Jurnal Info Sains : Informatika dan Sains
Volume 14, Number 04, 2024, DOI 10.54209/infosains.v14i04
ESSN 2797-7889 (Online)
https://ejournal.seaninstitute.or.id/index.php/InfoSains

Identification And Antioxidant Activity Test Of Graptophyllum Pictum L. Griff Var. Viride Leaf Extract

Desi Fajrianti[1], Chaidir[2], M.Hanafi[3]
Pharmacy, Universitas Pancasila, Lenteng Agung Raya Street No.56, RT.1/RW3, Srengseng Sawah, Jagakarsa, South Jakarta

Article Info
Keywords:
Graptophyllum pictum,
antioxidants,
flavonoids,
maceration,
TLC.

ABSTRACT
Flavonoids are one of the compounds that are effective as antioxidants and function to protect against exposure to free radicals. One of the plants that contain flavonoid compounds is the leaves of Graptophyllum pictum L. Griff Var. viride. This study was conducted to determine phytochemical screening, total flavonoids, total phenolics, and antioxidant activity analysis contained in the leaves of Graptophyllum pictum L.Griff Var. viride. This type of research is an experimental exploratory research. Extraction by multistage maceration using three solvents namely N-Hexan, ethyl acetate and 96% ethanol, antioxidant activity test with DPPH method, total flavonoid test using aluminum chloride colorimetric assay method, and total ph enol test using Folin-Ciocalteu method from Graptophyllum pictum L .Griff Var. viride leaves. The results of extraction with 400 g of Graptophyllum pictum L. Griff Var. viride leaf simplicia with N-Hexan, ethyl acetate, 96% ethanol respectively produced 4.5 g, 3.9 g, and 41.5 g of thick extract. The IC 50 values of N-Hexan, ethyl acetate, and ethanol extracts were 317.70 µg/mL, 227.30 µg/mL and 231.86 µg/mL, respectively. The total flavonoid content of N-Hexan, ethyl acetate, and ethanol extracts were 8.03; 55.52; 25.61mg quercetin equivalents/g extract, respectively. The total phenol content of N-Hexan, ethyl acetate, and ethanol extracts were 19,164; 16,135; 27,251 mg GAE/g extract, respectively.

Corresponding Author:
Desi Fajrianti
Pharmacy, Universitas Pancasila, Lenteng Agung Raya Street No.56, RT.1/RW3, Srengseng Sawah, Jagakarsa, South Jakarta
desifajrianti14@gmail.com

INTRODUCTION

As many as 17 million people die each year from degenerative diseases caused by oxidative stress. [1] Oxidative stress is caused by excess free radicals in the body. To prevent it, antioxidants are needed. [1] Antioxidants are substances needed by the body to neutralize free radicals and prevent damage caused by free radicals to normal cells, proteins, and fats. [2] Naturally, the body has antioxidants that function as a defense against free radicals. [1] Antioxidants in the body (endogenous antioxidants) are mostly in the form of enzymes such as the enzyme Super Oxide Dismutase (SOD), Glutathione Peroxidase (GPx) and catalase (CAT). [1,3,4] However, if there is excessive exposure to free radicals, the body needs exogenous antioxidants. [3,4] The use of synthetic antioxidants is feared to have side effects,

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this is the reason antioxidants from natural ingredients are considered safer. [5]

Secondary metabolite compounds in plants that have the potential to be sources of antioxidants are flavonoids. [6] The flavonoid group has a carbon skeleton consisting of two substituted benzene rings connected by a three-carbon aliphatic chain. [7] Indonesia is one of the countries with biodiversity that can be used as raw materials for modern and traditional medicines. [8] The purple plant (*Graptophyllum pictum* L. Griff) is one of the plants that is efficacious as a medicinal plant and is known to contain secondary metabolites in the form of flavonoids, where the most frequently used part is the leaves. [9] Phytochemical compounds from this plant are considered to help protect cells from oxidative stress caused by free radicals. [5] Based on previous studies, it was stated that 96% ethanol extract and chloroform fractions of purple leaves, ethyl acetate and n-butanol of purple plants contain secondary metabolites in the form of flavonoids of 402.88; 98.14; 374.45 and 345.95 mg/100 g QE, with antioxidant activity to reduce DPPH free radicals respectively from ethanol extract, ethyl acetate fraction, and n-butanol fraction respectively with IC50 values of 83.25; 271.04; and 385.82 ppm^3. This study was conducted to determine the phytochemical compounds, total flavonoids, total phenolics, and analysis of antioxidant activity contained in *Graptophyllum pictum* L.Griff Var. viride leaves.

Tools And Materials

The tools used in this study were glassware, micro pipettes, dropper pipettes, Hitachi brand UV-VIS spectrophotometer, analytical scales, cuvettes, test tubes, *water baths* and *vacuum pumps* . The materials used in this study were the leaves of *Graptophyllum pictum* L. Griff Var. viride. The chemicals used included n-hexane pa, ethyl acetate pa, ethanol 96% pa, quarsetin, Folin-Ciocalteu , 1,1-diphenyl-2-picrylhydrazine (DPPH), and distilled water.

RESEARCH METHODS

This research is an experimental exploration research conducted at the National Research and Innovation Agency (BRIN) Serpong, EBM scientific and technology Ltd, and the Pancasila University Laboratory. in August 2022 to July 2023.

Extraction

Extraction in this study was done by multilevel kinetic maceration using three types of solvents with different polarity levels, namely n-hexane pa, ethyl acetate pa, and ethanol 96% pa. A total of 400 grams of *Graptophyllum pictum* L.Griff Var. viride leaf simplicia powder was macerated using 4 liters of N-Hexane pa, then the macerate was filtered, then the filtered dregs were macerated again until a clear macerate color was obtained. The previously filtered dregs were macerated in the same way using a semipolar solvent, namely ethyl acetate pa and a polar solvent, namely ethanol 96% pa. Each macerate obtained was concentrated using *a water bath* to obtain a thick extract, then weighed and the yield value of each solvent was calculated.

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Phytochemical Test

Phytochemical tests aim to determine the metabolite compounds contained in the leaves of *Graptophyllum pictum* L.Griff Var. viride including alkaloids, saponins, flavonoids, tannins and phenols, triterpenoids, steroids, and quinones.

Flavonoid Test

Flavonoid test was conducted using aluminum chloride colorimetric assay method. Sample and quarsetin were weighed as much as 4 mg then dissolved in 4 ml of methanol to obtain a stock solution with a concentration of 1000 µg/ml. Pipette 250 µL of sample solution into a test tube, add 2 mL of distilled water and 150 µL of 5% $NaNO_2$, wait for 5 minutes, then add 150 µL of 10% AlCl3, $_{wait}$ for 6 minutes, then add 2 mL of 1M NaOH. Add distilled water to the boundary mark (5 mL). The solution was homogenized and its absorbance was measured at a wavelength of 510 nm using a UV-Vis spectrophotometer.[10]

Phenolic Test

Phenolic test was conducted using the Folin-Ciocalteu method. The stock solution of N-Hexane and ethyl acetate extracts was diluted to 5000 ppm while the ethanol extract was diluted to 4000 ppm, then 100 µL of each sample was put into each Eppendorf tube, 500 µL of 7.5% Folin-Ciocalteu solution was added and incubated for 8 minutes. Then 400 µL of 1% NaOH was added and incubated for 1 hour. The absorbance was recorded at a maximum absorption wavelength of 734 nm. [11]

Antioxidant Activity Test

Antioxidant activity test was carried out using the DPPH method. 4 mg of sample was dissolved with 4 ml of methanol (1000 ppm). then pipette 25 µL, 125 µL, 250 µL and 500 µL into opaque test tubes to make test solutions with concentrations of 10 ppm, 50 ppm, and 100 and 200 ppm. add 0.5 ml of DPPH that has been diluted with methanol to 2.5 ml into each tube. then incubated for 30 minutes at 37˚C. absorbance was measured with a UV-Vis spectrophotometer at a wavelength of 51.1 nm. [12]

RESULTS

Extraction

Table 1. Results of N-Hexane, Ethyl Acetate, and Ethanol Extraction of Leaves *Graptophyllum pictum* L. Griff Var. viride

Sample	Weight of materia (g)	Extract weigh (g)	Yield (%)	Ttexture	Color
Extract n-hexane	400	4.5	1.12	Thick	Brownish yellow
Extract ethyl acetate		3.9	0.97	Thick	Blackish green
Ethanol extract 96%		41.5	10.37	Thick	Blackish green

Extraction of 400 grams of *Graptophyllum pictum* L.Griff Var. viride leaves using 4

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liters of N-Hexane pa solvent obtained a yield value of 1.12% with a thick extract weight of 4.5 g which is brownish yellow, the yield value produced by ethyl acetate pa solvent was 0.97% with a thick extract weight of 3.9 g which is blackish green, and the yield value of ethanol solvent 96% pa was 10.37% with an extract weight of 41.5 g which is blackish green. The yield results can be influenced by the ratio of the solvent liquid, the polarity level of a solvent, and the extraction technique. These results indicate that the content of polar compounds has a yield 10 times greater.

Phytochemical Test

Table 2. Phytochemical Test Results of N-Hexane, Ethyl Acetate, and Ethanol Leaf Extracts *Graptophyllum pictum* L. Griff Var. viride

Information:

Compound Groups	Reagent	Indicator	Results					
			N-Hexane Extract	Note	Ethyl Acetate Extract	Note	Ethanol extract 96%	Note
Alkaloid	Bouchardat	Chocolate	Chocolate	+	Chocolate	+	Chocolate	+
	Dragendorf	Orange	Orange	+	Orange	+	Orange	+
Saponins	Aquadest + HCl 1N	Foam	Foam	+	Foam	+	Foam	+
Flavonoid	Concentrated HCl + Mg powder	Yellow or orange	Orange	+	Orange	+	Orange	+
Tannins and Phenols	FeCl3	green or blackish green	Blackish green	+	Blackish green	+	Blackish green	+
Triterpenoid	As . glacial acetate + H_2SO_4 P	Red	Brownish green	-	Brownish green	-	Red	+
Steroid	Chloroform + H_2SO_4 p +CH_3COOH	A brown layer that forms at the meeting of two layers	A brown layer that forms at the meeting of two layers	+	A brown layer that forms at the meeting of two layers	+	Chocolate layer which is formed at the meeting of two layers	+
Quinone	NaOH	Purplish Red	Yellow	-	Greenish Yellow	-	Greenish Yellow	-

+ : Contains compounds
- : Does not contain compounds

The results obtained from phytochemical screening of purple leaf extract were that there were secondary metabolites in the form of alkaloids, flavonoids, saponins, and steroids.

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Flavonoid Test

Table 3. Total Flavonoid Results of N-Hexane, Ethyl Acetate, and Ethanol Leaf Extracts *Graptophyllum pictum* L. Griff Var. viride

No.	Sample Name	mg quercetin equivalent/g extract
1	N-Hexane Extract	8.03±0.59
2	Ethyl acetate extract	55.52±1.00
3	Ethanol Extract 96%	25.61±0.41

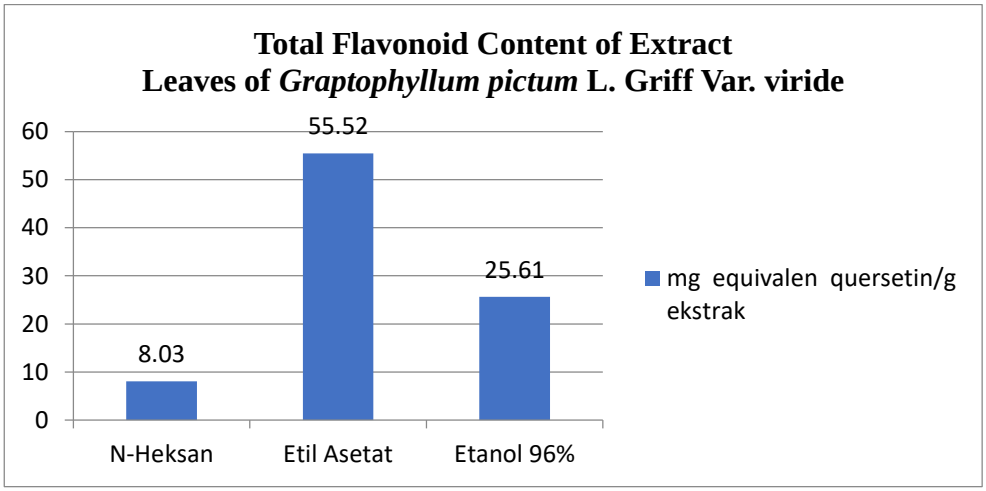

Figure 1. Graph of Total Flavonoids of N-Hexane, Ethyl Acetate, and Ethanol Extracts of Leaves *Graptophyllum pictum* L. Griff Var. viride

The highest flavonoid content was found in the ethyl acetate extract of *Graptophyllum pictum* L.Griff Var. viride leaves with a value of 55.52±1.00 mg quercetin equivalent/g extract, while the lowest flavonoid content was found in the N-Hexane extract of *Graptophyllum pictum* L.Griff Var. viride leaves with a value of 8.03±0.59 mg quercetin equivalent/g extract.

Phenolic Test

Table 4. Total Phenol Results of N-Hexane, Ethyl Acetate, and Ethanol Leaf Extracts *Graptophyllum pictum* L. Griff Var. viride

No.	Sample Name	mg GAE/g extract
1	N-Hexane Extract	19.164±0.133
2	Ethyl acetate extract	16.135±0.087
3	Ethanol Extract 96%	27,251±0,362

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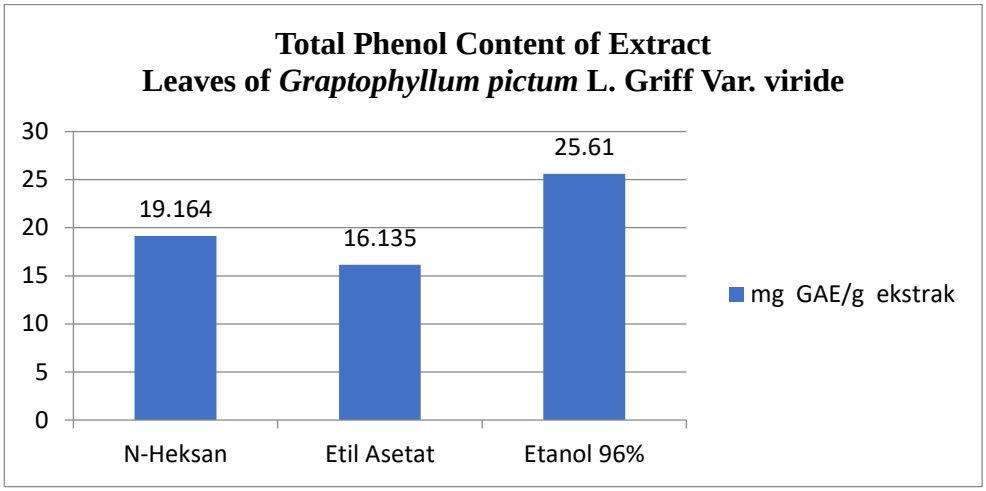

Figure 2. Graph of Total Phenol of N-Hexane, Ethyl Acetate, and Ethanol Leaf Extracts *Graptophyllum pictum* L. Griff Var. viride

Phenolic content was found in the ethanol extract of *Graptophyllum pictum* L.Griff Var. viride leaves with a value of 27.251±0.362 mg GAE/g extract, while the lowest phenolic content was found in the ethyl acetate extract of *Graptophyllum pictum* L.Griff Var. viride leaves with a value of 16.135±0.087 mg GAE/g extract.

Antioxidant Activity Test

Table 5. Results of Antioxidant Activity Tests of N-Hexane, Ethyl Acetate, and Ethanol Leaf Extracts *Graptophyllum pictum* L. Griff Var. viride

No.	Sample Name	Concentration IC50 (µg/mL)	Caption
1	N-Hexane Extract	317.70±0.46	Weak
2	Ethyl acetate extract	227.30±0.98	Weak
3	Ethanol Extract 96%	231.86±0.97	Weak

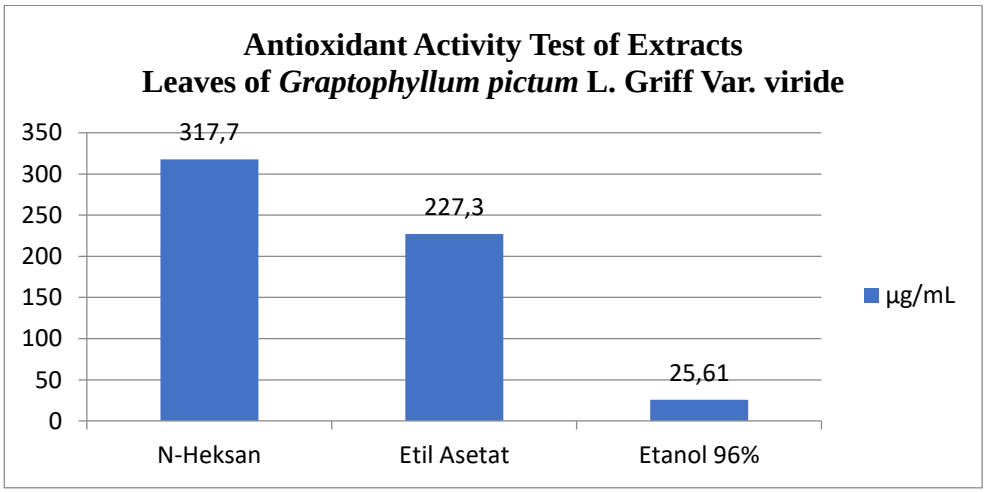

Figure 3. Graph of Antioxidant Activity Test Results of N-Hexane, Ethyl Acetate, and Ethanol Extracts of *Graptophyllum pictum* L. Griff Var. viride Leaves

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The smaller the IC50 value , the stronger the antioxidant activity. However, the larger the IC50 value , the weaker the antioxidant activity of a compound. Based on the results obtained, ethyl acetate extract has the best antioxidant activity compared to n-Hexane and ethanol extracts, which is 227.30 µg /mL.

CONCLUSION

Based on the research that has been done, it can be concluded that the highest phytochemical screening of ethyl-acetate, n-Hexane and ethanol extracts of *Graptophyllum pictum* L.Griff Var. viride leaves was found in ethyl-acetate extract with a value of 55.52 ± 1.00 mg quercetin equivalent/extract. The highest phenolic content was found in ethyl-acetate, n-Hexane, and ethanol extracts of *Graptophyllum pictum* L.Griff Var. viride leaves. Ethanol extract with a value of 27.251 ± 0.362 mg GAE / g extract. Ethyl-acetate extract *of Graptophyllum pictum* L.Griff Var. viride has the best antioxidant activity compared to n-Hexane and ethanol extracts, which is 227.30 µg / mL. Further research is needed on the content of compounds in parts of the *Graptophyllum pictum* L.Griff Var. viride plant other than the leaves.

THANK-YOU NOTE

This research was supported by research facilities, as well as scientific and technical support from the Serpong Advanced Characterization Laboratory and the Chemical Analysis Services Laboratory at the National Research and Innovation Agency.

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