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Antioxidant Capacity from Ethyl Acetate Fraction of Kenikir (*Cosmos Caudatus K*)

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ABSTRACT

Antioxidant is an activity that inhibits oxidation reactions due to exposure to free radicals, the search for antioxidant compounds continues to be developed from natural sources. The purpose is to identify new sources of safe and inexpensive agents through antioxidant capacity from ethyl acetate fraction on kenikir leaves was evaluated through enzymatic and non-enzymatic in vitro models. The research began with the method of separating compounds from ethanol extracts and ethyl acetate fractions, quantification of total phenolics and flavonoids, antioxidant capacity tests with DPPH, and tyrosinase enzyme. The results of the study produced isolate E which was observed in a single stain with Rf 0,80. The compound has a typical marker worth 1734.07 cm-1 with an indication of stretching vibration movement on C-O. It has a maximum wavelength of ± 216 nm, with an abundance of 49.75% and a retention time of 27.20 minutes. TPC and TFC test results, the water fraction is the most dominant value with 0.430 mgGAE/g and 0.056 mQE/g. DPPH assay results, isolate E has an IC50 value of 162.03 ppm and a %inhibition of 12.73%. The tyrosinase inhibition test of L-DOPA substrate was resulting an IC50 value of 77.51 ppm compared to kojic acid of 16.48 ppm.

Keywords: Cosmos caudatus; Antioxidant; Enzymatic; N0n Enzymatic; Inhibition; In vitro

INTRODUCTION

Free oxidative compounds from poor-quality environments brought on by weather contaminants like global warming and vehicle density reduce public health risks and increase the likelihood of free radical attack. The principle of compound reactions can be used to explain the reaction mechanism of biomarker compounds to free radical resistance activity (antioxidants). As food additives, antioxidants have been widely used to protect the body from free radical-induced oxidative degradation processes. Some naturally occurring chemicals in plants have begun to receive a lot of attention as safe antioxidants because they have been proven by humans for a long time. Therefore, it is desirable to discover and make use of more natural antioxidants. Because compounds with different functional groups may exhibit varying polarities, resulting in dispersed antioxidant activity depending on the assay, an approach with multiple assays to evaluate antioxidant potential would be more informative and even necessary (Demirkiran. 2013). Kenikir leaves have been widely used to treat a variety of disorders because of their high antioxidant content (Chan EWC et al., 2016).

Kenikir leaf is a herb with a high antioxidant content that has been widely used to treat a variety of diseases, are one example (Chan E. 2016). By directly eating the leaves as fresh vegetables, kenikir is frequently used as a food crop. According to Fita et al. (2015), the tropical climate and geographical conditions of Indonesia permit the cultivation and distribution of kenikir leaves. Herbal plants' secondary metabolites exhibit both reducing and anti-radical properties when reacting with radical compounds (Chookalaii et al., 2020; Llorent-Martnez et al., 2017).

The harvest time for kenikir leaves which ranges from 42 to 63 days after planting, is unaffected by the season. According to Griep et al., the plant can also lower the risk of heart disease, stroke, arthritis, inflammation, and cancer. 2011). Vegetables are rich in phytochemicals, vitamins, minerals, fiber, and phytochemicals. Their secondary metabolite compounds must be characterized because they have the potential to alter gene expression, epigenetic regulation, cell signaling, inflammation, antioxidant activity, detoxification, and immune function (Kang et al., 2011)

The pharmacological activity of kenikir leaf extract as an antioxidant, antihypertensive, and antimicrobial has significant potential, according to previous studies. (Cheng S et al, 2016). Previous research by Firdaus et al. (2021) utilized warm water as a solvent; ethanol, 50%; 96% ethanol, which without fractionation produced crude extracts. The test for total phenolic and flavonoid

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compounds revealed that 50% ethanol had the highest value and the highest antioxidant activity, with an IC50 value of approximately 5.976 0.08 g/ml. 100% methanol was used in Cheng, S. H. et al.'s (2016) different test methods; ethanol to 100%; ethanol at 95%; ethanol, 50%; 100% water. Methods for testing for antioxidants include DPPH, ABTS, FRAP, and -carotene bleaching assays. Additionally, total carotenoid, total phenol, and total flavonoid content were examined to validate antioxidant activity. With 1654.7 mgQE/100 g and 10.513 mgGAE/100 g, respectively, the water solvent was found to have the highest total flavonoid and total phenolic content. Antioxidant activity was highest in the extracts of kenikir leaves made with 50% ethanol and 100% methanol.

Since there is no one broad-spectrum system that can provide an all-encompassing, precise, and quantitative prediction of antioxidant capacity and antiradical efficiency (Shah et al., 2014), multiple methods are suggested for evaluating the antioxidant capacities of complex samples. These measurements typically involve a variety of reaction mechanisms. The issue of collaborating enzymatic and non-enzymatic approaches is essential because there are differences between reagent-based inhibition mechanisms and enzyme-assisted ones. According to previous research, there are no related studies that have combined experimental studies of dominant compounds isolated from ethyl acetate fraction in kenikir leaves with combinations of enzymatic and non-enzymatic compounds under in vitro analysis. The purpose is to identify new sources of safe and inexpensive agents through antioxidant capacity from ethyl acetate fraction on kenikir leaves was evaluated through enzymatic and non-enzymatic in vitro models.

METHODOLOGY

Materials

Kenikir leaves, water, distilled water, 96% ethanol (theiknis), n-hexane, ethyl acetate, toluene, formic acid, silica gel 60 (Merck), cerium sulfate, gallic acid, ascorbic acid, thymol, quercetin, ABTS solution, foline-ciocalteau reagent, 2N HCl, filter paper, aluminum foil, Cerium sulfate spray receptor (Ce2(SO4)3, DPPH spray receptor, tyrosinase enzyme, DMSO, phosphate buffer (pH 6. 5), kojic acid, L-DOPA.

Methods

Plant Determination. Determination of kenikir leaves was carried out at the Phytochemistry Laboratory, Department of Pharmaceutical Biology, UGM**.**

Extraction and Fractionation. Ten kilograms of fresh kenikir leaves were dried in an oven at 40^0-50^0C for two days. 500 grams of dried kenikir leaves were collected and macerated in ten parts ethanol to a technical concentration of 96% (1:10, powder: solvent). The solid-liquid method was used for fractionation, with n-hexane and ethyl acetate as solvents. The ethanol extract of kenikir leaves was fractionated using a solid-liquid approach using an organic solution (technical) of n-hexane and a sample-to-solvent ratio of 1:3.

Isolation and Elucidation. The ethyl acetate fraction was isolated using TLC preparative. Toluene was used as the mobile phase: ethyl acetic acid derivation: Formic acid (3:1.5:0.5) was used in a total 50ml of chromatography chamber, which had a size of 20 x 20 centimeters and an elution distance of 17 centimeters. Using a TLC system containing three distinct eluents, the isolated band from the preparative TLC was scraped and dissolved. If only one spot was observed, it means that the separation method using TLC with three mobile phase systems has a good separation. GCMS is used to predict the structure of phytochemicals. UV Vis, which can identify chromophore groups in a chemical compound and predict functional groups using FT-IR wave numbers, can also be used to confirm compounds from the separation of the ethyl acetate fraction.

Total Phenolic Content Flavonoid. The Folin-Ciocalteu method, as described by (Shui et al.,), was used to determine TPC. 2005), with a few minor adjustments. The standard compound was used with a concentration variation of 10 ppm, and the sample was prepared at a concentration of 1000 ppm; 25 ppm; 50 ppm; 75 ppm; 100 ppm. The test arrangement contained a 300 µl sample, 3.7 ml distilled water, 250 µl folin reagent, and 750 µl Na2CO3. Before measurement, the mixture was incubated for 120 minutes at room temperature. At a wavelength of 743 nm, an ultraviolet-visible spectrophotometer (UV-Vis) was used to measure absorbance. The results were averaged after each sample was examined three times. A gallic acid calibration curve was used to determine TPC, which was expressed as mg gallic acid equivalent (GAE) per gram of dry plant material (mg GAE/g db).

Total Flavonoid Content

Aluminum chloride colorimetric assay using quercetin as a standard was used to measure total flavonoids (Cheng et al., 2016) with a slight change. 4.5 mL of aluminum chloride reagent, 10% aluminum chloride (0.1 mL), 1 M potassium acetate (0.1 mL), 95% methanol (1.5 mL), and

Figure 1. Kenikir plant obtained from Mlati, Sleman, Yogyakarta region. The kenikir plants used have the correct taxonomy of *Cosmos caudatus (K.)*, according to the determination results from the Phytochemical Laboratory of the Department of Pharmaceutical Biology at UGM.

distilled water (2.8 mL) were added to the reacting tube. The absorbance of the response blend was estimated at 430 nm after hatching at room temperature for 30 min. The calibration curve of quercetin standards at concentrations of 10 ppm served as the basis for the calculation of the TFC in each solvent; 25 ppm; 50 ppm; 75 ppm; 100 ppm. The aluminum chloride reagent combination alone, without the expansion of test material, was utilized as a clear. The quercetin and gallic acid calibration curve was used to calculate the samples' TFC value, which was expressed as mg of quercetin/gallic acid equivalent (QE) per gram of dry plant material (mg QE/g db).

Inhibition Level using DPPH. Up to 0.4 mM DPPH solution was made, or 0.01577 g was dissolved in 10 ml of methanol until baseline was reached. After that, concentrations of 5, 10, 15, 20, and 25 ppm were added to the solution. Gallic acid and quercetin were compared using standard solutions. Test arrangement of ethanol extricates hexane part, ethyl acetic acid derivation division, and water part of 1000 ppm as stock arrangement. Ethanol was added to each concentration until the limit (10 mL) was reached. Then the test arrangement was pipetted as much as 1.5 mL of every variation, added 1.5 mL of DPPH arrangement, and methanol up to 5 ml. Samples were made up to three times in a row. The UV-Vis spectrophotometer was used to test the DPPH control solution, and the maximum wavelength was 515 nm. A spectrophotometer was used to measure the free radical agents' activity after the samples were incubated for 30 minutes at 37^0 Celsius (Ikhrar. 2019).

Inhibition Level using Enzymatic Assay. The test procedure was prepared according to (Batubara et al., 2010) with some modifications. The test solution was added with DMSO to a final concentration of 20 mg/ml. Then add 600 ppm in 50 mM phosphate buffer solution (pH6.5). The extract was then tested at various concentrations of 50, 100, 150, 200, 250 ppm. Kojic acid was used as a positive control with variations of 7.81, 15.63, 31.25, 62.5, and 125 ppm. 96 well plates were filled with 70 µL of the test solution and 30 µL of tyrosinase enzyme (Sigma, 333 units/ml in phosphate buffer) in triplicate. After incubating for 5 minutes at room temperature, 110 µL of L-DOPA substrate was added to each test variation and continued incubation for 30 minutes. Optical density was observed using UV-Vis spectrophotometry at a maximum wavelength of 510 nm.

RESULT AND DISCUSSION

Determination results at the Phytochemical Lab, Branch of Drug Science, UGM. showed that the used kenikir plant was *Cosmos caudatus* (K.), which belongs to the Tracheophyta Division and the Class: Nation: Magnoliopsida Tribe of Asterales: Genus: Asteraceae Cav Cosmos (Figure 1). The extraction strategy utilized was maceration. Because it requires minimal processing and equipment. The sample is soaked in a solvent during the maceration procedure. The leaves of kenikir were extracted into a concentrated form. With a yield of 8.688% or weighted 43.44 grams. The kenikir leaf extract is sticky and has a blackish-green color.

Seen in visible light Seen in UV254 light Seen in UV366 light

Figure 2. TLC results of spot visualization after isolation. For isolates D and E, a pink one-line stain at uv 366 nm was observed in the results of the KLT compound separation. On the KLT plate, at a distance of 6.5 cm, the Rf value is 0.8..

Using n-hexane and ethyl acetate as solvents, the solid-liquid method was used for fractionation. Non-polar compounds like chlorophyll are separated by fractionation with n-hexane, and semi-polar compounds are separated from the crude extract with ethyl acetate (Irianti). T et al., 2015). Accordingly, the n-hexane division (FH) has non-polar properties and the ethyl acetic acid derivation portion (FEA) has semi-polar properties (Irianti. T et al., 2019). The n-hexane fraction of 2509.7 mg had a yield of 25.1%, the ethyl acetate fraction of 965.9 mg had a yield of 9.7%, and the water fraction (FA) of 44.686 grams had a yield of 44.7%.

A mixture containing toluene: is used in the TLC-based mobile phase optimization process. etheric acid: formic acid (3:1.5:0.5). which is advantageous for the separation of the ethyl acetate and n-hexane fractions. The location of tender loving care spots can likewise be seen actually utilizing 254 nm and UV 366 nm lights, and synthetically utilizing shower reagents (Sopiah. B et al., 2019). Under UV 254 light, the plate will fluoresce, and the sample will become black or dark. The collaboration between UV light and the fluorescence marker on the plate causes the presence of spots under UV 254 and 366 lights. When an electron is excited from its base energy to a higher energy level and then returns to its original state with the release of energy , this process is known as visible light fluorescence (Gandjar. and Rohman, 2007).

Some plates were sprayed using sulfuric acid to degrade the compound. After spraying and heating, 3 bands that were previously invisible were visible. Thus, the results obtained were 9 bands with 2 dominant bands (bands D and E) of light green color. The weight of the isolate obtained for stain D = 15.9 mg and E = 15.4. Each band from A to I has Rf 0.19; 0.28; 0.63; 0.81; 0.88; 0.90; 0.94; 0.99. The Rf value is evidence of compound identification. If the Rf value is the same, it can be said that the compound has the same or similar characteristics as the comparator. A compound with a larger Rf value means it has a lower polarity, and so does the opposite (Fessenden and Fessenden, 1986).

After drying in a water bath, the weight of isolate E isolate was 29.3 mg per 100 mg of the ethyl acetate fraction, which was divided into several batches for each test. Isolates D and E had a pink one-line stain at uv 366 nm when KLT was used to separate the compounds (Figure 2). This indicates that the compound has been well separated because there is no additional stain after the cerium sulfate semptor. On the KLT plate, at a distance of 6.5 cm, the indicated rF value is 0.8. The stain will be lifted a little higher and be semi-polar to non-polar, indicating a low polarity due to the mobile phase's tendency to be non-polar. Because

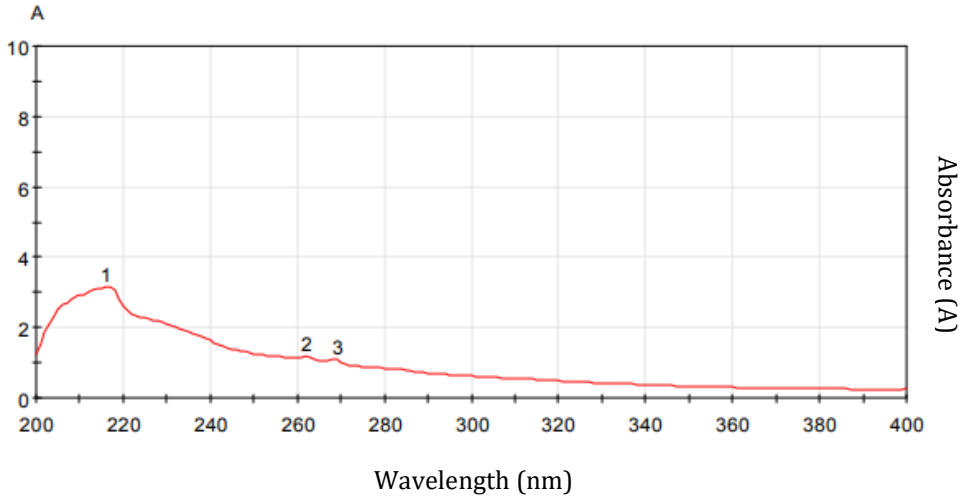


Figure 3. UV-Vis results of isolates E. It is known from the UV spectrum that isolate E has a maximum wavelength of ± 216 nm

Table I. GC-MS identification results of isolate 3 with %area >0.99 criteria

No	R.Time	Chemical Compound	Chemical Formula	Mol. Weight	SI	%.Area
1	5.78	3,3-Dimethoxy-2-butanone **(E1)**	$C_6H_{12}O_3$	132	661	1.01
2	21.29	Isoaromadendrene epoxide **(E2)**	$C_{15}H_{24}O$	220	788	1.64
3	21.74	Hexadecanoic acid, methyl ester **(E3)**	$C_{17}H_{34}O_2$	270	828	2.76
4	22.41	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde **(E4)**	$C_{23}H_{32}O$	324	762	1.41
5	27.04	Octadecanal, 2-bromo- **(E5)**	$C_{18}H_{35}BrO$	346	724	5.14
6	27.20	Diisooctyl phthalate **(E6)**	$C_{24}H_{38}O_4$	390	935	49.75
7	35.69	Stigmasterol **(E7)**	$C_{29}H_{48}O$	412	744	2.90
8	35.84	Cholesta-5,7-dien-3-ol, 4,4-dimethyl-, (3ß)- **(E8)**	$C_{29}H_{48}O$	412	675	4.52
9	36.93	?-Sitosterol **(E9)**	$C_{29}H_{50}O$	414	660	1.53
10	37.11	3-(1,5-Dimethyl-hexyl)-3a,10,10,12b-tetramethyl-1,2,3,3a,4,6,8,9,10,10a,11,12,12a,12b-tetradecahydro-benzo[4,5]cyclohepta[1,2-E]indene **(E10)**	$C_{30}H_{50}$	410	639	2.75

quercetin will appear at 3.7 to 4.1 cm or (rF = 0.46 to 0.5) when compared to the standard spot, it is concluded that the separated isolate has distinct physicochemical properties from the standard quercetin.

UV-vis spectroscopy is useful for the majority of organic and biological chemists when studying molecules with conjugated pi systems. The assimilated frequencies are bigger in this gathering because the energy hole for the -π* progress is more modest than that of a secluded two-fold bond. According to Clayden J et al. (2012), chromophores are molecules or molecular components with a high UV-vis absorbance. In light of the UV range, it is realized that confine E has the greatest frequency at 216 nm (Figure 3). Due to benzene's strong absorption at 202 nm and 1,3-butadiene's UV light absorption at 217 nm, these findings point to the presence of benzene. This is primarily because of the expansion of formed bonds and bathochromic shift (Filippi. C. et al., 2012).

Since they can retain UV beams, flavonoids are phenolic subordinates with a formed aromatic ring framework. In the presence of reducing radicals, flavonoids neutralize radicals to oxidize and stabilize reactive oxygen species (Herlina. N et al., 2018). One of the flavonol bunches that is

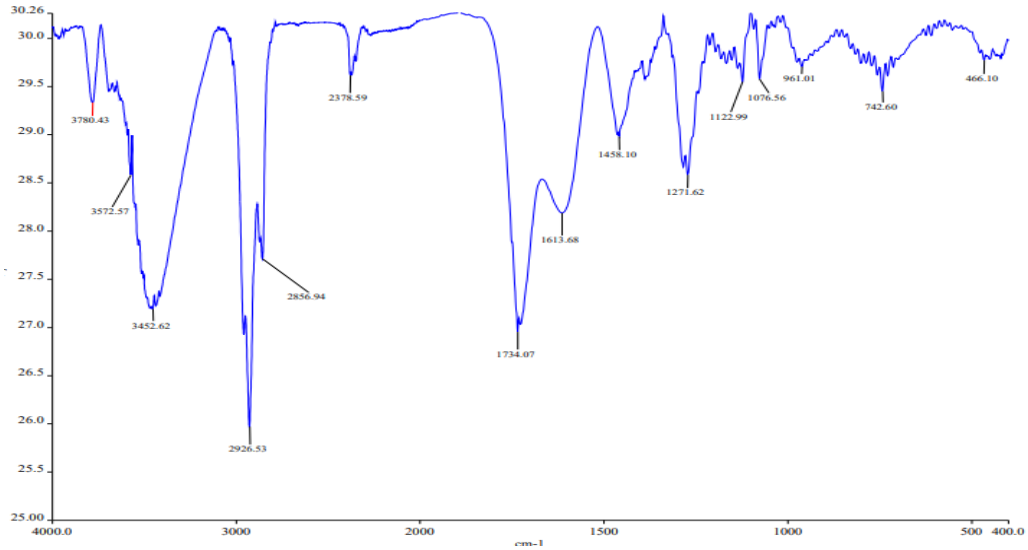


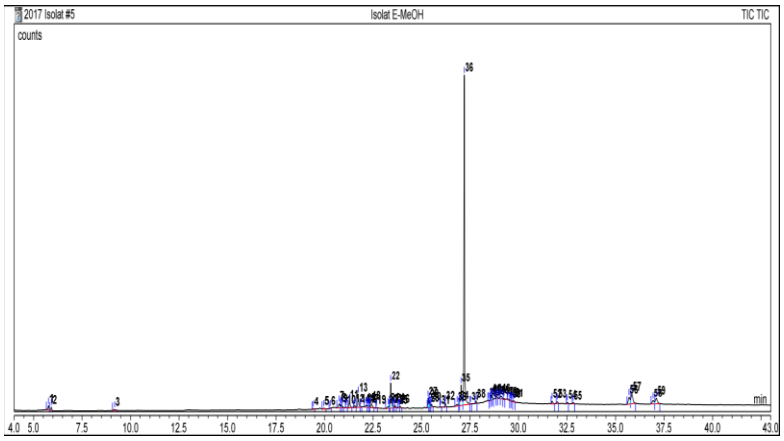
Figure 4. FT-IR results of isolates E. FT-IR analysis by pressing the material into a solid tablet using KBr plates and measuring wave numbers between 500 and 4000 nm.

Table II. TPC and TFC value of each sample

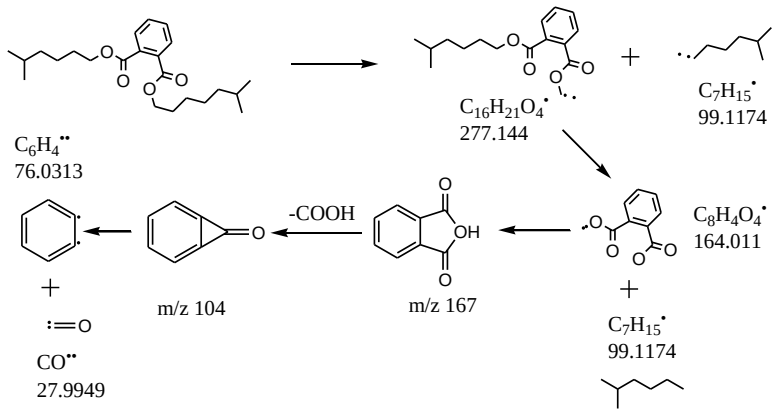
Sampel	TPC (ppm)	TPC (mgGAE/g)	SD	TFC (ppm)	TFC (mgQE/g)	SD
Ekstrak	141,851	0,211	0,120	18,361	0,046	0,006
Fraksi Etil Asetat	101,851	0,152		22,790	0,057	
Fraksi Heksan	180,370	0,268		24,742	0,061	
Fraksi Air	288,888	0,430		22,409	0.056	
Sample	**TPC (ppm)**	**TPC (mgGAE/g)**	**SD**	**TFC (ppm)**	**TFC (mgQE/g)**	**SD**
Ethanol Extract	141,851	0,211	0,120	18,361	0,046	0,006
Ethyl Acetat Fraction	101,851	0,152		22,790	0,057	
Hexane Fraction	180,370	0,268		24,742	0,061	
Water Fraction	288,888	0,430		22,409	0.056	

regularly utilized is quercetin, which has a ketone bunch subbed on carbon 4 in the heterocyclic C ring and a hydroxyl bond subbed on carbons 5 and 7 in the aromatic ring A. Gallic acid are corrosively responded with the Folin-Ciocalteu reagent to create a dazzling yellow tone, showing that it contained phenol. To produce a blue color, it was then added to the Na_2CO_3 solution. $AlCl_3$ forms a bright yellow complex that is stable in visible light when hydroxyl on carbon 3 or 5 and ketone on carbon 4 are combined (Hikmawati. N et al., 2021). The expansion of the Na_2CO_3 arrangement was important to make buildings because the phenolic compounds just responded with the Folin-Ciocalteu reagent under basic circumstances, bringing about proton separation into phenolic particles (Ahmad. A. R et al., 2015).

Isolate E (Figure 4) analyzed using the IR spectrum showed a sharp and strong absorption in the range of 1076.56 cm^{-1}; 1122.99 cm^{-1} with a model of in-plane bending vibrations on the C-H bond of the benzene ring. The absorption at 1271.62 cm^{-1} also indicates in-plane bending vibrations of benzene and alkyl C-H bonds. The value of 1613.10 cm^{-1} indicates the presence of the C=C stretching functional group of a benzene ring. 1734.07 cm^{-1} indicates stretching vibration movement on C-O (Bin Du et al., 2014). There is an -OH group in the compound tested, indicated by the presence of a strong and widened absorption at 3452.62 cm^{-1}. There is also absorption at 1458.10 cm^{-1} and 961.01 cm^{-1} of the CH2-CH2 and CH-CH functional groups in bending (Fessenden. 1986).



5a. MS spectrogram from Isolate E.

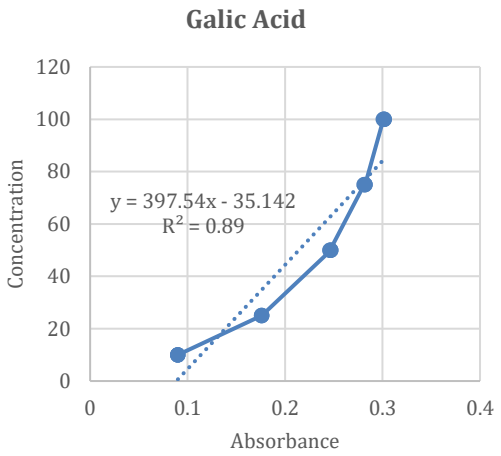


5b. Fragmentation pattern of diisooctyl phthalate compound (Banda et al., 2022).

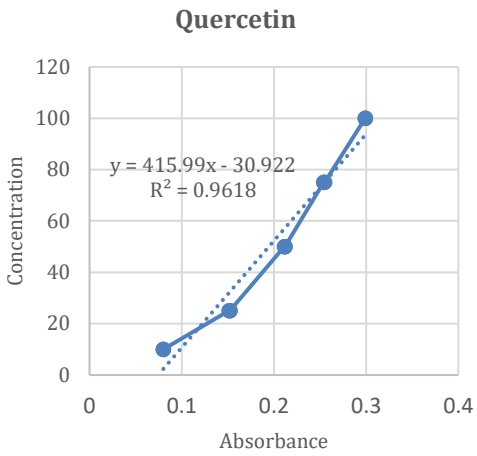
Figure 5. Spectrogram analysis (a) and fragmentation pattern (b). From compound isolate E, a total of 59 compounds were obtained, with peak number 36 appearing to be the most dominant in the spectra. Peaks 167, 149, and 57 of the MS fragmentation of isolate E with diisooctyl phthalate share similarities. The loss of H_2O molecules from the product at m/z = 167 results in the formation of the base ion (protonated phthalic anhydride) with m/z = 149.

Isolates E total weight of 15.4 mg were injected into the GC – MS. The sample preparation carried out is the use of methanol solvent, run time of 38.9 minutes, injection volume of 2 µl, dilution factor of 1, and injection sample weight of 1 µg. The literature used is mainlib with a 3-hit system. In the results of isolate E, a total of 10 compounds were obtained (Table IV). The use of gas chromatography is very useful in purifying the components of a mixture. Gas chromatography separates the mixture according to the rate of movement in the stationary phase carried by the mobile phase (Ladwani et al., 2018). This difference in migration is caused by differences in the interaction of these compounds (in the mixture) with the stationary phase and mobile phase. In the GC-MS spectra of isolate E (Figure 5a), it can be analyzed if peak number 36 has the most dominant intensity with 42.04% abundance and 27.20 minute retention time. Diisooctyl phthalate compounds are antibiotics that can be found in the form of derivatives in water hyacinth plants (*Eichhornia crassipes*). TLC-based fractionation and methanol extraction method. (Shanab. 2010). These compounds also identified on seaweed (*Ulva lactuca*) has displayed antibacterial action against Proteus mirabilis, E. coli, and Kleb. pneumonia, which causes infections of the urinary tract (El-Shouny et al., 2017)

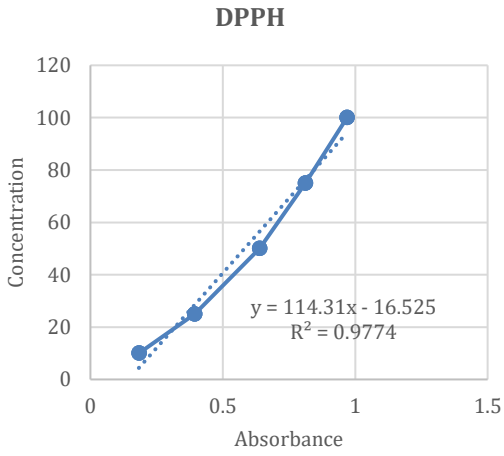
The fragmentation scheme on Diisooctyl Phthalate results shows that it has the highest ion abundance (base peak) at m/z = 149.1. The base peak is the most stable fragment in a molecule, and the intensity of other fragments is also relative to



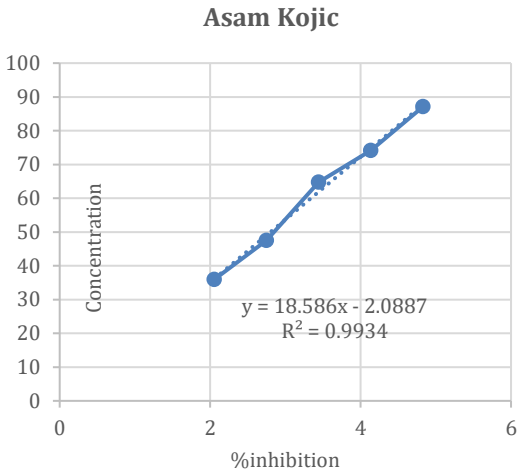
6a. Galic Acid Linear regression curve



6b. Quercetin Linear regression curve



6c. DPPH Linear regression curve



6d. Kojic acid Linear regression curve

Figure 6. Linear regression curve of (a) Galic acid, (b) quercetin, (c) DPPH, and (d) Kojic Acid. It can be concluded that a valid standard compounds are used for antioxidanc capacity, TFC and TPC test.

Table III. IC50 value of DPPH antioxidant activity

Sample	%Inhibition	IC50 (ppm)	St. Dev	Range
Ethanol Extract	14,260	138,700	0,070	100-150 ppm
Ethyl Acetate Fraction	38,840	50,850	0,200	50-100 ppm
Hexane Fraction	18,790	160,330	0,060	150-200 ppm
Water Fraction	79,610	3,540	0,000	<50 ppm
Isolate	12,730	162,030	0,200	150-200 ppm
Quercetin	64,510	2,360	0,400	<50 ppm
Gallic Acid	60,360	5,200	0,130	<50 ppm

the base peak, indicating that its stability is relative. The separation of one electron from the C-O bond results in the formation of a radical compound with m/z values of 277 and 167, which is the first step in the analysis of the pattern of fragmentation found in diisooctyl Phthalate compounds. The -COOH compound is separated next, resulting in a benzene with a m/z value of 76.

The broadest tops in confine E are like the mass range of Diisooctyl phthalate ($C_{24}H_{38}O_4$). Similarities at peaks 167, 149, and 57 can be seen in the MS fragmentation data of isolate E with diisooctyl phthalate. The loss of H2O molecules from the product at m/z = 167 results in the formation of the base ion (protonated phthalic anhydride) with m/z = 149 (Figure 5b)

Table I shows that in TPC and TFC test results, the water fraction is the most dominant value with 0.430 mgGAE/g and hexane fraction as much as 0.056 mQE/g using gallic acid and quercetin as a standard compound (Figure 6a,b). From these results, any fraction of kenikir leaves have a high potent for antioxidant activity because of high TPC and TFC values related to the previous study which states that the existence of antioxidant activity is due to the main components in kenikir leaves consisting of flavonoid groups, phenolic acids, chlorogenic acids and terpenoids (essential oils) in all parts of the plant (Moshawih. S et alk., 2017).

The DPPH method's antioxidant activity test is based on the purple color loss caused by antioxidants' reduction of DPPH. The Inhibitory Concentration (IC_{50}) value is typically used to represent a substance's DPPH radical scavenging activity. IC_{50} is the inhibition level when the centralization of cell reinforcement mixtures can lessen half of the underlying DPPH extremist action (Litescu et al., 2010). The linear regression equation between the test sample's concentration and the sample's percentage of DPPH radical capture can be used to calculate the IC_{50} value (Figure 6c). The reading's sensitivity will be maximized at the maximum wavelength, resulting in a very small reading error. This study utilized extricates, hexane divisions, ethyl acetic acid derivation parts, water portions, and separates to decide the IC_{50} esteem as per Table II.

When the antioxidant activity was tested using the DPPH method, it was found that Isolate E had the highest IC_{50} value 162.03 ppm with a level inhibition of 12.73%, while the quercetin comparison compound had the lowest IC_{50} value of 2.36 ppm with a % inhibition of 64.61 percent. If the IC_{50} value is less than 50 ppm, the antioxidant power is said to be very strong (Firdaus. 2021). The obtained isolate has an IC_{50} value that is greater than quercetin's. This suggests that the antioxidant capacity of the isolate is lower than that of the antioxidant quercetin in its pure form.

L-tyrosine is hydroxylated to 3,4-dihydroxyphenylalanine L-DOPA (monophenolase activity), and the latter is then oxidized to dopaquinone (diphenolase activity) in melanin synthesis due to the oxidase activity between the enzyme and the metal (Kim et al., 2013). Figure 6b depicts a linearity value (R^2) of 0.9934 on the standard curve, which means the category of very good linearity and reliability. Because of its function as a chemical compound that inhibits tyrosinase, kojic acid is used as a standard or positive control. It has been clinically used to treat hyperpigmentation (Ko et al., 2011). Tyrosinase inhibitors that work to reduce skin pigmentation disorders are expected to be safe and effective (Kang. 2012). Along these lines, it is important to search for new competitors showing viable tyrosinase hindrance however without secondary effects; As a result, metabolites biosynthesized by plants have emerged as promising alternatives to synthetic analogs, and this study's isolates may even function as free radical scavenger. In this study, L-DOPA was used as a substrate for the tyrosinase enzyme test solution because it is the pathway by which the tyrosinase enzyme converts melanin into dopaquinone.

The isolate does have an inhibition level of as much as 33.15% with an IC_{50} value is 77.51 ppm carries out the tyrosinase enzyme inhibition mechanism, so it is a smaller value than kojic acid as a tyrosinase enzyme inhibitor compound for the prevention of hyperpigmentation. Ethyl acetate fraction, ethanol extract, and isolate produce inhibition ability of 97.30 ppm; 85.48 ppm; 77.51 ppm is categorized as good because it is still in the range of values of 50-100 ppm based on research (Syarifah et al., 2021) but still below the inhibition ability of kojic acid. The kojic acid standard has an IC50 value of 16.48 ppm, meaning that at that concentration the compound can carry out the tyrosinase enzyme inhibition mechanism.

CONCLUSION

Kenikir leaves' ethyl acetate fraction can be separated into two predominant spots, revealing 10 compounds (%area > 0.99). With a yield of 9.7%, 965.9 mg of ethyl acetate fraction was collected. Isolate E from the preparative KLT isolation had a single stain with an rF of 0.8. The compound has a typical marker worth 1734.07 cm-1 with a stretching vibration movement on C-O. It has a maximum wavelength of ± 216 nm, with an abundance of 49.75% and a retention time of 27.20 minutes. TPC and TFC test results, the water fraction is the most dominant value with 0.430 mgGAE/g and hexane fraction as much as 0.056 mQE/g. Isolate E has an IC_{50} value of 162.03 ppm and a % inhibition of 12.73% equal to DPPH. The tyrosinase inhibition test was resulting an IC_{50} value of 77.51 ppm compared to kojic acid of 16.48 ppm.

Table IV. IC50 value of antioxidant activity by enzym tyrosinase reaction

Sample	%Inhibition	IC50 (ppm)	St.Dev	Range
Ethyl Acetate Fraction	31,430	97.300	0.008	
Eethanol xtract	31,550	85.480	0.008	50-100 ppm
Isolate	33,150	77.510	0.005	
Kojic Acid	61,880	16.480	0.0052	<50 ppm

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CONFLICT OF INTEREST

Authors have no conflict of interest to declare.

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