radicals can accept electrons or donate electrons to other molecules and can act as oxidants or reductants (Jamshidi-Kia et al., 2020). Free radicals tend to cause damage in the body if a continuous chain reaction occurs (Radical et al., 2011). Therefore, molecules are needed that can counteract free radicals, one of which is antioxidants. Antioxidants are molecules that can interact and inhibit the initiation or spread of oxidation chain reactions produced by free radicals before important molecules are damaged. Antioxidants can inhibit the reactivity of free radicals through several mechanisms, one of which is hydrogen donation. Antioxidants are divided into natural and synthetic chemicals (Irivibulkovit et al., 2018). In general, antioxidants such as ascorbic acid (Vitamin C), α -tocopherol (Vitamin E), carotenoids and polyphenols, which can be found in fruits, vegetables, beverages, cereals and other commonly consumed food products, can support antioxidant defense (Lourenço et al., 2019).

Indonesian people believe that plants have properties in every part, such as roots, stems, and leaves, which can be used as medicine. Among the various plants that spread in nature, water hyacinth (*Eichhornia crassipes*) is one of the natural plant materials that can be used as a natural antioxidant. Previously, this plant was only used for feed formulation and phytoremediation of waters contaminated with metals (Surendraraj et al., 2013). However, this study was conducted using the DPPH method (1,1 diphenyl, - 2 picrylhydrazyl) because among these methods this method is the most widely used and the results of the analysis of antioxidant activity are high.

Meanwhile, the reason researchers took water hyacinth (*Eichhornia crassipes*) from the stem section is because there is still a lack of research conducted on this section. Based on the background above, the author was motivated to conduct research on the free radical scavenging activity of the ethanol extract fraction of water hyacinth stems (*Eichhornia crassipes*). *crassipes*) using the DPPH (1,1 Diphenyl-2 Picrylhydrazyl) method. This study is expected to provide information on the free radical scavenging activity of water hyacinth ethanol extract (*Eichhornia crassipes*). *crassipes*) and can be used as an additional guide for natural plant sources that have the potential as antioxidants.

METHODS

Extraction

The extraction method used was maceration with 96% pa ethanol solvent. The water hyacinth stem simplicial powder weighed as much as 1000 grams then put into a glass jar, and 96% pa ethanol solvent was added to the glass jar with a ratio of 1: 8 (w / v). The sample was soaked for 3 x 24 hours while stirring occasionally. Then the results of all filtrates were collected in 1 container to be evaporated using a rotary evaporator which aims to separate the 96% ethanol solvent from the dissolved compound so that a thick extract is obtained that is ready to be fractionated and its yield calculated.

Fractionation

Fractionation using the liquid-liquid partition method by inserting 1 gram of thick extract into a test tube, 10 test tubes were used, each tube was dissolved in 10 ml of n-hexane pa (non-polar) (Fauzziya et al., 2017) then centrifuged for 3 minutes at a speed of 3000 rpm. The insoluble part was replicated with n-hexane repeatedly until it was clear. The soluble n-hexane parts were combined and made into one as the n-hexane fraction, then left in a fume hood until it became a filtrate (Hesturini et al., 2022). The insoluble n-hexane part was then fractionated with 10 ml of ethyl acetate pa and the same procedure was carried out as in the fractionation with n-hexane. The soluble ethyl acetate portion is collected and concentrated as the ethyl acetate fraction, while the insoluble ethyl acetate portion is collected as the insoluble ethyl acetate fraction (residue). Then three fractions will be obtained, namely the n-hexane fraction, the ethyl acetate fraction and the insoluble ethyl acetate fraction which will then be tested for antioxidants and the yield calculated.

Thin Layer Chromatography and Vacuum Liquid Chromatography

Separation by TLC is used to determine the ratio and type of solvent in the separation process of n-hexane fractions to be used in liquid vacuum chromatography. The manufacture of KVC columns is carried out using the dry method to obtain maximum density. Liquid vacuum chromatography is carried out to obtain the best subfractions. The working principle of KVC is the partition and adsorption of compound components whose separation is assisted by the pressure of the vacuum device.

Table 1. TLC Analysis Eluent and Comparison

Fractions	Eluent	Eluent Comparison
1	N- Hexane	1
2	N- Hexane : Ethyl Acetate	8 : 2
3	N- Hexane : Ethyl Acetate	6 : 4
4	N- Hexane : Ethyl Acetate	4 : 6
5	N- Hexane : Ethyl Acetate	2 : 8
6	Ethyl Assets	1
7	Ethyl Acetate : Methanol	8 : 2
8	Ethyl Acetate : Methanol	6 : 4
9	Ethyl Acetate : Methanol	4 : 6
10	Ethyl Acetate : Methanol	2 : 8
11	Methanol : Chloroform	1

The elution results were observed under UV light 254 and 366 nm. Subfractions that have the same separation pattern (TLC profile) are grouped together. The results of the subfractions that have been grouped will be subjected to TLC again to continue to preparative TLC. (Rofifah Marlis et al., 2022). Preparative TLC aims to obtain the most active isolate from the subfraction. The mobile phase used is the best eluent and the stationary phase used is a special preparative silica gel plate G60 F254. Furthermore, the separation of the most active subfractions is carried out using preparative TLC. The resulting chromatogram is detected with visible light, UV254, UV366 and anisaldehyde spray reagent, then marked. The marked spots are scraped and collected, then dissolved with chloroform: methanol solution (1: 1), filtered, and dried (Br & Susanto, 2019). The separation results from TLC can be analyzed by calculating R_f (retardation factor).

Antioxidant Activity of Water Hyacinth

Antioxidant activity testing in this study used the DPPH (1,1 diphenyl-2-picrylhydrazyl) method. This test was conducted to compare the antioxidant activity of the extract, n-hexane fraction, ethyl acetate fraction and water-insoluble fraction based on the IC₅₀ value and determine the percentage inhibition value. Weighed as much as 1 mg of DPPH then dissolved with methanol and put into a 10 mL measuring flask to the limit mark then shaken until

homogeneous. DPPH solution was taken as much as 100 μ L then put into a test tube, added as much as 100 μ L of methanol, then measured the absorbance at a wavelength of 515-520 nm, then plotted the maximum absorbance. Each sample was taken as much as 100 μ L then put into a microplate. Then 100 μ L of DPPH solution was added to each microplate, then left for 30 minutes. After that, measure the absorbance of each sample using spectrophotometry.

FTIR

The isolated compound was weighed as much as 1 mg, then 100 mg KBr was added as a background for solid sample analysis, homogenized using a mortar and then pressed into pellet form. After that, it was analyzed using FTIR at a wavelength of 400-4000 cm^{-1} . (Indah et al., 2018). The FTIR method is used to identify and characterize functional groups in the isolated results.

GC and MS

The instrumental analysis approach using Gas Chromatography-Mass Spectrometry (GC-MS) characterization aims to identify compounds. The main points in this identification method are based on two orthogonal identifiers: fragmentation patterns in MS and retention indices.

RESULT AND DISCUSSION

Extraction

The filtrate obtained from the maceration process was then evaporated using a rotary evaporator to obtain a thick extract of water hyacinth stems. The thick extract of water hyacinth stems obtained was dark green in color as much as 27.60 grams with a yield of 2.76%. One of the parameters of extract quality is the yield of the resulting extract. Yield is a comparison between the extract obtained and the initial simplicia. The yield uses a percentage unit (%), the higher the yield value produced indicates the greater the value of the extract produced. The yield of an extract can be influenced by several factors, one of which is the extraction method used (Wijaya et al., 2018). The results of the thick extract obtained are stored in a container covered with aluminum foil and stored in a refrigerator to avoid fungal contamination that can damage the water hyacinth stem extract sample.

Fractionation

Fractionation was carried out using the liquid-liquid partition method using n-hexane and ethyl acetate solvents. The use of n-hexane solvents aims to extract nonpolar compounds in the solvent. Ethyl acetate solvents are used to extract semi-polar compounds (Sugiarti et al., 2020). The results of the fractionation process obtained 3 types of fractions, namely the n-hexane fraction (FNH), the ethyl acetate fraction (FEA), and the insoluble fraction (FTL). The weight of the fraction and the yield obtained from each fraction can be seen in the table.

Table 2. Fractionation Yield

Heavy extract (Gram)	Fractions	Weight Fraction (Gram)	Produce (%)
25	n- hexane fraction	5.5483	22.20%
	Faction ethyl acetate	1.8278	7.31%
	Faction not late	8.3688	33.47%

Thin Layer Chromatography and Vacuum Liquid Chromatography

The separation of n-hexane fractions was carried out using the vacuum liquid chromatography (VLC) method. Optimization of the eluent to be used as the VLC process was carried out by looking at the good compound separation pattern using TLC. Eluent optimization used eluents with a combination of comparisons listed in table 3.1. Fraction separation aims to separate compounds with different levels of polarity into simpler ones.

The separation of n-hexane fractions using the KCV method used a sample of 4 grams so that the silica gel used for the manufacture was adjusted until the n-hexane fraction was mixed homogeneously. The separation of n-hexane fractions using the KCV method produced 30 subfractions, which were then analyzed using TLC to combine several similar and similar subfractions by looking at the pattern or profile of the compound, spot color, and spot height. TLC was carried out using a mobile phase eluent of n-hexane: ethyl acetate (9: 1) which can be seen in appendix 6.3. The results of weighing the subfractions and the yield obtained after combining 9 subfractions, namely subfractions A to I, can be seen in the table.

Table 3. Subfraction Yield

Subfraction	No Cup	Initial weight	Final weight	Results (%)
Subfractions 1-3	A	52.56	52.75	4.89%
Subfraction 4-5	B	56.88	57.54	16.60%
Subfractions 6-8	C	52.36	52.74	9.56%
Subfraction 9	D	55.41	55.51	2.56%
Subfraction 10-11	English	56.44	56.68	5.83%
Subfraction 12-14	F	48.20	48.39	4.53%
Subfractions 15-17	G	47.73	48.18	11.13%
Subfraction 18-20	H	94.68	95.26	14.46%
Subfraction 21-30	I	94.43	96.49	51.50%

Purification of subfraction B to obtain isolates using preparative thin layer chromatography (P-TLC) method according to the results obtained after KCV with solvent polarity that can attract more compounds. This is because the composition of the mobile phase used is n-hexane: ethyl acetate (8: 2) which can be fractionated in solvents. The combination of 9 subfractions can be seen in table 4.3. Subfraction B has good separation results compared to other subfractions, seen in visible light, UV 254 nm appears active in brownish green, UV 366 nm appears active in red, blue, and green, and spraying using anisaldehyde spotting after heating is dark and red.

Antioxidant Activity of Water Hyacinth

This test aims to compare the antioxidant power between extracts and fractions based on the IC₅₀ value. Based on the table above, it shows that the ethanol extract of water hyacinth stems has an IC₅₀ value of $4,712 \pm 91.51 \mu\text{g} / \text{ml}$, which means that the antioxidant activity in it is weak because the IC₅₀ value is $> 200 \text{ ppm}$. The results of the n-hexane fraction and ethyl acetate fraction each have IC₅₀ values of $1,145 \pm 122.20 \mu\text{g} / \text{ml}$ and $2,415 \pm 72.68 \mu\text{g} / \text{ml}$, which means that both fractions have weak antioxidant activity with IC₅₀ values $> 200 \text{ ppm}$. However, the antioxidant activity of the extract when compared with the results of the n-hexane fraction showed a decrease in the IC₅₀ value, indicating that the n-hexane fraction has better antioxidant activity than the extract and ethyl acetate fraction. This is because after the fractionation process, the secondary metabolite compounds contained therein undergo reduction and separation according to the solubility level of each compound.

Antioxidant activity testing using the DPPH method on the combined KVC subfractions aims to determine the comparison of antioxidant activity in each subfraction A, B, C, D, E, F, G, H and I. The concentration used was 1000 ppm, where this concentration can show the percentage of inhibition with the ability of antioxidant activity against DPPH free radicals. The concentration used was 1000 ppm, where this concentration can show the percentage of inhibition with the ability of activity as an antioxidant against DPPH free radicals. Based on subfractions A and B have good antioxidant activity against DPPH radicals based on high inhibition percentage values. Subfraction A has the highest activity of $73.769 \pm 4.755\%$, followed by subfraction B with an inhibition percentage value of $37.794 \pm 1.073\%$. In both subfractions, the yield produced by subfraction A is smaller than that of subfraction B, so the compounds will be more difficult to separate. The results of the inhibition percentage and SD values in the separated subfraction B approached purity. Subfraction B is a combination of fractions number 4 and number 5 from the results of the KVC process using a solvent ratio of n-hexane: ethyl acetate (6: 4). From these results, separation was carried out on subfraction B to obtain pure compounds using the TLC-Preparative method.

FTIR

Identification of isolate B8 from subfraction B was analyzed using FTIR to determine the functional group of a compound contained in the sample. The absorption produced by the FTIR spectrum was used to strengthen the suspicion of the compound contained in the active isolate of water hyacinth stem extract. Isolate B8 was measured at a wavelength range of 400 cm^{-1} to 4000 cm^{-1} . The results of the identification of isolate B8 using FTIR can be seen in the table.

Table 4. FTIR Elucidation

Functional Group	Wave Number		Reference
	Target Compound	Literature	
CH (stretch)	2952 2922	3000 – 2850	(Pavia et al., 2014)
methyl O-CH ₃ ether (stretch)	2851	2850 - 2815	(Nandiyanto and others , 2019)
CH aliphatic			(Colorful and others , 2024)
C=O	1733	1750 – 1730	(Pavia et al., 2014)
Carboxylic acid (stretch)	1716 1700	1725 – 1700	(Nandiyanto and others , 2019)
(-C=N-) no There is	1685 1670	1690 – 1590	(Nandiyanto and others , 2019) (Pavia et al., 2014)
Secondary amines, NH bend	1636	1650 - 1550	(Nandiyanto and others , 2019)
CH methylene bend	1457	1490 - 1410	(Nandiyanto and others , 2019)
tert - butyl (multiplet)	1375	1395 - 1385	(Nandiyanto and others , 2019)
TOGETHER	1243 1221 1198 1165 1116 1072 1057 1019	1300 - 1000	(Nandiyanto and others , 2019) (Pavia et al., 2014) (Nurfirzatulloh and others , 2023)
Alkene -C ₆ H ₆ (oops) bend)	971 777 766 719	1000 – 650	(Nandiyanto and others , 2019) (Pavia et al., 2014)

GC-MS

Analysis was carried out on the fragmentation of the mass peaks obtained, the results of the analysis obtained compound 1-tetradecanol with a mass peak similarity level of 72.7%, namely at peaks 41, 55, 69, 83, 97, 111, 125, and 126. The compound 1-tetradecanol has a molecular weight of 214.39 m / z with the molecular formula C₁₄H₃₀O which is an oxygenated monoterpene compound. 1-tetradecanol is a straight chain saturated fatty alcohol. According to research by Brat'ka et al., (2022) 1-tetradecanol was reported to increase melatonin penetration through the skin. The highest penetration of melatonin through mouse skin was observed in decanol, and with increasing carbon chain length it decreased slightly. 1-tetradecanol is the highest among other alcohols after 48 hours and has been shown to be effective against bacteria that cause periodontitis in rabbits. The compound 1-tetradecanol shows anti-inflammatory effects (Omotoso et al., 2024). And according to research by Ajjiah et al., (2023) compound 1-tetradecanol also shows antifungal effects. Analysis of target compounds from screening results on the MassBank database page: (<https://massbank.eu/MassBank/Search>).

The results of the analysis of the chromatogram data of the second compound in isolate B8 showed a peak area of 19.92% with a retention time of 6.59 minutes. From these data, an analysis of the mass peak fragmentation obtained was carried out, the results of the analysis obtained a palmitic acid compound with a mass peak similarity level of 92.30%, namely at peaks 39, 41, 43, 69, 73, 99, 115, 129, 143, 171, 213, and 256. The palmitic acid compound has a molecular weight of 256 m / z with the molecular formula C₁₆H₃₂O₂. The results of the analysis of the compound are strengthened by the presence of functional groups in the FTIR analysis results. The results of the FTIR analysis show the presence of a carbonyl functional group C = O, carboxylic acid stretching, methylene functional group. The palmitic acid compound is the main saturated fatty acid (Ezealisiji, 2023). According to research conducted by Tyagi & Agarwal, (2017). Palmitic acid compounds have antioxidant, hypocholesterolemic, nematocide, pesticide, lubricant and

5-alpha hemolytic activities are reductase inhibitors. This is proven in research.

The third compound in isolate B8 showed a peak area of 7.81% with a retention time of 19.14 minutes. From these data, an analysis of the mass peak fragmentation obtained was carried out, the results of the analysis obtained compound 2,6-Di-Tert-Butyl-4-Methylphenol with a mass peak similarity level of 42.85%, namely at peaks 55, 57, 147, 161, 177, and 205. The compound has a molecular weight of 220 m/z with a molecular formula of $C_{15}H_{24}O$. The results of the analysis of the compound are strengthened by the presence of functional groups in the FTIR analysis results. The results of the FTIR analysis showed the presence of carbonyl functional groups $C=O$, carboxylic acid stretching, aliphatic CH, methylene bending CH, tert-butyl.

CONCLUSION

The n-hexane fraction of ethanol extract of water hyacinth stem (*Eichhornia crassipes* (Mart.) Solms) has the highest antioxidant activity against DPPH based on the IC₅₀ value. The activity of isolate B8 from the n-hexane fraction of ethanol extract of water hyacinth (*Eichhornia gondok* crassipes (Mart.) Solms) was $648.87 \pm 33.06 \mu\text{g} / \text{ml}$. This result is still quite good when compared to the IC₅₀ value of the extract and n-hexane fraction $> 200 \mu\text{g} / \text{ml}$. The functional group of the active ethanol extract of isolate B8 BEG (*Eichhornia crassipes* (Mart.) Solms) was $648.87 \pm 33.06 \mu\text{g} / \text{ml}$. crassipes (Mart.) Solms) consisting of functional groups CH, CH aliphatic, CH₃, CO, C=C aromatic, carboxylic acid strain, methylene CH bend, tert-butyl. Cucumber skin extract (*Cucumis sativus* L.) contains secondary metabolites in the form of steroids, alkaloids, phenols, flavonoids, and saponins. Cucumber skin extract (*Cucumis sativus* L.) contains secondary metabolite compounds in the form of steroids, alkaloids, phenols, flavonoids, and saponins. sativus L.) has antibacterial activity against *Staphylococcus epidermidis* with an MIC value of 2.5% and an MBC value of 5%. The most optimal formula is F₃ with a combination of 10% stearic acid, 3% TEA, and 4.5% extract and meets all the requirements of the cream evaluation test. All three formulations have very strong antibacterial activity against *Staphylococcus epidermidis*.

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CONFLICT OF INTEREST

We declare that we don't have any conflict of interest.

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