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Antioxidant Activity of Flavonoids from *T. catappa* Linn Fruit Flesh Fractionated using Continuous Column chromatography

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ABSTRACT. *Terminalia catappa* L. is traditionally used as antioxidant anti-inflammatory, antimicrobial, hepatoprotector, vermifuge, anticancer and antidiabetic. Especially the flesh of *T. catappa* fruit has potential as an antioxidant. There has been no research that conducts a fractionation process using column chromatography to find antioxidant active fractions from fruit flesh of *T. catappa.* Therefore, this research aims to find the antioxidant active fraction of the fruit flesh *T. catappa*. The research steps began with the extraction process using methanol and continued with fractionation using n-hexane and ethyl acetate. The ethyl acetate fraction (with antioxidant activity IC_{50} = 246.3 ppm) was subjected to the first column chromatography with a gradient of increasingly polar eluents. The fractionation results obtained 4 subfractions with the highest antioxidant activity found in subfraction 3 (FG3) with IC_{50} = 81.9 ppm containing phenolic, flavonoids, and terpenoids compounds. FG3 was fractionated again using second column chromatography. The fractionation results obtained 2 subfractions with antioxidant activity IC_{50} = 76.936 ppm (FG1P) and 82.745 ppm (FG2P). FG1P contains phenolic secondary metabolites, namely flavonoids of the isoflavone class. The results provide information that the compounds acting as antioxidants in *T. catappa* fruit flesh are flavonoids presumably from the isoflavone class. The discovery of flavonoid compounds that act as antioxidants in *T. catappa* fruit flesh can be developed in the pharmaceutical industry.

Keywords: Antioxidant, fruit flesh, Lisoflavone, *T. catappa*

INTRODUCTION

Therapeutic plants, in addition to essential metabolites, also contain secondary metabolites such as flavonoids, alkaloids, phenolic compounds, steroids, glycosides, and tannins. These compounds are extremely important alternative therapies for wound healing, whether individually or in combination. Herbal plants are easily affordable and accessible to everyone, especially in the third world, and can be used as antioxidants against free radicals which cause many diseases in humans (Diab et al., 2021; Mwangi et al., 2024)

The *T. catappa* (Combretaceae) commonly referred to as tropical almond or Indian almond is traditionally used as an anti-inflammatory, antimicrobial, hepatoprotective, vermifuge, antioxidant, anticancer, and antidiabetic agent (Chinaka et al., 2018; Mwangi et al., 2024). A study on the *T. catappa* leaves methanol extract showed 70.4% ± 4.9 DPPH scavenging activity and 99% ± 1.6 for the same concentration of extract and L-ascorbic acid respectively. According to the study, the *T. catappa* methanol extract had an IC_{50} of 5.6 μg/ml for DPPH scavenging activity. The *T. catappa* L. also contained scavenging activity for reactive oxygen species such as hydroxyl, superoxide, and peroxide radicals (Huang et al., 2018; Mwangi et al., 2024). Studies have shown that the leaves and barks of the plant exhibit antioxidant, hepato-protective, anti-inflammatory, antidepressant, antifungal, and chemopreventive activities. As a constituent in topical applications for wound healing, it has previously been reported that the *T. catappa* extracts exhibited reduction in wound healing properties in rats by 97% compared to betadine ointment. The ethanol extract of the *T. catappa* leaves displayed anti-inflammatory activity in animal models (Mwangi et al., 2024; Huang et al., 2018)

In the flesh of *T. catappa* fruit, the extraction results using water solvents obtained a yield of 12.912% with secondary metabolite content: alkaloids, sterols and steroids, flavonoids, tannins and phenolic (Krishnaveni & Dhanalakshmi, 2015)

The *T.catappa* plant is empirically efficacious for treating inflammation, headaches, diarrhea, hypertension and dysentery (Cock, 2015). Several studies have studied the compound content and antibacterial activity of the *T.catappa* plant, both in the leaves, stems and fruit of the *T.catappa* (Dewi et al., 2022). The secondary metabolite compounds that

have been identified in *T.catappa* leaves mostly come from the flavonoids and glycosides such as apigenin, quercetin, and isoflavones as well as the tannin compound group such as punicalin and punicalagin (Ohara et al., 2020) . Saponin, terpenoid and fatty acid compounds in *T.catappa* have also been reported. Fatty acid components such as n-hexadecanoic acid, octadecanoic acid, and other fatty acids in *T.catappa* leaves are known to have high levels (Yakubu et al., 2021).

The use of *T.catappa* fruit can be used as an antioxidant, antibacterial, and anticancer. Based on research, it is proven that *T.catappa* fruit has an antibacterial capacity of 17.90 mm and 15.55 mm against staphylococcus epidermidis and salmonella typhi (Istarina et al., 2015). *T.catappa* fruit has anticancer activity as evidenced by Chasani, 2022, for the ethyl acetate fraction of *T. catappa* fruit flesh, using the MTT Assay method with an IC_{50} value of 165.37 ppm (Chasani et al., 2022).

The phytochemical screening of *T. catappa* fruit, using ethyl acetate extracts, revealed the presence of various phytochemical components. Extracts were found to contain alkaloids, flavonoids, tannin, steroids, and terpenoids (Amelia et al., 2024). Antioxidant activity of *T. catappa* extract using the 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) radical scavenging test. The IC_{50} value for the ethyl acetate extract of *T.catappa* fruit is 2515.1 µg/mL (Amelia et al., 2024). The leaf extracts of *T. catappa* show strong 2,2- Diphenyl-1-picrylhydrazyl (DPPH) scavenging and antioxidative activities. Conversely, the seed extracts only exhibited strong inhibition of conjugated diene hydroperoxide formation and very low DPPH scavenging activity (Venkatalakshmi et al., 2016). The seed flour presented antioxidant activity (46.05 mg.100-1 g ± 0.23), but did not present inhibitory power against the tested bacterial strains. *T. catappa* fruit can be an alternative new ingredient with natural antioxidant action. *T. catappa* apart from containing oil, also contains secondary metabolites which are widely used from the roots, stems, leaves to the flesh of the fruit. Research on *T.catappa* has been widely published, as an antibacterial (Istarina et al., 2015), and antifungal (Yastanto & Indrati, 2024).

The increasing demand for antioxidants (Ji et al., 2023), especially natural antioxidants, and the potential of *T catappa* as a new source of antioxidants , necessitate further research to obtain more specific information, particularly on the parts of *T. catappa* whose potential has not yet been studied

Extensive research has been conducted on the *T. catappa* species, particularly on its leaves and bark, to examine its pharmacological potential and other uses, as well as research on the use of its seeds as a source of oil for various applications. However, there has been no research on the flesh of *T. catappa* fruit, especially regarding its pharmacological potential, particularly as an antioxidant (Widyaningsih et al., 2018). Until this year there has been no research that has carried out fractionation of the ethyl acetate fraction of *T.catappa* fruit flesh to study compounds that act as antioxidants. Therefore, this research has carried out fractionation of ethyl acetate extract from *T.catappa* fruit flesh using column chromatography to obtain information on the active antioxidant fraction. Fractionation using column chromatography will separate compounds that are not active as antioxidants, so that active antioxidant fractions can be obtained which will enable further research to be carried out to obtain pure antioxidant compounds. Fractionation in this study used column chromatography, which was carried out in stages until fractions with high antioxidant activity were obtained. The results of this study are the first report detailing stepwise fractionation using column chromatography on *T. catappa* fruit flesh.

EXPERIMENTAL SECTION

Material

The ingredients used in this study were *T. catappa* fruit flesh, methanol pa, *n*-hexane pa, ethyl acetate pa, chloroform pa, glacial acetic acid pa, acetone pa, Dragendorff's reagent, filter paper, $AlCl_3$ reagent, ammonia, I_2, 5% $FeCl_3$ reagent, dimethyl sulfoxide (DMSO), silica gel G_{60}, TLC plate G_{254}, 2,2-diphenyl-l-picrylhydrazyl (DPPH) and glacial acetic acid

Instrumentation: The tools used in this research were glassware, rotary vacuum evaporator, Buchner funnel, digital scales, TLC plate, blender, sample vial 10 mL, aluminum foil, UV lamp with wavelengths of 254 nm and 366 nm, spatula, column chromatography, Shimadzu UV-Vis 1800 spectrophotometer and Shimadzu FT-IR spectrophotometer.

Methods

Sample preparation

T. catappa fruit (5000 g) was peeled and the flesh was taken, then cut into small pieces and dried. After that, it was mashed using a blender and extracted by maceration using methanol for 3 days and filtered using a Buchner funnel. The methanol extract was then evaporated and the yield was calculated. The concentrated methanol extract was then fractionated with n-hexane, and the n-hexane fraction and residue were obtained. The residue from fractionation with n-hexane was fractionated again using ethyl acetate solvent, and ethyl acetate fraction and residue were obtained. The ethyl acetate fraction and residue were evaporated and weighed.

Sample fractionation by column chromatography

Silica gel G-60 was saturated with *n*-hexane until it became slurry outside the column, then slowly poured into the column through the edge of the column tube and left for 2 days. The next step is to take 1.2 grams of the ethyl acetate fraction and dissolve it in ethyl acetate. The dissolved fraction was then mixed with 30 grams of G-60 silica gel and stirred until

homogeneous with the silica gel. Then the ethyl acetate fraction which has been saturated with silica gel is slowly poured into the column through the edge of the column tube. It was eluted using the eluent ethyl acetate: chloroform: methanol (1:8:1) to obtain subfractions resulting from the elution. Each sub-fraction was identified using TLC with the eluent ethyl acetate: chloroform: methanol (1:8:1). The elution process continues with a more polar eluent ratio when no sub-fractions from the column are detected on the TLC plate. The eluent used is a mixture of methanol: ethyl acetate (4:1) and the subfractions obtained are collected again. The volume collected from column chromatography was 5 mL per subfraction. Each subfraction was identified using TLC and subfractions with the same spot pattern were combined. The TLC data of the sub-fractions obtained were identified using a UV lamp at wavelengths of 254 nm and 366 nm. Spots that have the same Rf value are combined into combined subfraction groups. The groups of subfractions obtained were tested for antioxidant activity using the DPPH method.

Antioxidant activity test with the DPPH method

Preparation of DPPH solution: 1.9716 mg of DPPH was dissolved with methanol in a flask to 100 mL, so that a DPPH solution with a concentration of 0.05 mM was obtained. Determination of maximum λ: 4 mL of 0.05 mM DPPH solution was added to 1 mL of methanol and then allowed to stand for approximately 30 minutes in a dark place. Measured using a UV-Vis spectrophotometer with a wavelength of 500-600 nm. Determination of operating time: 4 mL of 0.05 mM DPPH solution was added to 1 mL of the test solution with the lowest concentration in various fractions. After that, the absorbance of the solution was measured with a UV-Vis spectrophotometer at the maximum wavelength, and the stability time was searched at intervals of 5 minutes, until a stable absorbance was obtained (Kamoda et al., 2021).

Measurement of antioxidants in various fractionations of the ethyl acetate fraction by column chromatography

Blank absorbance: 4 mL of DPPH solution with a concentration of 0.05 mM was added with 1 mL of methanol solvent, then incubated at 37 ^{0}C for the operating time, after that the solution was put into a cuvette and its absorbance was measured on a UV-Vis spectrophotometer with the maximum wavelength that has been obtained. Sample absorbance: As much as 25 mg of various subfractions were dissolved with methanol in a flask to 50 mL, so that 500 ppm mother liquor was obtained. If the extract is insoluble in methanol, DMSO is added, then a concentration series of 50, 60, 70, 80, 90 ppm is made. Prepared test tubes for each concentration that had been coated with aluminum foil, then each test tube was filled with 1 mL of extract and 4 mL of DPPH was added. The treatment was repeated twice. After that, it was incubated at 37 ^{0}C during the operating time, then the solution was put into the cuvette and the absorbance was measured at the maximum wavelength that had been obtained. The absorbance data obtained at each concentration of each subfraction was calculated for the value (%) of its antioxidant activity. This value is obtained by the equation below:

$$\% \text{ Inhibition} = \frac{A0-A1}{A0} \times 100\%$$

A0 = Absorbance at DPPH without sample/blank

A1 = Absorbance at DPPH after adding sample/ sample

Data on antioxidant measurement results were statistically analyzed using linear regression.

Phytochemical screening

Samples are tested with certain reagents to determine the content of chemical compounds. The analysis was carried out to determine the presence of phenolic, alkaloids, flavonoids, terpenoid and saponins. The flavonoid test was carried out using the TLC method. The eluted sample was tested using $AlCl_3$ reagent. Positive results are indicated by yellow spots. The terpenoid test was carried out using the TLC method. The eluted sample is detected using I2 vapor. Positive results for terpenoids are indicated by spots that turn brown by I2 vapor. The phenolic test was carried out using the TLC method. The eluted samples were tested using 5% $FeCl_3$ (w/v). Positive phenolic results are indicated by spots that change color to green, red, purple or black. The saponin test was carried out by dissolving the sample in 10 mL of distilled water and shaking vigorously. Positive results for saponin are indicated by the formation of stable foam for approximately 10 minutes.

Active compound identification

Fractions that had the highest antioxidant activity were identified by their compound groups using Phytochemical Screening, UV-VIS spectrophotometers and FT-IR spectrophotometers.

RESULTS AND DISCUSSION

Sample preparation of compounds in *T.catappa* fruit flesh was carried out by maceration method with methanol solvent repeatedly, with the aim of optimizing the maceration extraction process in binding the compounds contained in the sample. Maceration was chosen using methanol because this solvent has a high extraction ability and can attract secondary metabolites. Fractionation using n-hexane aims to attract non-polar compounds and fractionation using ethyl acetate for extracting semi-polar compounds. Semi-polar compounds have strong potential for pharmacological activity, one of which is as antioxidants compared to non-polar fractions, which are mostly dominated by fatty acids. Semi-polar solvents (such as ethyl acetate) are effective at extracting a broad range of bioactive metabolites, including phenolic acids, flavonoids, and certain terpenoids. The ethyl acetate solvent was chosen because it is a semipolar solvent, so it is expected to

be able to extract polar and nonpolar compounds. A significant majority of naturally occurring, plant-derived antioxidant compounds (such as phenolics and flavonoids) possess polar or semi-polar characteristics. Their ability to act as effective antioxidants is often linked to these properties, which enable them to be extracted using semipolar and polar solvents. (Sinulingga et al., 2024 ; Widyawati et al., 2014)

Fractionation of Ethyl Acetate Fraction by First Column Chromatography

The ethyl acetate fraction was analyzed by thin layer chromatography using the eluent ethyl acetate : chloroform : methanol (1:8:1) which produces the best component separation. This eluent mixture is used as the mobile phase in fractionation using column chromatography. The ethyl acetate fraction was fractionated by column chromatography to separate the active compound with the highest antioxidant activity. The basic principle of column chromatography is separation based on the principle of adsorption. The stationary phase used was silica gel G-60. Factors affecting the successful isolation of a compound using column chromatography are the selection of the mobile phase or eluent. In accordance with the solubility principle, like dissolves like, namely polar solvents will dissolve polar compounds and non-polar solvents will dissolve non-polar compounds.

The elution process was carried out using a gradient method, starting with ethyl acetate : chloroform : methanol (1:8:1) eluent, then varied with a more polar eluent. The mobile phase can flow through the column due to the gravitational force to produce bands in the ethyl acetate fraction. The ethyl acetate fraction is dissolved with the eluent and undergoes separation, so that it can be collected in the form of subfractions when it leaves the column. The subfraction obtained was 61 test tubes. Eluent variations used in column chromatography can be seen in **Table 1.**

Table 1. Variation of column chromatographic eluent of the ethyl acetate fraction of *T.catappa* fruit flesh

Eluent	Comparison	Number of est tube
EA : C : M	1:8:1	35
EA : C : M	1:7:1	1
EA : C : M	1:6:1	1
EA : C : M	1:5:1	1
EA : C : M	1:4:1	1
EA : C : M	1:3:1	6
EA : C : M	1:1:1	14
EA : M	1:4	2

Note: EA = Ethyl acetate
C = Chloroform
M = Methanol

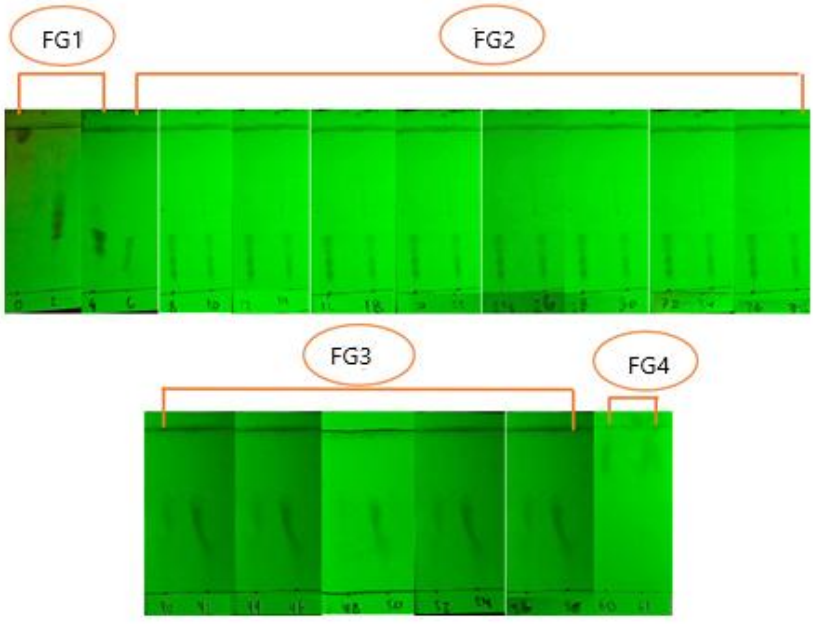

Figure 1. Chromatogram of subfraction 0-60 UV 254 nm

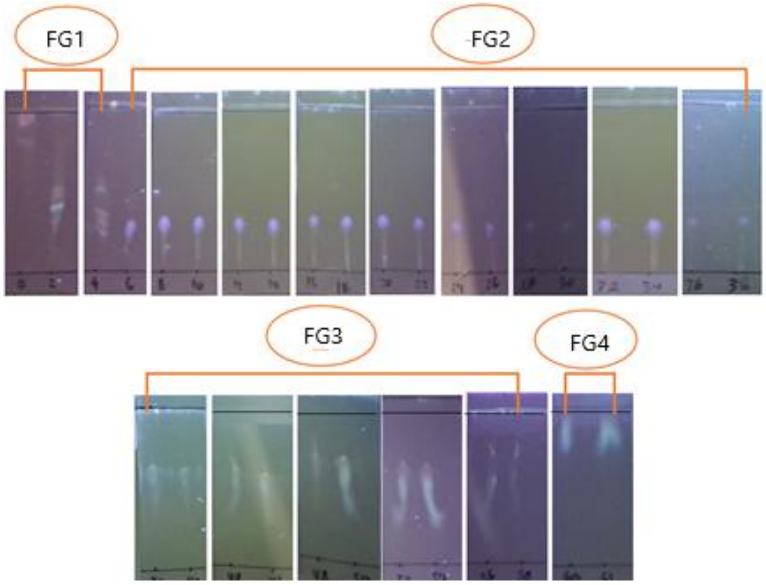

Figure 2. Chromatogram of subfraction 0-60 UV 366 nm

Table 2. Column chromatography subfraction results based on similarity of Rf values

Sample	Mass (gram)	Test tube number	Yield (%)
Sub fraction 1 (FG1)	0.56	1-5	46.89
Sub fraction 1 (FG2)	0.17	6-39	13.89
Sub fraction 3 (FG3)	0.21	40-58	17.28
Sub fraction 4 (FG4)	0.26	59-61	21.93

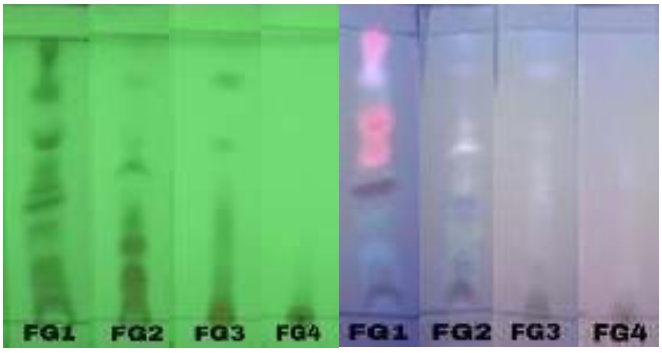

Figure 3. Chromatogram of the combined fraction from 2nd column chromatography using UV 254 nm and 366 nm

TLC test was carried out on all subfractions using eluent for column chromatography. Obtained 4 subfraction groups. The results of the TLC test on subfractions can be seen in **Figure 1** dan **Figure 2**.

Test tube number 1-5 are grouped into subfraction 1 FG1), test tube number 6-39 are grouped into subfraction 2 (FG2), test tube number 40-58 are grouped into subfraction 3 (FG3), and test tube number 59-61 are grouped into subfraction 4 (FG4) . The subfractions were concentrated using a rotary evaporator and weighed, so that the weight data for each subfraction was obtained as shown in **Table 2**. Each subfraction was carried out by TLC using ethyl acetate : chloroform : methanol (1:8:1) eluent. The results of the TLC can be seen in **Figure 3.**

Antioxidant Activity Test Results First Column Chromatography with The DPPH Method

The maximum wavelength is determined by using a control solution, namely DPPH solution with methanol as the solvent. Subsequent absorption measurements were carried out in the range of 400-600 nm. The measurement result of the 0.05 mM DPPH wavelength is 516 nm. These results are in accordance with the reference that DPPH has maximum absorbance in the wavelength range of 515-520 nm. The parameter used to determine antioxidant activity by the DPPH method is IC_{50}. The IC_{50} value is the sample concentration that is able to reduce free radicals by 50%. The smaller the IC_{50} value, the higher the free radical scavenging, and the

greater the IC_{50} value, the lower the free radical scavenging. The IC_{50} value can be obtained from the linear regression equation on the graph between the relationship between the concentration of the test solution (ppm) and the percent attenuation (Sinala & Dewi, 2019). Antioxidant activity of the ethyl acetate fraction; subfractions 1, 2, 3, 4; were analyzed using DPPH at the obtained stability times using a UV-Vis spectrophotometer with a wavelength of 516 nm. The results can be seen in **Table 3**.

Based on **Table 3**, FG3 has the highest antioxidant activity compared to FG 1, FG 2 and FG4. The secondary metabolite test results from FG3 compared to the ethyl acetate fraction are shown in **Table 4**. FG3 has much higher antioxidant activity than the ethyl acetate fraction, IC_{50} 81,09 ppm. Based on data from **Table 3** and **Table 4**, the FG3 test results contained three secondary metabolites, namely flavonoids, terpenoids, and phenolics, which are three secondary metabolites suspected of playing a role in the high antioxidant activity of FG3 (IC_{50} = 81,9 ppm). The alkaloids and saponins contained in the ethyl acetate fraction are suspected to be the cause of the low antioxidant activity of the ethyl acetate fraction (IC_{50} = 246.3 ppm).

Fractionation of Ethyl Acetate Fraction by Second Column Chromatography

FG3, which has the highest antioxidant activity, was separated again using a second column chromatography.The results of FG3 column chromatography using the eluent ethyl acetate: chloroform: methanol (1:1:1) obtained 19 fractions as shown in **Figure 4.**

The isolates resulting from column chromatography were grouped into 2 combined isolates, namely combined fraction 1 (FG1P) which was a group from fractions 1-10 and combined isolate 2 (FG2P) which was a group from fractions 11-19, as seen in **Figure 5.** Based on the TLC results using methanol and chloroform eluent (1:1), FG1P obtained one spot at Rf 0.5 while FG2P obtained 2 spots at Rf 0.5 and 0.05. TLC results using variations of ethyl acetate: chloroform: methanol (1:1:1) also showed that FG1P obtained one spot at Rf 0.53 while FG2P obtained 2 spots at Rf 0.55 and 0.03. A single chromatogram pattern in analysis using TLC cannot be concluded that FG1P is a pure compound, however by using two eluents with different polarities for the elution process it turns out that FG1P still shows a single yield spot. This result suggests that FG1P is relatively pure (Al Bara et al., 2021).

Table 3. Antioxidant activity

Sample	IC_{50} (ppm)
FG 1	527.5
FG 2	283.8
FG 3	**81.9**
FG 4	1001.4
Ethyl acetate	**246.3**

Table 4. Secondary metabolites in FG3 and ethyl acetate fraction

Compound	Reagent	FG3	ethyl acetate fraction
Flavonoids	$AlCl_3$	+	+
Alkaloids	Dragendorf	–	+
Terpenoids	Uap I_2	+	+
Phenolic	$FeCl_3$ 5%	+	+
Saponins	Aquades	–	+
Steroids	Lieberman-Bunchard	–	-

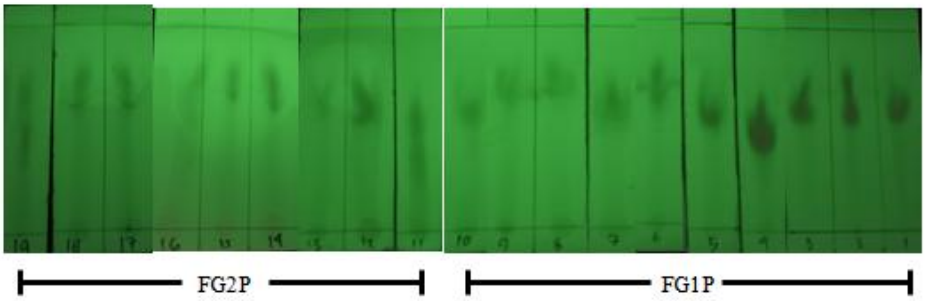

Figure 4. Chromatogram pattern with UV 254 nm

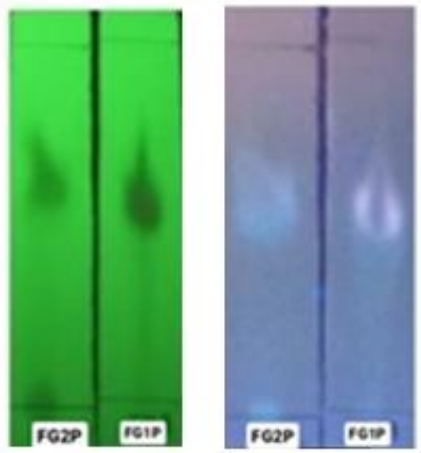

Figure 5. Chromatogram pattern of TLC test results for FG1P and FG2P

FG1P and FG2P Antioxidant Activity Test

The maximum wavelength for testing the antioxidant activity of FG1P and FG2P was obtained with an absorbance of 0.239 at a wavelength of 516 nm. Meanwhile, the maximum wavelength of DPPH is in accordance with theory, namely 515-520 nm (Molyneux, 2004). The analysis results show that the sample that has a higher antioxidant activity value is FG1P with an IC_{50} value of 76.936 ppm, while FG2P has a lower antioxidant activity value with an IC_{50} value of 82.745. Meanwhile, ascorbic acid has an IC_{50} value of 15.829 ppm. The results of the IC_{50} value analysis can be seen in **Table 5**. The higher antioxidant activity value of FG1P compared to FG2P is thought to be because FG1P is relatively purer than FG2P. This can be seen in the TLC data that FG2P still shows the second point detected at Rf 0.05.

The secondary metabolite test results from FG1P showed that only 2 secondary metabolites were contained in FG1P, namely flavonoids and phenolics. When compared with the secondary metabolite test results from FG3 and the ethyl acetate fraction as shown in **Table 6**, it shows that the terpenoids contained in FG3 are no longer present in FG1P. These results provide information that what is thought to play a role in the antioxidant activity of the ethyl acetate fraction of *T.catappa* fruit flesh are secondary metabolite compounds from the flavonoid and phenolic groups.

Testing of flavonoid metabolite compounds is based on the formation of $AlCl_3$ complexes with ortho hydroxy groups and hydroxy ketone groups in flavonoid compounds. For example, in the reaction of the quercetin compound with the $AlCl_3$ reagent (Ramadhan et al., 2021). The reaction between quercetin and $AlCl_3$ reagent can be seen in **Figure 6.**

The positive result in the phenolic test was because the color changed from brownish yellow to blackish blue. This happens because $FeCl_3$ reacts with the hydroxyl group (Alviani et al., 2022). The metabolite compound test reactions that occur can be seen in **Figure 7.**

Table 5. IC_{50} values of isolates

Number	Sample	IC_{50} (ppm)
1	**FG1P**	**76.936**
2	FG2P	82.745
3	Ascorbic acid	15.829

Table 6. Data on secondary metabolite test results of FG1P, FG3 and ethyl acetate fraction from methanol extract of *T.catappa* fruit flesh

Test type	Reagent	FG1P	FG3	ethyl acetate fraction
Flavonoids	$AlCl_3$	+	+	+
Alkaloids	Dragendorf	-	–	+
Terpenoids	I_2 vapor	-	+	+
Phenolics	$FeCl_3$ 5%	+	+	+
Saponins	distilled water	-	–	+
Steroids	Lieberman-Bunchard	-	–	-

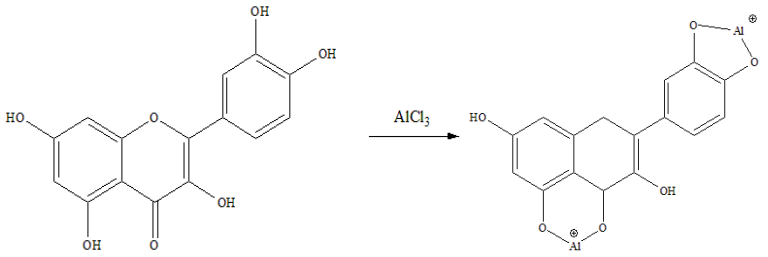

Figure 6. Reaction between quercetin and AlCl3 reagent (Ramadhan, 2021)

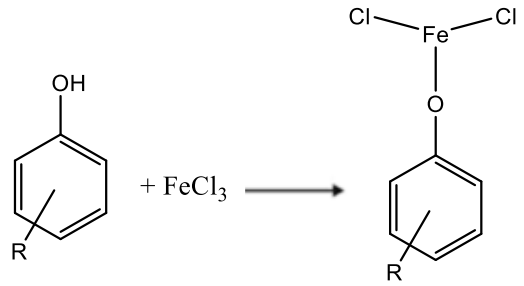

Figure 7. Phenolic secondary metabolite test reaction

Flavonoids are a family of polyphenolic compounds which found in a wide variety of vascular plants and their role in plants is as antioxidants, antimicrobials, photoreceptors, attractants, repellents, and light filters (Pietta, 2000). The classification of flavonoids is based on their heterocyclic C-ring structure, and is categorized mainly into flavanols, flavones, flavones, isoflavones, flavonols, and flavanonols (Kumar & Pandey, 2013; Banjarnahor & Artanti, 2014). The results of secondary metabolite testing of FG1P showed the presence of flavonoids and phenols. It is estimated that the positive phenol test result was due to the presence of flavonoids. Therefore, the positive test results for the presence of phenols reinforce the assumption that there are flavonoid compounds in the FG1P fraction. This is reinforced by the FTIR and UV test results as shown in **Figure 8** and **Figure 10.**

Identification of FG1P using UV and FT-IR Spectrophotometer

The results of identification carried out using a UV spectrometer are presented in **Figure 8.** The results show that the sample shows band I at a wavelength of 321.8 nm and band II at a wavelength of 249 nm. This shows that the FG1P sample is thought to be a flavonoid from the isoflavone group (Markham, 1988). As seen in **Figure 9** is the basic structure of isoflavones.

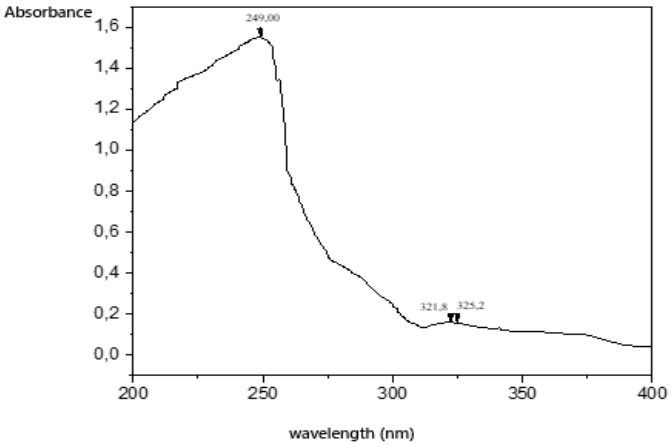

Figure 8. UV spectrophotometer spectrum of sample FG1P

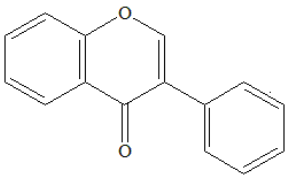

Figure 9. Basic structure of isoflavones

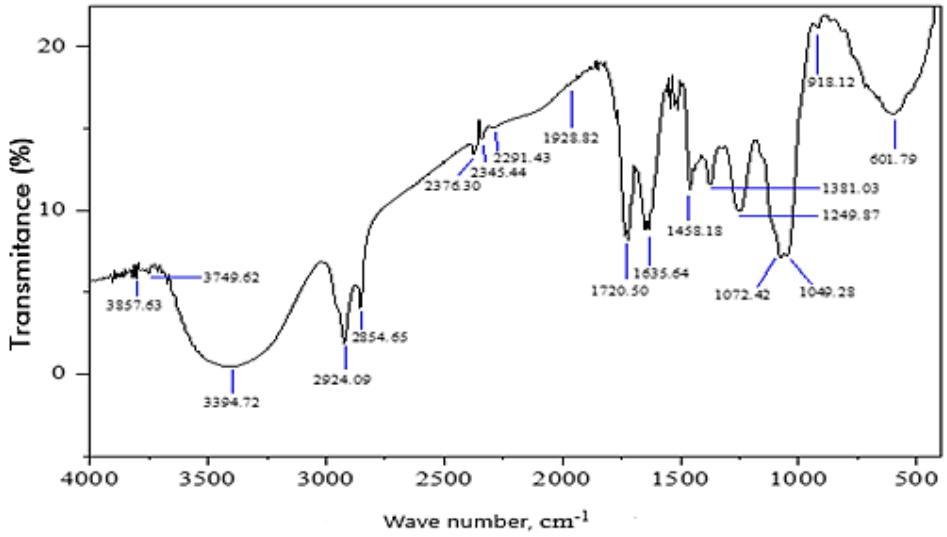

Figure 10. FT-IR spectrum of FG1P sample

The results of identifying the FG1P sample using an FT-IR spectrophotometer can be seen in **Figure 10**. The results of analysis using an FT-IR spectrophotometer on the FG1P sample showed strong absorption at a wave number of 3394.72 cm^{-1} indicating the presence of a hydroxyl group (O-H). Strong absorption at wave number 2924.09 cm^{-1} which indicates the presence of a methyl group. The medium absorption is at a wave number of 1720.5 cm^{-1} which indicates the presence of a C=O (carbonyl) group, while at a wave number of 1635.64 cm^{-1} shows C=C of aromatic compounds. The medium absorption shows the presence of C-H bonds at a wave number of 1458.18 cm^{-1} which indicates CH_2 and a wave number of 1381.03 cm^{-1} which indicates CH_3. Medium absorption is also found at a wave number of 1249.87 cm^{-1} which indicates the presence of an asymmetric C-O-C group and 1072.42 cm^{-1} which indicates the presence of a symmetric C-O-C group (Silverstein & Webster, 1996). The results of this test strengthen the suspicion that FG1P is dominated by flavonoids compounds.

Phenolic compounds have excellent antioxidant properties. These compounds include flavonoids, flavanols, anthocyanins, anthraquinones, as well as benzoic and acetic compounds. The high potential of flavonoids in inhibiting free radicals is related to their ability to transfer hydrogen atoms from hydroxyl groups to free radicals and ultimately stabilize them (Hassanpour & Doroudi, 2023). Additionally, they have the ability to chelate metals and prevent the oxidation of low-density lipoproteins (Heim et al., 2002). The flavonoid content in *T.catappa* fruit flesh is indicated by its reddish-brown color, particularly in mature ketapang fruit. This reddish-brown color indicates the presence of flavonoids (Orillaneda et al., 2022).

This study provides new information that *T.catappa* fruit flesh possesses strong antioxidant activity, and the compounds responsible for this antioxidant activity are isoflavone flavonoids. With the discovery of isoflavone flavonoids in *T.catappa* fruit flesh from this research, it is hoped that this information will be useful for utilizing ketapang fruit flesh as an antioxidant agent and further developed in the pharmaceutical industry.

CONCLUSIONS

Fractionation using column chromatography of the ethyl acetate fraction from the methanol extract of *T.catappa* L. fruit flesh, obtained groups of phenolic compounds (FG1P), namely flavonoid presumably from the isoflavone class which have antioxidant activity, IC_{50} = **76.936** ppm. The results of this study provide important information about secondary metabolites that act as antioxidants from the fruit flesh of *T. catappa* L. The discovery of flavonoid compounds that function as antioxidants in T. catappa fruit pulp can be developed in the pharmaceutical, food, and other industries.

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REFERENCES

Alviani, S., Adelia, A., Fajri, R., Amri, Y., & Amna, U. (2022). Skrining fitokimia ekstrak daun benalu kopi (*Scurrula Parasitica* L.) dataran tinggi Gayo (Phytochemical screening of coffee mistletoe leaf extract (*Scurrula Parasitica* L.) from the Gayo highlands). *QUIMICA: Jurnal Kimia Sains Dan Terapan*, *4*(1), 9–14. https://doi.org/10.33059/jq.v4i1.4360

Al Bara, B., Rivianto, F.A., Nurlaela, N., & Sulastri, S. (2021). Isolasi Senyawa Alkaloid Bahan Alam. *Jurnal Health Sains*, *2*(7), 858–870. https://doi.org/10.46799/jhs.v2i7.217

Amelia, F., Parbuntari, H., Iryani, Sudji, I. R.,

Rahmayani, S., Ramadhani, A. N., & Ananda, S. (2024). Phytoconstituents of *Terminalia catappa* linn fruits extract exhibit promising antidiabetic activities against α-amylase and α-glucosidase in vitro and in silico. *Informatics in Medicine Unlocked*, *47*(April), 101509. https://doi.org/10.1016/j.imu.2024.101509

Banjarnahor, S. D. S., & Artanti, N. (2014). Antioxidant properties of flavonoids. *Medical Journal of Indonesia*, *23*(4), 239–244. https://doi.org/10.13181/mji.v23i4.1015

Chasani, M., Widyaningsih, S., Rastuti, U., Ramadhan, A., & Aziz, I. A. (2022). Anti-cervical cancer test of the ethyl acetate fraction of *Terminalia catappa* Linn fruit flesh against HeLa cells. *Molekul*, *17*(2), 212–218. https://doi.org/10.20884/1.jm.2022.17.2.5492

Chinaka, C. N., Ezealisiji, K. M., & Akpofure, R. E. (2018). Phyto-chemical characterization of the leaf extracts of *Terminalia catappa* L. (*Combretaceae*) using ultra violet-visible, fourier transform infrared and gas chromatography-mass spectroscopic techniques. *Journal of Pharmacognosy and Phytochemistry*, *7*(1), 2017–2023.

Cock, I. E. (2015). The medicinal properties and phytochemistry of plants of the genus *Terminalia* (*Combretaceae*). *Inflammopharmacology*, *23*(5), 203–229. https://doi.org/10.1007/s10787-015-0246-z

Dewi, P., Putri, A. R., Bintari, S. H., & Mubarok, I. (2022). Uji efektivitas ekstrak buah ketapang (*Terminalia catappa*) terhadap bakteri *Escherichia coli* dan *Bacillus subtilis* (Testing the effectiveness of ketapang fruit extract (*Terminalia catappa*) against *Escherichia coli* and *Bacillus subtilis* bacteria). *Life Science*, *11*(1), 47–59. http://journal.unnes.ac.id/sju/index.php/LifeSci

Diab, T. A., Donia, T., & Saad-Allah, K. M. (2021). Characterization, antioxidant, and cytotoxic effects of some Egyptian wild plant extracts. *Beni-Suef University Journal of Basic and Applied Sciences*, *10*(1). https://doi.org/10.1186/s43088-021-00103-0

Hassanpour, S. H., & Doroudi, A. (2023). Review of the antioxidant potential of flavonoids as a subgroup of polyphenols and partial substitute for synthetic antioxidants. *Avicenna Journal of Phytomedicine*, *13*(4), 354–376. https://doi.org/10.22038/AJP.2023.21774

Heim, K. E., Tagliaferro, A. R., & Bobilya, D. J. (2002). Flavonoid antioxidants: Chemistry, metabolism and structure-activity relationships. *Journal of Nutritional Biochemistry*, *13*(10), 572–584. https://doi.org/ 10.1016/S0955-2863(02)00208-5

Huang, Y. H., Wu, P. Y., Wen, K. C., Lin, C. Y., & Chiang, H. M. (2018). Protective effects and mechanisms of *Terminalia catappa* L. methenolic extract on hydrogen-peroxide-induced oxidative stress in human skin fibroblasts. *BMC Complementary and Alternative Medicine*, *18*(1), 1–9. https://doi.org/10.1186/s12906-018-2308-4

Istarina, D., Khotimah, S., & Turnip, M. (2015). Aktivitas antibakteri ekstrak metanol buah ketapang (*Terminalia catappa* Linn.) terhadap pertumbuhan *Staphylococcus epidermidis* dan *Salmonella typhi* (Antibacterial activity of methanol extract of ketapang fruit (*Terminalia catappa* Linn.) against the growth of *Staphylococcus epidermidis* and *Salmonella typhi*). *Protobiont*, *4*(3), 98–102.

Ji, X., Liang, J., Wang, Y., Liu, X., Li, Y., Liu, Q., & Liu, R. (2023). *Synthetic antioxidants as contaminants of emerging concern in indoor environments : Knowns and unknowns*. https://doi.org/10.1021/acs.est.3c06487

Kamoda, A. P., Nindatu, M., Kusadhiani, I., Astuty, E., Rahawarin, H., & Asmin, E. (2021). Uji aktivitas antioksidan alga cokelat *saragassum* sp. dengan metode 1, 1-difenil-2-pikrihidrasil (DPPH) (Antioxidant activity test of brown algae *Saragassum sp*. using the 1, 1-diphenyl-2-picryhydryl (DPPH) method). *PAMERI: Pattimura Medical Review*, *3*(1), 60-72.

Krishnaveni, M., & Dhanalakshmi, R. (2015). Phytonutrient analysis in *Terminalia Catappa* fruit flesh, nut, shell. *International Journal of Current Pharmaceutical Review and Research*, *6*(1), 28–35.

Kumar, S., & Pandey, A. K. (2013). Chemistry and biological activities of flavonoids: An overview. *The Scientific World Journal*, *2013*. https://doi.org/10.1155/2013/162750

Markham. (1988). Cara mengidentifikasi flavonoid. In *ITB Press* (Issue November).

Molyneux, P. (2004). The use of the stable free radical Diphenylpicryl-hydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin Journal of Science and Technology*, *26*(December 2003), 211–219. https://doi.org/10.1287/isre.6.2.144

Mwangi, W. C., Waudo, W., Shigwenya, M. E., & Gichuki, J. (2024). Phytochemical characterization, antimicrobial and antioxidant activities of *Terminalia catappa* methanol and aqueous extracts. *BMC Complementary Medicine and Therapies*, *24*(1), 1–11. https://doi.org/10.1186/s12906-024-04449-7

Ohara, R., Périco, L. L., Rodrigues, V. P., Bueno, G., Zanatta, A. C., Campaner dos Santos, L., Vilegas, W., Constatino, F. B., Justulin, L. A., & Hiruma-Lima, C. A. (2020). *Terminalia catappa* L. infusion accelerates the healing process of gastric ischemia-reperfusion injury in rats. *Journal of Ethnopharmacology*, *256*(February),

112793. https://doi.org/10.1016/j.jep.2020.112793

Orillaneda, K. N. O., Caracal, E. S., Campo, I. D. E. A. Del, Flores, A. K. T., Malda, L. M., Morales, C. E. B., & Reniedo, C. J. D. (2022). Phytochemical screening, antioxidant, and antibacterial property of Talisay (*Terminalia catappa*) leaves ethanolic extract against *Staphylococcus aureus* used as formulated ointment. *International Journal of Pharma Medicine and Biological Sciences*, *11*(4), 89–95. https://doi.org/10.18178/ijpmbs.11.4.89-95

P, V., V, V., & P, B. (2016). Phytopharmacological significance of *Terminalia Catappa* L.: an Updated review. *International Journal of Research in Ayurveda & Pharmacy*, *7*(2), 130–137. https://doi.org/10.7897/2277-4343.07272

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

Ramadhan, H., Rezky, D. P., & Susiani, E. F. (2021). Penetapan kandungan total fenolik flavonoid pada fraksi etil asetat kulit batang kasturi (*Mangifera casturi* Kosterman) (Determination of total phenolic flavonoid content in the ethyl acetate fraction of castor bean bark (*Mangifera casturi* Kosterman)). *Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia*, *8*(1), 58. https://doi.org/10.20473/jfiki.v8i12021.58-67

Silverstein, R. M., & Webster, F. X. (1996). Spectrometric identification of organic compounds 6th Edition. In *John Wiley & Sons Ltd* (Vol. 6, pp. 1–482).

Subandrate, S., Sinulingga, S., Adma, A. C., Monanda, M. D. A., Fatmawati, F., Safyudin, S., & Oswari, L. D. (2024). Effect of solvent polarity on secondary metabolite content and Î±-glucosidase enzyme IC_{50} of *Dendrophthoe pentandra* (L). Miq leaves extract. *Jurnal Ilmu Kefarmasian Indonesia*, *22*(1), 1-7.

Widyaningsih, S., Chasani, M., Diastuti, H., & Fredyono, W. N. (2018). Liquid soap from nyamplung seed oil (*Calophyllum inophyllum* L) with ketapang (*Terminalia catappa* L) as antioxidant and cardamom (*Amomum compactum*) as fragrance. *Molekul*, *13*(2), 172. https://doi.org/10.20884/1.jm.2018.13.2.461

Widyawati, P. S., Budianta, T. D. W., Kusuma, F. A., & Wijaya, E. L. (2014). Difference of solvent polarity to phytochemical content and antioxidant activity of *Pluchea indicia* less leaves extracts. *International Journal of Pharmacognosy and Phytochemical Research*, *6*(4), 850-855.

Yakubu, Y., Lee, S. Y., & Shaari, K. (2021). Chemical profiles of *Terminalia catappa* Linn nut and *Terminalia subspathulata* King fruit. *Pertanika Journal of Tropical Agricultural Science*, *44*(4), 795–823. https://doi.org/10.47836/PJTAS.44.4.06

Yastanto, A. J., & Indrati, R. (2024). Suplementasi ekstrak daun ketapang (*Terminalia catappa*) pada potato sucrose agar (PSA) untuk menggantikan dichloran rose bengal chloramphenicol (DRBC) pada praktikum mikrobiologi pangan dan pengolahan (Supplementation of ketapang leaf extract (*Terminalia catappa*) on potato sucrose agar (PSA) to replace dichloran rose bengal chloramphenicol (DRBC) in food microbiology and processing practicums). *Jurnal Pengelolaan Laboratorium Pendidikan*, *6*(2), 116–125. https://doi.org/10.14710/jplp.6.2.116-125