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EXTRACT AND FRACTIONS EXPLORATION OF DAYAK ONION (*Eleutherine Americana Merr.*) WITH ANTIOXIDANTS POTENTIAL USING GC-MS and FTIR METHODS

Eksplorasi Ekstrak dan Fraksi-Fraksi Bawang Dayak (*Eleutherine Americana Merr.*) yang Berpotensi sebagai Antioksidan dengan Metode GC-MS dan FTIR

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ABSTRACT

Free radicals can cause various diseases in the body if not treated immediately. Large levels of free radicals may trigger damage to normally functioning tissues, lipid layers, blood vessels, DNA synthesis disturbances, and even destruction of cells. The objective is to evaluate the antioxidants potential of dayak onion extract and fractions. FTIR spectrophotometers, gas chromatography-mass spectrometry (GC-MS) and an antioxidants multimode reader are some of the research methods. The outcomes of this research are identifying plants that showed the dayak onion extract of the species *Eleutherine Americana Merr.* It contains compounds such as flavonoids, alkaloids, tannins, and saponins. In addition, the components found in the extract, water fraction, ethyl acetate fraction, and n-hexane fraction are octadecanoic acid 298 m/z, 9-octadecanoic acid 282 m/z, undecanoic acid 214 m/z, hexadecanoic acid 368 m/z, and nonadecanoic acid 326 m/z. Therefore, the antioxidants test using the DPPH method showed that the best IC_{50} value is for ethyl acetate with IC_{50} 20.56 ppm.

Keywords: *Free Radicals, GC-MS, FTIR, DPPH*

ABSTRAK

Radikal bebas dapat menyebabkan berbagai macam penyakit di dalam tubuh apabila tidak segera dilakukan penanganan lebih lanjut, jika radikal bebas memiliki jumlah yang besar dapat berpengaruh rusaknya jaringan normal, gangguan produksi DNA, pembuluh darah, lapisan lipid hingga kerusakan sel. Tujuan menguji aktivitas ekstrak dan fraksi-fraksi dari bawang dayak sebagai antioksidan. Metode penelitian Spektrofotometer FTIR, Gas Chromatography-Mass Spectrometry (GC-MS, dan Antioksidan multimode reader. Hasil penelitian determinasi tanaman memiliki spesies *Eleutherine americana merr*, kandungan senyawa yang terdapat pada ekstrak bawang dayak flavonoid, alkaloid, tanin dan saponin. Selain itu, komponen yang terdapat di ekstrak, fraksi air, fraksi etil asetat dan fraksi n-Heksana adalah Asam oktadekanoat 298 m/z, Asam 9-oktadekanoat 282 m/z, Asam undekanoat 214 m/z. Asam heksadekanoat 368 m/z, Asam nonadekanoat 326 m/z. sedangkan uji antioksidan metode DPPH dengan nilai IC_{50} terbaik adalah etil asetat IC_{50} 20,56 ppm.

Kata Kunci: *Radikal Bebas, GC-MS, FTIR, DPPH*

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INTRODUCTION

Academics and scientists have been discussing free radicals in Indonesia a lot. Free radicals can cause many different kinds of diseases in the body; consequently, that is harmful if more therapy is not started immediately. Free radicals are responsible for abnormalities in the body, which are caused by lifestyle choices such as fast food consumption and bad ventilation, which can lead to pollution and problems with daily activities[1]. Free radicals can damage tissues that are at healthy levels, cause issues with the production of DNA, damage blood vessels and lipid layers, and even cause damage to specific cells [2].

Free radicals are atoms or molecules that contain a variety of unpaired electrons. Due to the fact that free radicals contain paired electrons, they are considered very reactive and will seek electrons from other compounds, such as proteins, lipids, carbohydrates, and DNA, trying to neutralize. The proliferation of free radicals can be decreased by antioxidants chemicals that transfer one or more electrons. The presence of antioxidants in food can be used as therapeutic agents as a result. Dayak onion is a specific type of antioxidants that is naturally generated. [2].

In Indonesia, some plants exhibit biodiversity. The development of biodiversity can be used as a treatment for diseases. Secondary metabolite compounds like phenolics, flavonoids, and coumarins in plants can protect against oxidative stress by maintaining a balance between free radicals and antioxidants. [3]. Dayak onion is a local plant of Central Kalimantan. It has long been traditionally applied by people there for the treatment of many kinds of diseases, such as diabetes, hypertension, cholesterol, breast and colon cancer, decreased postpartum abdominal pain, and curing boils. Dayak onion extract has been tested and found to contain secondary metabolite components such as alkaloids, flavonoids, tannins, phenols, steroids, and saponins.[2]

Few studies used GC-MS (Gas Chromatography-Mass Spectrometry) to analyze the measurement of the compound components in extracts and fractions of natural plants that have the potential to be antihypertensive and effective in histopathological tests and kidney cell-damaged.[4]

Phytochemical screening for compounds such as flavonoids, phenolics, tannins, resins, and glycosides is known to have significant health benefits due to their strong antioxidants content and was analyzed using GC-MS instruments from extracts that can identify 80 compounds, reported that there were several among them, some have high antioxidants activity.[5]

Dayak onion have a high antioxidants potential because phenolic compounds form phenoxide ions by release one electron to free radicals. The naphthoquinone group is one of the antioxidants that can act as an electron acceptor, which gives Dayak onion a high antioxidant value and the potential used as a natural antioxidant source.[6]

Dayak onion has also been studied as a natural antioxidant using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method, and the ethyl and n-hexane fractions used have very strong antioxidants activity with IC_{50} values of 10.7 and 26.98 respectively. The tubers of Dayak onion have secondary metabolite compounds that are suspected to have high antioxidants activity.[7]

The researchers determined the antioxidant tests of n-hexane, ethyl acetate, water fraction, and Dayak garlic extract based on the information obtained from the sources previously aimed at the active compounds from the extract and fractions. Researchers used gas chromatography, mass spectrometry, and infrared spectrophotometer methods[8].

MATERIALS AND METHODS

From June to September of 2024, an experimental study was carried out at Ahmad Dahlan University and the Laboratory of the School of Pharmacy Foundation of Semarang. The plant identification number for this study is 029/EL-AFM/VI/2024 [9].

Materials and Equipment

The items used in the research were equipment for a set of glassware, stirring rod, watch glass, beaker, scissors, cannula, filter paper, coarse cloth, fine cloth, oven, analytical balance, rotavapor, spectrophotometer, Synergy HTX Multimode Reader,

and GC-MS (Shimadzu). The materials used are aqua destilata, 96% alcohol, Dayak onion, filter paper, water, ethyl acetate, and n-hexane.

Determination

The objective is to ensure that the plant materials are selected properly and to obtain the species that match the plant [10] Identification of Dayak onion plants was determined in the Biology Laboratory at the School of Pharmacy Foundation in Semarang.

Methods

1. Extract and Fractions

A glass jar contains 100 grams of Dayak onion powder, and 1000 ml of 96% alcohol solvent is added, with a sample-to-solvent ratio of 1:10. Remaserate is done by immediately soaking for 7x24 hours, followed by the second to fourth soak with solvent replacement every 1x24 hours, accompanied by stirring for approximately 5-10 minutes and then filtering. A rotary evaporator was employed to concentrate the macerate, which was then evaporated using a water bath to produce a thick extract of Dayak onion. Subsequently, the optimal amount of the extract used water, ethyl acetate, and n-hexane. [11].

2. FTIR (***Spectrophotometer)***

The FTIR spectrophotometer is an instrument that measures the vibrational spectrum of molecules and can be used to determine the structure of chemical compounds. The sample used in this study is 2 mg of Dayak onion extract, which was then tested using the FTIR spectrophotometer. This research was conducted at the Chemistry Laboratory of the School of Pharmacy, Yayasan Pharmacy Semarang [12].

3. GC-MS (*Gas Chromatography-Spectrometry Mass)*

The profile of the Dayak onion extract compounds was determined by GC-MS testing, and it can be assumed that they have potential cholesterol-inhibiting effects. The results were presented as a chromatogram with one graph and several peaks, each peak representing a different type of compound. The sample is injected into a column measuring 30 m x 0.25 mm i.d. with a thin film of 0.25 µM. The carrier gas used is helium at a flow rate of 1 ml/min. The injector is set at a temperature of 200°C, and the column temperature is programmed from 50 to 250°C at a rate of 10°C/min using the injection method. In mass spectrometry, an ionization voltage of 70 eV, a temperature of 250 °C, and a mass range of 50-600 are used. This resulted in a chromatogram and mass spectrum of the unknown compound, which was then compared with the spectrum of known compounds. This research was conducted at the Chemistry Laboratory of Ahmad Dahlan University in Yogyakarta (Suma *et al* 2019).

4. DPPH

The DPPH test is delivered with the Synergy HTX Multimode Reader. A DPPH solution with an 80 ppm concentration was prepared (5 mg of DPPH was weighed and dissolved in 10.0 ml of methanol, then diluted by pipetting 4 ml of the DPPH solution into 25.0 ml of methanol) contained a 100 ppm sample extract solution. A standard vitamin C solution was developed at concentrations of 2, 4, 6, 8, and 10 ppm. The plate occurred on a 96-well plate containing samples, standards, and blanks. The dilution method was used to obtain sample concentrations directly in the wells, obtaining concentrations of 100, 50, 25, 12.5, and 6.25 ppm for each sample. A 1:1 mixture of samples and standards was made with a DPPH solution. The mixture was incubated for 30 minutes in a space with light protection at ambient temperature after mixing. The absorbance was measured at a wavelength of 516.4 nm, and the percent of inhibition was determined using the formula above. A linear regression of concentration % inhibition was performed, and the IC_{50} value was calculated [14].

$$\%\ Inhibition = \frac{(A\ Blanko - A\ Sample)}{A\ Blanko} \times 100\%$$

RESULTS

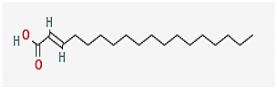
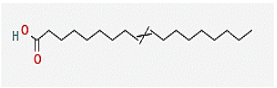
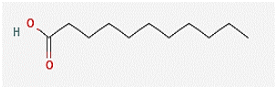
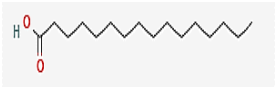
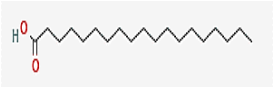
DETERMINE PLANT

The Dayak onion plant, obtained from Central Kalimantan, is frequently used in the community as an effective modern and traditional medicine. Dayak onion contains many therapeutic effects. The identification of Eleutherine Americana Merr, also known as Dayak onion, as a plant species is a source of encouragement for research on this plant. [15]. The experiments occurred at the Stifar Foundation's Pharmaceutical Biology Laboratory in Semarang, It can be seen in the table 1

Table 1. FTIR Spectrum Analysis

Stages	Wave number	Functional group
Extract	3248	O-H
	1629	C=O
	1647	C=C
	1101	C-O
Fraction Water	3289	O-H
	1636	C=O
	1021	C-O
Fraction Ethyl Acetate	2922	O-H
	1625	C=O
	1584	C=C
	1204	C-O
Fraction n-Hexane	2919	O-H
	1649	C=O
	1254	C-O

Table 2. GC-MS Analysis

Compound	% Area	R.Time	m/z	Molecular Formula	Structure
Octadecanoic acid	33.30	11.473	298	$C_{18}H_{36}O_2$	
9-octadecanoic acid	34.84	10.794	282	$C_{18}H_{34}O_2$	
Undecanoic acid	33.47	10.189	214	$C_{11}H_{22}O_2$	
Hexadecanoic acid	34.99	10.166	368	$C_{16}H_{32}O_2$	
Nonadecanoic acid	24.38	11.468	326	$C_{19}H_{38}O_2$	

Dayak onion is a plant from Central Kalimantan. For years, citizens were employing this plant as a medicinal herb. Dayak onions potential as a multifunctional medicinal plant has to be developed to render it an achievable modern therapeutic drug [16]. Dayak onion contains chemical compounds such as flavonoids, alkaloids, tannins, and saponins [17].

The results of the GC-MS analysis showed that the main components in the extract (solvent) of Dayak onion are undecanoic acid (214 m/z), 9-octadecenoic acid (oleic acid) (282 m/z), and octadecanoic

acid (methyl stearate) (298 m/z) (Zhang and Huang, 2014). The three compounds belong to the group of saturated fatty acids that have the same main chain group. The presence of these three main compounds was confirmed through functional group analysis used the FTIR method in tables 1 and 2. The major components in the water fraction of Dayak onion are hexadecanoic acid (palmitic acid) (m/z 256), 9-octadecenoic acid (oleic acid) (m/z 282), and octadecanoic acid (stearic acid) (m/z 284). The chemical compound was confirmed by the FTIR spectrum measurement shown in Table 1 and Table 2. Meanwhile, GC-MS fragmentation showed that the most prevalent elements in Dayak onions ethyl acetate fraction are hexadecanoic acid (368 m/z), 9-octadecenoic acid (oleic acid) (282 m/z), and octadecanoic acid (ethyl stearate) (312 m/z). The analysis of the functional groups conducted with FTIR shows the presence of the three compounds in Table 1 and Table 2. The results of the GC-MS analysis indicate that the most abundant components in the n-Hexane fraction of Dayak onions are hexadecanoic acid (368 m/z), 9-octadecenoic acid (oleic acid) (282 m/z), and nonadecanoic acid (m/z 326). FTIR analysis shows functional groups that support the presence of the compound. The results are presented in Table 1 and Table 2. Compounds with more non-polar characteristics are mostly found in the non-polar solvent fraction.

Table 3. The antioxidants result of Dayak onion extract

Concentration (ppm)	Absorbance	%Inhibition	IC_{50} ppm
102.72	0.153	57.14	
51.36	0.181	49.21	
25.68	0.197	44.72	58,03
12.84	0.204	42.95	
6.42	0.210	41.08	

Table 4. The antioxidants result of Dayak onion fraction water

Concentration (ppm)	Absorbance	%Inhibition	IC_{50} ppm
105.4	0.185	48.18	
52.7	0.197	44.72	
26.35	0.206	42.20	122,02
13	0.213	40.24	
6.59	0.215	39.68	

Table 5. The Antioxidants result of Dayak onion fraction ethyl acetate

Concentration (ppm)	Absorbance	%Inhibition	IC_{50} ppm
108.7	0.117	67.13	
54.35	0.142	60.22	
27.18	0.167	53.22	20,56
13.59	0.183	48.65	
6.79	0.200	43.88	

Table 6. The antioxidants result of Dayak onion fraction n-Hexane

Concentration (ppm)	Absorbance	%Inhibition	IC_{50} ppm
101.9	0.155	56.68	
50.95	0.181	49.39	
25.48	0.197	44.72	59,98
12.74	0.207	41.92	
6.37	0.216	39.50	

The research utilizes the Synergy HTX Multimode Reader to investigate the novelty of each study, with the antioxidants activity test employing the DPPH solution. The choice of the DPPH method is due to its simplicity, ease, speed, and the requirement for a small amount of sample. DPPH has a mechanism of action in which antioxidant compounds react with DPPH radicals through the receipt of hydrogen atoms [18]. Based on the information obtained, the more highly concentrated the extract and fraction, the higher the percentage of inhibition [19]. The percentage of antioxidants measured that activity in the extract samples and dayak onion fractions, the IC_{50} value can be determined, which is the quantity required to decrease DPPH by 50%. As a result of this, the lower IC_{50} value, the more effective chemical compounds with antioxidants abilities are at reducing the effectiveness of DPPH as a free radical [20]. The antioxidants activity of Dayak onion extract has an IC_{50} value of 58.03 ppm; the water fraction has an IC_{50} of 122.02 ppm, the ethyl acetate fraction has an IC_{50} of 20.56 ppm, and the n-hexane fraction has an IC_{50} of 59.98 ppm. Compared several test samples, as known Dayak onions extract, water fraction, ethyl acetate fraction, and n-hexane fraction, the results of the antioxidants activity indicated that the best result is the ethyl acetate fraction with an IC_{50} of 20.56 ppm.

CONCLUSION

This study reported that the antioxidants in Dayak onions is derived from the plant Eleutherine Americana Merr. The research mainly focused on the antioxidant compounds found in Dayak onion. The results demonstrated a strong antioxidants of Dayak onion. The phytochemical analysis result indicated the presence of different phytochemical classes, including flavonoids, alkaloids, tannins, and saponins, which are well-known for their antioxidants properties and ability to enhance health. Furthermore, GC-MS analysis of ethanol extract, water fraction, ethyl acetate fraction, and n-hexane fraction contain octadecanoic acid 298 m/z, 9-octadecanoic acid 282 m/z, undecanoic acid 214 m/z, hexadecenoic acid 368 m/z, and nonadecanoic acid 326 m/z and the DPPH antioxidants test showed that ethyl acetate is the highest IC_{50} value of 20.56 ppm. Therefore, these compounds may enhance the antioxidants effect found in this research. Findings from the research showed that Dayak onion could be an effective starting point for the development of modern therapies targeting illnesses caused by free radicals.

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