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Free Radical Scavenging Activity of Ethanol Extract and 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 (Eichornia 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 (Eichornia 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 (Eichornia 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 (Eichornia 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 all, 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 Rf (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 µL then put into a test tube, added as much as 100 µL 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 µL then put into a microplate. Then 100 µL 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 ± 91.51 µg / ml, which means that the antioxidant activity in it is weak because the IC50 value is > 200 ppm. The results of the n-hexane fraction and ethyl acetate fraction each have IC50 values of 1,145 ± 122.20 µg / ml and 2,415 ± 72.68 µg / ml, which means that both fractions have weak antioxidant activity with IC50 values > 200 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 ± 4.755%, followed by subfraction B with an inhibition percentage value of 37.794 ± 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-CH3 ether (stretch) **CH aliphatic**	2851	2850 - 2815	(Nandiyanto *and others* , 2019) (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 -C6H6 (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 Brať 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 Ajijah 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, nematicide, 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 ± 33.06 µg / ml. This result is still quite good when compared to the IC50 value of the extract and n-hexane fraction > 200 µg / ml. The functional group of the active ethanol extract of isolate B8 BEG (Eichhornia crassipes (Mart.) Solms) was 648.87 ± 33.06 µg / ml. crassipes (Mart.) Solms) consisting of functional groups CH, CH aliphatic, CH3, CO, C=C aromatic, carboxylic acid strain, methylene CH bend, tert-butyl.

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CONFLICT OF INTEREST

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

REFERENCES

A. De Caro, C. (2015). UV/VIS Spectrophotometry. *Mettler -Toledo International* , *September 2015* , 4–14. http://lcwu.edu.pk/ocd/cfiles/Chemistry/MSc/Chem-C-410/Fundamentals_UV_VIS.pdf.

Abubakar , A.R., & Haque , M. (2020). Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes. *Journal of Pharmaceutical & Biotechnological Sciences* , *12* (1), 1–10. https://doi.org/10.4103/jpbs.JPBS_175_19

Agustina , E., Andiarna , F., Lusiana , N., Purnamasari , R., & Hadi , MI (2018). Identity of Active Compounds from Water Apple Leaf Extract (Syzygium aquatica) by Comparison of Several Soluble Plates in the Maceration Method. *Biotropic: Journal of Tropical Biology* , *2* (2), 108–118. https://doi.org/10.29080/biotropic.2018.2.2.108-118

Ajijah , N., Fiodor , A., Dziurzynski , M., Stasiuk , R., Pawlowska , J., Dziewit , L., & Pranaw , K. (2023). Biological control potential of Pseudomonas protegens ML15 against Botrytis cinerea, the cause of gray leaf rot disease, a fungus on post-harvest tomato (Solanum lycopersicum var. cerasiforme). *Frontiers in Plant Science* , *14* (December), 1–18. https://doi.org/10.3389/fpls.2023.1288408

Alara , OR, Abdurahman , NH, & Ukaegbu , CI (2021). Extraction of phenolic compounds: A review. *Current Research in Food Science* , *4* (December 2020), 200–214. https://doi.org/10.1016/j.crfs.2021.03.011

Andriani, D., & Murtisiwi, L. (2020). Antioxidant Activity Test of 70% Ethanol Extract of Butterfly Pea Flower (Clitoris ternatea L) from Sleman Area using DPPH Method. *Pharmacon: Indonesian Journal of Pharmacy* , *17* (1), 70–76. https://doi.org/10.23917/pharmacon.v17i1.9321

Ben Bakrim, W., Ezzariai, A., Karouach, F., Sobeh, M., Kibret, M., Hafidi, M., Kouisni, L., & Yasri, A. (2022). Eichhornia crassipes (Mart.) Solms: A Comprehensive Review of Chemical Composition, Traditional Uses, and Value-Added Products. *Frontiers in Pharmacology* , *13* (March), 1–21. https://doi.org/10.3389/fphar.2022.842511

Br, R., & Susanto , FXH (2019). *POTENTIAL OF ANTIBACTERIAL AND ANTIRADICAL ACTIVITY FRACTIONS FROM FALOAK STEM BARK (Sterculia* . *23* (1), 25–28.

Braťka , P., Fenclová , T., Hlinková , J., Uherková , L., Šebová , E., Hefka Blahnová , V., Hedvičáková , V., Žižková , R.,

Litvinec , A., Trč , T., Rosina, J., & Filová , E. (2022). Preparation and Biological Testing of New Wound Dressings with Encapsulated Antibacterial and Antioxidant Substances. *Nanomaterials* , *12* (21). https://doi.org/10.3390/nano12213824

Coêlho , ML, Islam, MT, Laylson Da Silva Oliveira, G., Oliveira Barros De Alencar , MV, Victor De Oliveira Santos, J., Campinho Dos Reis, A., Oliveira Ferreira Da Mata, AM, Correia Jardim Paz, MF, Docea , AO, Calina , D., Sharifi - Rad, J., & Amélia De Carvalho Melo-Cavalcante, A. (2022). Cytotoxic and Antioxidant Properties of the Natural Bioactive Monoterpenes Nerol, Estragole, and 3,7-Dimethyl-1-Octanol. *Advances in Pharmacology and Pharmaceutical Sciences* , *2022*. https://doi.org/10.1155/2022/8002766

Coniwanti, P., Novalina, S., & Putri, IK (2009). Production of Water Hyacinth Pulp Through Organosolv Process. *Journal of Chemical Engineering* , *16* (4), 34–41.

DE OLIVEIRA, THB, DE GUSMÃO, NB, DA SILVA, LAO, & Coelho, LCBB (2021). Free radicals and actinobacteria as a gold mine of antioxidant compounds that are underexplored. *Anais Da Academia Brasileira de Ciencias* , *93* , 1–23. https://doi.org/10.1590/0001-3765202120201925

Dersseh , M.G., Melesse , A.M., Tilahun , S.A., Abate , M., & Dagnew , D.C. (2019). Water hyacinth: A review of its impacts on hydrology and ecosystem services-Lessons for Lake Tana management. *Hydrological Extremes and Climate Variability: Monitoring, Modeling, Adaptation, and Mitigation* , *August 2020* , 237–251. https://doi.org/10.1016/B978-0-12-815998-9.00019-1

Dewi, IS, Saptawati, T., & Rachma, FA (2021). Phytochemical Screening of Ethanol Extract of Peel and Seed of Dutch Eggplant (Solanum betaceum Cav.). Phytochemical Screening of Ethanol Extract of Peel and Seed of Dutch Eggplant (Solanum Betaceum Cav.). *Proceedings of the National Seminar of UNIMUS* , 1210–1218.

Eden, W.T., Wahyuono, S., Cahyono, E., & Astuti, P. (2023). Antioxidant activity of Eichhornia crassipes (mart) solms as sunscreen. *AIP Conference Proceedings* , *2614* (1), 30019. https://doi.org/10.1063/5.0128018

Elvira, K., Fachriyah, E., & Kusrini, D. (2018). Isolation of Flavonoid Compounds from Water Hyacinth (Eichhornia spp.) crassipes) and Antioxidant Test with DPPH (1,1-Diphenyl-2-Picrylhydrazyl) Method. *Journal of Chemical Science and Applications* , *21* (4), 187–192. https://doi.org/10.14710/jksa.21.4.187-192

Ezealisiji Kenneth Maduabuchi , & Okoh Precious Tobechukwu . (2023). Advanced phytochemical and chemometric profiling of bioactive medicinal components of n-hexane Xylopia aethiopica seed extract using FTIR and GC-MS techniques. *GSC Biological and Pharmaceutical Sciences* , *22* (1), 247–256. https://doi.org/10.30574/gscbps.2023.22.1.0031

Fadhli, H., Ikhtiarudin, I., & Lestari, P. (2020). Isolation and Test of Antioxidant Activity of Methanol Extract from Senduduk Bulu Fruit (Klidemia hirta (L.) D.Don). *Pharmacy: Indonesian Journal of Pharmacy* , *17* (2), 92–100. https://doi.org/10.23917/pharmacon.v17i2.9846

Fauzziya, R., Nurani, LH, & Sulistyani, N. (2017). Search for active antibacterial compounds of cayenne pepper leaf extract (Capsicum frutescens L.) against Klebsiella pneumoniae and the mechanism of cell leakage. *Trad Med J* , *22* (3), 2017. https://pdfs.semanticscholar.org/8004/81b46fa3163129387efbe3e945181f419921.pdf

Flieger , J., & Flieger , M. (2020). and Goutweed (Aegopodium podagraria L.). *Molecules* , *25* , 2–17.

Ganorkar, P. V, Jadeja, G. C., & Desai, M. A. (2023). Microwave-Assisted Extraction of Shikimic Acid from Different Morphological Parts of Water Hyacinth (Eichhornia crassipes). *Chemical Engineering and Processing - Process Intensification* , 109455. https://doi.org/10.1016/j.cep.2023.109455

Gulcin , İ. (2020). Antioxidants and antioxidant methods: an up-to-date review. In *Archives of Toxicology* (Vol. 94, Issue 3). https://doi.org/10.1007/s00204-020-02689-3

Handayani, Y., Susiloningrum, D., & Ismah, K. (2024). *DETERMINATION OF TOTAL PHENOL CONTENT OF TEMU KUNCI LEAVES (Boesenbergia rotunda (L.) Mansf.) USING ULTRASOUND ASSISTED EXTRACTION METHOD USING* . *8* (2), 182–189.

Hasanah, M. (Mauizatul), P, MR (Muhamad), & Amelia, K. (Kiki). (2016). Antioxidant Potential of Extract and Fraction of Water Hyacinth Leaves (Eichhornia spp.) Crassipes (Mart.) Solms) Originating from One of the Swamps in Palembang, Indonesia. *Journal of Science Research* , *18* (3), 168247. https://www.neliti.com/publications/168247/

Hesturini, RJ, Pertiwi, KK, Astari, MN, & Febriana, AA (2022). Journal of Pharmaceutical Science and Practice ANALGESIC AND TOXICITY TEST OF n-HEXANE FRACTION OF TREMBESI LEAVES (Samanea saman (Jacq.) Merr.) IN MICE (Mus musculus L.) ANALGESIC AND TOXICITY TEST OF n-HEXANE FRACTION OF TREMBESI LEAVES (Samanea saman (Jacq.) Merr.) IN MICE. *Jfsp* , *8* (1), 2579–4558. http://journal.ummgl.ac.id/index.php/farmasi

Hidayat, R., & Patricia Wulandari. (2021). Extraction Methods: Maceration, Percolation and Decoction. *Eureka Herba Indonesia* , *2* (1), 73–79. https://doi.org/10.37275/ehi.v2i1.15

Honour, JW (2006). *Gas Chromatography-Mass Spectrometry BT - Hormone Assay in Biological Fluids* . 53–74. https://doi.org/10.1385/1-59259-986-9:53

Indah, F., Sari, P., & Asriza, O. (2018). *Jengkol Peel Biosorbent (Pithecellobium) jiringa) as Pb Metal Absorbent in Post-*

Tin Mining Dam Water. Kacang Ngapi (Pithecellobium) jiringa) Peel Biosorbent as Pb Metal Absorbent in Post-Tin Mining Dam Water . *4* (2), 83–89.

Irawan, A. (2019). Spectrophotometer Calibration as a Quality Assurance of Measurement Results in Research and Testing Activities. *Indonesian Laboratory Journal* , *1* (2), 1. https://doi.org/10.22146/ijl.v1i2.44750

Irivibulkovit , K.S., Ouanthavong , S.N., & Ameenoi , Y.S. (2018). Sirivibulkovit K et al 2018. *Analytical Sciences* , *34* (July), 795–800.

Jamshidi - Kia, F., Wibowo, J.P., Elachouri, M., Masumi, R., Salehifard-Jouneghani, A., Abolhassanzadeh, Z., & Lorigooini, Z. (2020). The fight between plants as antioxidants and free radicals in the human body. *HerbMed Journal of Pharmacology* , *9* (3), 191–199. https://doi.org/10.34172/jhp.2020.25

Jangnga, ID, Kambaya, PP, & Kosala, K. (2018). Antibacterial Activity Test and Thin Layer Chromatography Bioautography Analysis of Ethanol Extract of Srikaya Leaves (Annona Squamosa L) Against Enterococcus Faecalis In Vitro. *ODONTO: Dental Journal* , *5* (2), 102. https://doi.org/10.30659/odj.5.2.102-109

Jayanthi , P., & Lalitha , P. (2011). *Determination of in vitro reducing power of aqueous solutions* . *4* (11), 4003–4005.

Karno, Koesmantoro, H., Sunaryo, & Prasetyo, A. (2020). *Water Hyacinth Biogas Monograph with Polyethilane Digester* (November Edition). https://www.researchgate.net/publication/345545836

Mrs. Yunita Handoyo, D., & Pranoto, ME (2020). The Effect of Variation in Drying Temperature on the Production of Neem Leaf Simplicia (Azadirachta) Indica). *Tincture Pharmacy Journal* , *1* (2), 45–54. https://doi.org/10.35316/tinctura.v1i2.988

Leksono, WB, Pramesti, R., Santosa, GW, & Setyati, WA (2018). Types of Methanol and N-Hexane Soluble Plates on Antioxidant Activity of Gelidium sp. Seaweed Extract from Drini Beach, Gunungkidul - Yogyakarta. *Journal of Tropical Oceanology* , *21* (1), 9. https://doi.org/10.14710/jkt.v21i1.2236

Lourenço , S.C., Moldão -Martins, M., & Alves, V.D. (2019). Natural plant-derived antioxidants: From sources to food industry applications. *Molecules* , *24* (22), 14–16. https://doi.org/10.3390/molecules24224132

Paru, JKS, & Destiani, DP (2018). Antioxidant Activity Test of Vitamins A, C, E using the DPPH Method. *Farmaka* , *15* (1), 53–62.

Marteau, C., Guitard, R., Penverne, C., Favier, D., Nardello-Rataj, V., & Aubry, J. M. (2016). Effect of increasing ortho-propenyl substituents on the antioxidant activity of natural phenols. *Food Chemistry* , *196* , 418–427. https://doi.org/10.1016/j.foodchem.2015.09.007

Munteanu , IG, & Apetrei , C. (2021). Analytical methods used in determining antioxidant activity: A review. *International Journal of Molecular Sciences* , *22* (7). https://doi.org/10.3390/ijms22073380

Musfiroh , I., Nur Hasanah , A., Faradiba , GA, Ayumiati , I., Mutakin , M., & Muchtaridi , M. (2019). Modification of Extraction Method in Determination of Simethicone Suspension Using FTIR Method. *Indonesian Journal of Pharmaceutical Science and Technology* , *6* (3), 125. https://doi.org/10.24198/ijpst.v6i3.22355

Mutmainnah, PA, Hakim, A., & Savalas, LRT (2017). Identity of Derivative Compounds from Fractionation of Artocarpus Odoratissimus Root Wood. *Journal of Science Education Research* , *3* (2). https://doi.org/10.29303/jppipa.v3i2.89

Muzdalifa, D., & Jamal, S. (2019). Antioxidant Activity Test of Robusta Coffee Bean Skin Fraction Extract (Coffea) canephora Pierre ex A.Froehner) Effect of DPPH Reagent (1,1- Diphenyl-2- Picrylhydrazyl). *Indonesia Natural Pharmaceutical Research Journal* , *4* (2), 41–50.

Nahor, EM, Rumagit, BI, & Tou, HY (2020). Comparison of Ethanol Extract Yield of Andong Leaves (Cordyline) futicosa L.) Using Maceration and Soxhlet Extraction Methods. *2020 National Seminar* , 40–44.

Nandiyanto, ABD, Oktiani, R., & Ragadhita, R. (2019). How to read and interpret organic material spectroscopes. *Indonesian Journal of Science and Technology* , *4* (1), 97–118. https://doi.org/10.17509/ijost.v4i1.15806

Nurfirzatulloh, I., Suherti, I., Insani, M., Shafira, RA, Abriyani, E., Buana University, M., Karawang, P., University, D., Perjuangan, B., & Abstrak, K. (2023). Identity of Tannin Functional Groups in Several Plants Using the Scientific *Journal Instrument* Ftir *Wahana Pendidikan, February* , *9* (4), 201–209. https://doi.org/10.5281/zenodo.7678425

Omotoso , DR, Olubowale , VO, Aina , FM, & Daramola , OOO (2024). Phytochemical Profile of Basella alba Using Gas Chromatography-Mass Spectrometry. *Journal of Tropical Natural Products Research* , *8* (6), 7561–7565. https://doi.org/10.26538/tjnpr/v8i6.36

Pavia, D.L., Lampman, G.M., & Kriz, G.S. (2014). Ultraviolet Spectroscopy. In *Paint Testing Manual* . https://doi.org/10.1520/stp37187s

Pietta , PG (2000). Flavonoids as antioxidants. *Journal of Natural Products* , *63* (7), 1035–1042. https://doi.org/10.1021/np9904509

Putri, SS, Suryati, C., & Nandini, N. (2020). Journal of Science and Health. *Journal of Science and Health* , *3* (1), 242–247.

Radical, F., Activity, S., Alsophila, OF, & Sm, J. (2011). FREE RADICAL SCAVENGER ACTIVITY OF FERN STEM (Alsophila glauca J. Sm). *Traditional Medicine Journal, 16(3)* , *16* (3), 156 – 160.

Ranti , Y.paula . (2021). Tropical biopharmaceuticals Tropical Biopharmaceuticals . *Journal of Tropical*

Biopharmaceuticals , *2* (2), 158–169.

Retno Sari. (2023). Evaluation of Oral Vitamin E Preparations as Antioxidants Using the DPPH (Diphenyl picrylhydrazyl) Method. *Scientific Periodical of Pharmaceutical Chemistry* , *10* (1), 13–17. https://doi.org/10.20473/bikfar.v10i1.47115

Rofifah Marlis et al., H. (2022). IDENTIFICATION AND TESTING OF ANTIOXIDANT ACTIVITY OF METHANOL EXTRACT OF AGARWOOD LEAVES (Gyrinops versteegii (Gilg.) Domke) COASTAL VARIETY USING DPPH METHOD. *Department of Chemistry* , *10* (1), 1–52. https://doi.org/10.21608/pshj.2022.250026

Rufchaei , R., Abbas- Mohammadi , M., Mirzajani , A., & Nedaei , S. (2022). Evaluation of Chemical Compounds and Antioxidant and Antimicrobial Activities of Eichhornia Crassipes (Water Hyacinth) Leaves. *Jundishapur Journal of Natural Pharmaceutical Products* , *17* (1), 1–11. https://doi.org/10.5812/jjnpp.101436

Sami, F., Soekamto, N., Firdaus, & Latip, J. (2019). *Abstract This study aims to investigate the antioxidant activity of methanol, ethyl acetate, and n-hexane extracts of brown algae species* . *4* (1), 1–6.

Santiago, M., & Strobel, S. (2013). Thin layer chromatography. In *Methods in Enzymology* (1st ed., Vol. 533). Elsevier Inc. https://doi.org/10.1016/B978-0-12-420067-8.00024-6

Silvia, S.D., Nadia Rahma, E., Celloce Njurumana, V., & Yanuarti, R. (2022). Total Flavonoid Testing and Antioxidant Activity Test of Active Fraction of Kaik-Kaik Root Leaves (Uncaria cordata (Lour.) Merr) Which Have Potential as Diarrhea Medicine. *Journal of Biology and Learning (JB&P)* , *9* (2), 105–112. https://doi.org/10.29407/jbp.v9i2.18618

Sugiarti, L., Andriyani, DM, Pratitis, MP, & Setyani, R. (2020). Antibacterial Activity of N-Hexane Fraction, Ethyl Acetate and Ethanol Water Extract of Parijoto Leaves (Medinilla) Speciosa Blume) Against Propionibacterium Acnes and Staphylococcus Epidermidis. *Journal of Pharmacy Scholar* , *4* (2), 120–130. https://doi.org/10.31596/cjp.v4i2.105

Surendraraj , A., Farvin , K. H. S., & Anandan , R. (2013). Antioxidant potential of water hyacinth (Eichornia crassipes): In vitro antioxidant activity and phenolic composition. *Journal of Aquatic Food Products Technology* , *22* (1), 11–26. https://doi.org/10.1080/10498850.2011.621582

Susanto, SW, & Ranggaini, MD (2022). Antioxidant activity of ethanol extract of temulawak rhizome Roxb. and ascorbic acid (with DPPH, FRAP, and H2O2 methods). *Integrated Dentistry Journal* , *4* (1), 81–88. https://e-journal.trisakti.ac.id/index.php/jkgt/article/view/14276

Tyagi, T., & Agarwal, M. (2017). Phytochemical screening and GC-MS analysis of ethanol extract of ACN. *Journal of Pharmacognosy and Phytochemistry* , *6* (1), 195–206.

Udayani, NNW, Wiguna, PDS, Cahyaningsih, E., & Wardani, IGAAK (2023). Phytochemical Screening and Antioxidant Activity of Citrus Mistletoe Leaf Extract (Dendrophthoe) glabrescens (Blakely) Barlow) with n-Hexane and Ethanol Solvents *Scientific Journal of Medicines* , *9* (2), 150–157. https://doi.org/10.36733/medicamento.v9i2.7136

Uludag , N., & Serdaroğlu , G. (2022). Efficient study of C-2 cyanomethylation in indole synthesis: Electronic and spectroscopic (FT-IR, NMR, UV-Vis) characterization, antioxidant activity, and theoretical calculations. *Journal of Molecular Structure* , *1247*. https://doi.org/10.1016/j.molstruc.2021.131416

Warnis, M., & Artika, L. (2021). Comparison of Yield and Chemical Content of Cashew Leaf Extract (Anacardium) Occidentale L.) With Several Types of Solvents. *JKPharm Journal of Pharmaceutical Health* , *3* (1), 63–69. https://doi.org/10.36086/jkpharm.v3i1.907

Warsi, & Sholichah , AR (2017). Phytochemical screening and antioxidant activity of ethanol extract and ethyl acetate fraction of basil leaves (Ocimum basilicum L.) by DPPH radical scavenging method. *IOP Conference Series: Materials Science and Engineering* , *259* (1). https://doi.org/10.1088/1757-899X/259/1/012008

Weng , XC, & Huang, Y. (2014). Structure-antioxidant activity relationships of inhibited phenolic compounds. *Grasas y Aceites* , *65* (4). https://doi.org/10.3989/gya.0225141

Wijaya, H., Novitasari, & Jubaidah, S. (2018). Comparison of Extraction Methods on Harvest Results of Sea Cucumber Leaf Extract (Sonneratia) caseolaris L. Engl). *Manuntung Scientific Journal* , *4* (1), 79–83.

Xu, Z., Gao, P., Liu, D., Song, W., Zhu, L., & Liu, X. (2022). Chemical Composition and In Vitro Antioxidant Activity of Sida rhombifolia L. Volatile Organic Compounds. *Molecules* , *27* (20). https://doi.org/10.3390/molecules27207067

Yang, B., Chen, Y., & Shi, J. (2019). Reactive oxygen species (ROS)-based nanomedicine [Review article]. *Chemical Reviews* , *119* (8), 4881–4985. https://doi.org/10.1021/acs.chemrev.8b00626