Jurnal Farmasi Indonesia: Volume 18 Nomor 1 (2026)
https://doi.org/10.35617/jfionline.v18i1.480

Antioxidant Activity of Green and Purple Kale Leaves *(Brassica oleracea* L.) Ethanol Extract

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Artikel Penelitian

Abstract: *Kale (Brassica oleracea L.) is a plant from the Brassicaceae family that is often consumed by the public. Kale leaves contain vitamin C, phenolic compounds, and pigments that have the potential as antioxidants. Kale leaves that are often consumed by the public are green kale and purple kale. This study aims to determine the comparison of antioxidants between green kale and purple kale using the DPPH method. Green and purple kale leaf Simplicial powder was macerated using 96% ethanol solvent. The extraction results were then tested qualitatively for their phytochemical content using phytochemical screening. Antioxidant activity was tested qualitatively and quantitatively (DPPH method). The results showed that the antioxidant activity (IC_{50}) of Vitamin C, purple kale, and green kale leaves extract successively are 3.19 µg/ml (very strong), 100.03 µg/ml (strong), and 144,35 µg/ml (moderate). The antioxidant activity of purple kale leaves is stronger than green kale. leaves.*

Keywords: *antioxidant, DPPH, green kale, purple kale*

Abstrak: Kale (*Brassica oleracea* L.) merupakan tanaman. dari famili Brassicaceae yang sering dikonsumsi oleh masyarakat. Daun kale mengandung vitamin C, senyawa fenolik, dan pigmen yang berpotensi sebagai antioksidan. Daun kale yang sering dikonsumsi masyarakat adalah kale hijau dan kale ungu. Penelitian.. ini bertujuan.. untuk mengetahui perbandingan antioksidan antara kale hijau dan kale ungu dengan metode... DPPH. Serbuk simplisia daun kale hijau dan ungu dimaserasi menggunakan pelarut etanol 96%. Hasil ekstraksi kemudian diuji kandungan fitokimianya secara kualitatif menggunakan skrining fitokimia. Aktivitas.. antioksidan. diuji secara kualitatif dan kuantitatif menggunakan metode. DPPH. Hasil menunjukkan bahwa aktivitas antioksidan yang dilihat dari nilai IC_{50} dari Vitamin C, ekstrak. etanol kale ungu, dan kale hijau. Berturut-turut adalah 3.19, µg/ml (antioksidan sangat kuat), 100.03 µg/ml (antioksidan kuat), dan 144.35 µg/ml (antioksidan sedang). Aktivitas antioksidan daun Kale ungu yang lebih kuat dibandingkan dengan daun Kale hijau.

Kata kunci: antioksidan, DPPH, kale hijau, kale ungu

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https://doi.org/10.35617/jfionline.v18i1.480

Introduction

The development of modern technology and science has caused changes in people's lifestyles that have a negative impact on health. Improper diet, lack of exercise, lack of rest, smoking, and alcohol consumption. Deteriorating environmental conditions, such as heavy pollution, can affect people's quality of life (1). The evolution of lifestyle in everyday life can trigger the emergence of various degenerative diseases (2). Based on WHO data, non-communicable diseases (NCDs) or degenerative diseases cause 73% of deaths and 60% of all illnesses worldwide. Degenerative diseases that occur can be caused by high concentrations of free radicals in the body (3).

Free radicals are unstable and reactive atoms or molecules because they consist of one or more unpaired electrons in their outermost layer. These radicals can trigger chain reactions in the body that can cause continuous and ongoing damage that can cause various health problems, such as respiratory tract, lung, and heart infections, as well as the development of very dangerous cancers (4). An imbalance in the number of free radicals that exceeds the body's ability to neutralize them causes oxidative stress (5). Radical-induced oxidative stress can affect the development of various degenerative diseases, such as cancer (6), (7), and ischemia (8).

Oxidative stress can be prevented by increasing the body's antioxidant status. Insufficient antioxidant levels in the body require additional external antioxidants that can protect against free radical attacks (5). Antioxidants are compounds needed to ward off free radicals and prevent cells from damage. Antioxidants will provide one electron to free electrons and free radical compounds so that they become stable. Antioxidants will then protect body cells from oxidative damage by free radicals and ROS (9). Antioxidants can be divided into three groups: primary antioxidants (such as superoxide dismutase, catalase, and glutathione peroxidase), secondary antioxidants (such as vitamin C, vitamin E, carotenoids, and flavonoids), and tertiary antioxidants methionine sulfoxide reductase (10).

Kale (*Brassica oleracea* L.) is a plant that is widely distributed in Indonesia, including Bogor, Bandung, and Malang. Kale contains antioxidant compounds such as flavonoids, beta-carotene, quercetin, glucosinolates, carotenoids, vitamin C, and anthocyanins. Based on the FRAP assay, Kale leaves have an antioxidant activity = 29.35 ± 2.00 µmol Fe2+ g−1 DW due to their total polyphenol content (11). Long-term dietary administration (90 days in Rats) of Kale leaves increased antioxidant status based on Trolox equivalents antioxidant capacity (TEAC) and had no side effects on blood biochemical markers (12). Based on the DPPH method, the IC50 of the Kale market extract (99.397µg/ml) is greater than that of hydroponic kale leaves (142.184 µg/ml) (13). The antioxidant effect also causes protection from liver damage induced by paracetamol (14). The protective function of water spinach leaves occurs in several organs and other biological activities, such as anticancer, cardiovascular, and digestive protection (15).

Kale leaves have various colours such as white, yellow, purple, green, red, and grey. The colour of kale leaves is influenced by the intensity of 3 colour pigments, namely chlorophyll, carotenoids, and anthocyanins (16). Green kale leaves and purple kale are two types of kale leaves that are commonly found in Indonesia. In previous studies, purple kale has been observed, but green kale has not been fully elucidated. Based on the background above, this study aims to compare the antioxidant activity of green and purple kale leaf extracts using the DPPH method.

Materials and Methods

Materials

This study used materials, namely kale leaf samples obtained from the Bumiaji Agrotourism Plantation, Batu City, Malang, East Java, Indonesia, 96% ethanol (p.a, Merck, Germany), 2% HCl (Nitra Kimia Indonesia), Dragendroff's reagent (Merck, Germany), Meyer's reagent (Nitra Kimia Indonesia), 1% $FeCl_3$ (Merck, Germany) $CHCl_3$ (Merck, Germany), silica gel (Merck, Germany), aluminium plate (Local), ethyl acetate (Merck, Germany), n-hexane (Merck Germany), TLC plate (Merck Germany), DPPH powder (sigma-Aldrich), and vitamin C (Nutrimax C max) comparison

solution. This study used several laboratory tools, namely maceration tubes, glassware, microscopes, rotary evaporators, capillary tubes, dropper pipettes, volume pipettes, and UV-Visible spectrophotometers (Genesys 10S, Thermo Scientific).

Methods

Green and purple kale leaves (*Brassica oleracea* L.) were obtained from Bumiaji Agrotourism Plantation, Batu City, Malang, East Java, and determined at the UPT Laboratory Herbal Materia Medika, Batu City, Malang (Determination Number: 074/158/102.20-A/2023).

Plant Preparation

The plants used as samples in this study were green and purple kale plants, whose leaves were taken. Green and purple kale leaves were dried by air drying and ground into powder. Leaves and simplex were observed organoleptically, macroscopically, and microscopically. Green and purple kale leaf powder was macerated using 96% ethanol (1:5) and macerated up to 3 times (13) with modification. The macerate was filtered, and the macerate results were concentrated using a rotary evaporator.

Phytochemical Screening

Alkaloid Test

Green and purple kale leaf extract (1 ml) was added with 2% HCl, and the solution was divided into two tubes. Tube 1 contained 2-3 drops of Dragendorff's reagent, and tube 2 contained Meyer reagent. A positive alkaloid result occurs when Dragendorff's reagent forms a brick red, red, or orange precipitate, while Meyer's reagent forms a white or yellowish precipitate (17).

Flavonoid Test

Green and purple kale leaf extract (1 ml) was given 10 drops of HCl. Positive results from flavonoids are indicated by the formation of orange, pink, or red colours (17).

Tannin Test

Green and purple kale leaf extract (1 ml) was added to 20 ml of water, boiled, filtered, and 10 drops of 1% $FeCl_3$ were added. The greenish brown extract contains tannins (17).

Polyphenol Test

Green kale and purple kale leaf extracts (1 ml) were reacted with 1% $FeCl_3$. Positive results are the formation of green, red, purple, dark blue, blue, black-blue, or black-green (17).

Saponin Test

Green and purple kale leaf extract (0.5 mg) was added to 0.5 ml of hot water, shaken vigorously for 10 seconds until foam formed, then 1.5 N HCl was added, and the mixture was allowed to stand for 10 minutes; if the foam did not disappear, the extract was positive for Saponin (17).

Triterpenoid Test

$CHCl_3$ was added to the green kale and purple kale leaf extracts, then three drops of Lieberman-Burchard reagent were added. A positive reaction was indicated by the formation of magenta color (17).

Thin Layer Chromatography (TLC) Analysis

Green and purple leaf extracts were dissolved sufficiently in ethanol. This TLC analysis used silica gel on an aluminium plate as the stationary phase and n-hexane: ethyl acetate (7:3) as the mobile phase. The extract was spotted on a thin layer chromatography (TLC) plate and eluted to the boundary mark. After elution, the TLC plate was removed, dried, and sprayed with (18). DPPH solution. The spots on the TLC plate with antioxidant activity turned yellow (18).

Antioxidant Activity Test

Antioxidant activity is calculated based on the inhibition of DPPH radical absorption on a UV-Vis spectrophotometer at a maximum wavelength of 517 nm. The results of the absorption measurements are then used to calculate the percentage of inhibition. The percentage of inhibition is the percentage that indicates free radical activity (19).

Data Analysis

The data from the antioxidant activity test were analyzed descriptively, qualitatively, and

quantitatively. Qualitative data were obtained from the results of TLC observations, while quantitative data were obtained from the calculation of the percentage (%) of inhibition and the determination of IC_{50}. The percent DPPH inhibition was calculated by the equation % inhibition = (A0 – A1)/A0 x 100, where A0 was the absorbance of the control and A1 was the absorbance of the reaction mixture.

The percentage of inhibition that has been obtained is then searched for the IC_{50} value, which is the concentration of the sample solution that can inhibit 50% of DPPH radicals. The IC_{50} value is obtained from the correlation between the percentage of inhibition and the concentration of the extract plotted in the regression equation: y = ax + b. The IC_{50} value can be calculated by the equation (Sumardi et al., 2024): $IC_{50} = (50\text{-}a)/b$, where Y was % inhibition (50), *a* was the intercept, *b* was the slope, and x was the sample concentration.

A sample is categorized as a very strong antioxidant if the IC_{50} value is <50 µg/ml, a strong antioxidant if the IC_{50} value is between 50-100 µg/ml, a moderate antioxidant if the IC_{50} value is between 101-250 µg/ml, a weak antioxidant if the IC_{50} value is between 251-500 µg/ml, and inactive as an antioxidant if the IC_{50} value is >500 µg/ml (20).

Result and Discussion

The results of organoleptic, macroscopic, and microscopic observations of the simplex, and the yield of green kale and purple kale leaf extracts are shown in **Table 1** and **Figure 1**.

Table 1. Organoleptic, Microscopic, and Microscopic Observation of Green and Purple Kale (*Brassica oleracea* L.) Leaves

Sample	Green Kale Leaves	Purple Kale Leaves
Organoleptic Observation	The edges of the leaves are serrated with leaf curves that resemble lettuce leaves, the leaves are green, the smell is aromatic, and the taste is slightly sweet	The edges of the leaves are serrated with leaf curves that resemble lettuce leaves, the leaves are purple in color, the smell is aromatic, the taste is slightly sweet
Water Content	1,16%	1,12%
% Extract Yield	16,7%	19,16%

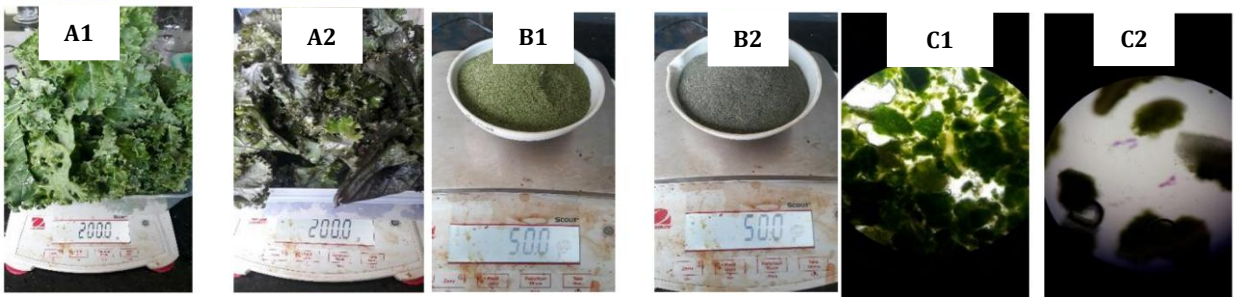

Figure 1. Physical and Microscopic Observation of Kale: A1. Green Kale Leaves, A2. Purple Kale Leaves, B1. Green Kale Leaves Powder, B2. Purple Kale Leaves Powder, C. Microscopic Observation of Green Kale Leaves (C1) and Purple Kale Leaves (C2) (P: 4 x 10).

Antioxidant activity testing of green and purple kale (*Brassica oleracea* L.) leaves was conducted to determine the potential of antioxidants in the plant in counteracting free radicals and how the antioxidant activity of both is compared with the characteristics of different pigment content between green kale and purple kale. The determination results obtained at the UPT. Herbal Materia Medica Laboratory showed that the plant is a kale (*Brassica oleracea* L.) plant

https://doi.org/10.35617/jfionline.v18i1.480

from the Sabellica variety with a morphology in the form of single leaves and rosettes, with a fairly large size in green and purple colors.

Based on the results of organoleptic, macroscopic, and microscopic observations, green and purple kale leaves have the same leaf shape, with distinctive aromatics and different pigments. Green kale leaf powder contains bright green pigments, while purple kale leaf powder is purple. Green kale contains chlorophyll pigments, while purple kale contains anthocyanins. Previous studies have shown that white kale and purple kale contain less chlorophyll than green leaves, and the anthocyanin content is highest in purple leaves. The difference in color is determined by differences in the regulation of DEG genes that determine chlorophyll and anthocyanin biosynthesis (16).

To ensure the quality of the resulting herbal medicine and extract, several parameters are used, such as the water content of the herbal medicine and the percentage of extract yield. Based on the requirements of the Indonesian Pharmacopoeia, the water content of good herbal medicine is less than 10% to prevent the herbal medicine from the growth of fungi and bacteria that can reduce the stability of active compounds in the extract (21).

The results showed that the water content of green kale leaf herbal medicine powder was 1.16% while the purple kale leaf herbal medicine powder was 1.12%. These results indicate that the water content of both herbal medicine powders has met the requirements of the Indonesian Pharmacopoeia. The kale leaf herbal medicine was dried by air drying. Although the air-drying method produces a higher water content of herbal medicine than the oven, the air-drying method is the most effective method for maintaining total phenol and antioxidant capacity (22).

Based on the percentage yield analysis, it was found that the green and purple kale leaf extracts obtained in this study met the Indonesian Pharmacopoeia standards of not less than 10% (>10%) (21). The yield values of green and purple kale leaf extracts were 16.7% and 19.16%, respectively. Yield is the ratio of the weight of the extract obtained from the extraction results to the weight of the raw materials used. The percentage yield indicates the amount of bioactive content obtained in the extraction process.

The amount of yield is influenced by several factors such as the extraction method, length of time, and solvent used. Extraction of green and purple kale leaves using 96% ethanol solvent. Ethanol is a polar solvent and can be used in preliminary extraction. Ethanol is able to penetrate cell wall materials so that it can carry out cell diffusion and attract bioactive compounds quickly (17). The maceration extraction method with ethanol solvent produces high phenolic content and antioxidant capacity (23) (24).

Based on the phytochemical screening shown in Table 2, it is known that green kale and purple kale leaves contain alkaloids, flavonoids, tannins, polyphenols, saponins, and triterpenoids.

Table 2. Phytochemical Screening Results of Ethanol Extracts of Green and Purple Kale (*Brassica oleracea* L.) Leaves

Class of Compound	Phetochemical Screening Result		Visible Characteristic
	Green Kale Leaves	Purple Kale Leaves	
Alkaloid	+	+	A brick red precipitate
Flavonoid	+	+	A yellow solution
Tanin	+	+	A greenish brown solution
Polyphenol	+	+	A blackish green solution
Saponin	+	+	The presence of stable foam
Triterpenoid	+	+	The presence of a purple-red solution

https://doi.org/10.35617/jfionline.v18i1.480

Based on phytochemical screening, the ethanol extract contains alkaloids, flavonoids, terpenoids, glycosides, steroids, carbohydrates, and phenolic compounds.

Antioxidant Activity

Antioxidant testing in this study was carried out qualitatively using TLC and quantitatively using a UV-Vis Spectrophotometer using DPPH. The results of the qualitative antioxidant test were shown by the results of the TLC separation under UV light shown in **Figure 2**.

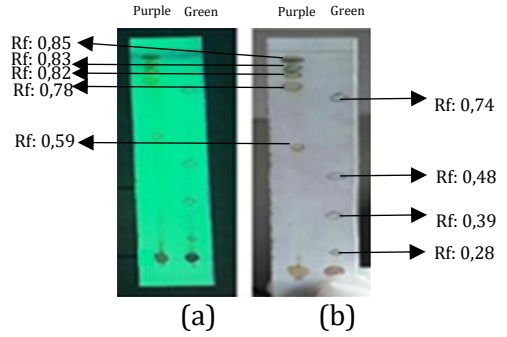

Figure 2. Results of Thin Layer Chromatography Separation under UV Light 475 nm: (a) TLC results of Green and Purple Kale Leaf Ethanol Extract before DPPH spraying, and (b) TLC results after DPPH spraying.

DPPH is a stable free radical with a characteristic purple colour in solution due to the presence of an unpaired electron. The separated compounds on the TLC plate are then exposed to a DPPH solution (sprayed or dipped) (25). Based on the results of qualitative antioxidant tests using TLC, the presence of yellow spots after spraying DPPH under UV light indicates the antioxidant activity of green and purple kale leaves. Yellow spots are produced as a reaction between DPPH molecules and compounds that can donate hydrogen atoms in green and purple kale leaf extracts, resulting in DPPH molecules being reduced to form yellow DPPH-H (26). Rf value of purple kale consists of 0.59, 0.78, 0.82, 0.83, and 0.85. Rf value of green kale consists of 0.28, 0.39, 0.48, and 0.74.

Besides the qualitative method, the antioxidant activity test was also compared using a quantitative method using Spectrophotometry analysis. The comparison of the results of the antioxidant activity test of green and purple kale leaves quantitatively is shown in **Table 3**, **Figures 3** and **4**.

Table 3. Results of Antioxidant Activity Test of Green and Purple Kale Leaves (*Brassica oleracea* L.)

Vitamin C		
Concentration (µg/ml)	Mean of Absorbance ± SD	% Inhibition
2	0,111 ± 0,007	25
4	0,047 ± 0,009	68,24
6	0,023 ± 0,003	84,46
8	0,020 ± 0,118	86,49
10	0,014 ± 0,005	90,54
Regression Equation y = 7,4665x + 26,147		
R^2 = 0,962		
IC_{50} = 3,19		
Antioxidant activity: Very Strong		
Ethanol Extract of Green Kale Leaves		
Concentration (µg/ml)	Mean of Absorbance ± SD	% Inhibition
10	0,233 ± 0,021	2,10
20	0,212 ± 0,005	10,93
40	0,197 ± 0,007	18,91
80	0,158 ± 0,006	22,61
160	0,113 ± 0,002	52,52
Regression Equation: y = 0,3204x + 3,7488		
R^2 = 0,966		
IC_{50} = 144,35 (Moderate)		

Ethanol Extract of Purple Kale Leaves		
Concentration (µg/ml)	Mean of Absorbance ± SD	% Inhibition
10	0,174 ± 0,078	10,69
20	0,165 ± 0,043	22,90
40	0,148 ± 0,012	30,84
80	0,118 ± 0,006	44,86
160	0,066 ± 0,001	69,16
Regression Equation: y = 0,3342x + 16,569		
R^2 = 0,9966		
IC_{50} = 100,03		
Antioxidant activity: Strong		

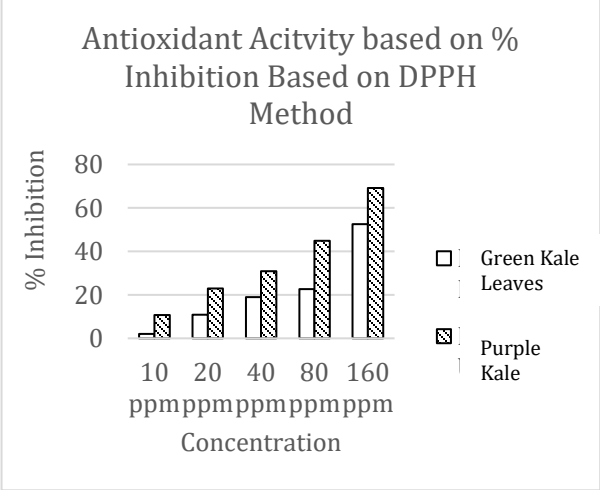

Figure 3. Graph of Antioxidant Activity of Ethanol Extract of Green Kale and Purple Kale Leaves Based on Inhibition Percentage Using the DPPH Method.

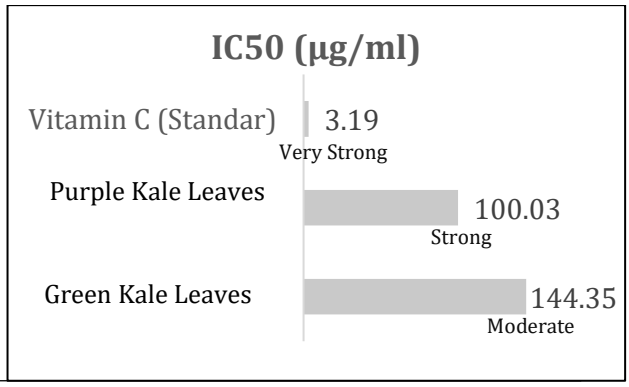

Figure 4. IC_{50} Value Graph

The results obtained from measuring antioxidant activity are in the form of absorbance values. The higher the concentration, the lower the absorbance. The percentage of inhibition (% Inhibition) is calculated based on the absorbance value obtained. The percentage of inhibition is the ability of the sample to inhibit radical activity. This study shows that the greater the concentration of green and purple kale leaf extract, the greater the percentage of inhibition or inhibitory effect on free radical activity. This is shown by the linear regression equation for both kale leaf samples. This study shows that the higher the concentration of ethanol extract of green and purple kale leaves, the lower the DPPH levels detected through the absorbance value, which indicates higher antioxidant activity (27).

Based on the observation of the IC_{50} value and antioxidant category (20), it is known that the highest antioxidant activity is shown by the standard, namely Vitamin C (IC_{50} = 3.19 µg/ml), which shows very strong antioxidant activity. Comparison of the IC_{50} values of green and purple kale leaf extracts shows that purple kale leaves have higher antioxidant activity. (IC_{50} = 100.03 µg/ml) with a strong antioxidant category compared to green kale leaves (IC_{50} = 144.35 µg/ml), with a moderate antioxidant category.

In this research, Vitamin C acted as a standard with the highest antioxidant capacity compared to others. Vitamin C (L-ascorbic acid or ascorbate) is a cofactor that participates in many biochemical processes (28). Vitamin C plays an important role in protecting against oxidative stress in various tissues (29). Oxidative stress refers to conditions of imbalance between the production of reactive oxygen species (ROS) and antioxidant defence (30). The antioxidant mechanisms of Vitamin C are based on hydrogen atom donation to lipid radicals, quenching of singlet oxygen, and removal of molecular oxygen (31). Kale has a

Vitamin C content equivalent to 112,1 mg in 100 g fresh leaves (32). Information regarding the vitamin C content between purple and green kale is still limited.

Besides vitamin C, kale also contains some active metabolites that have antioxidant activity, such as phenolic content (such as gallic, protocatechuic, p-hydroxybenzoic), quercetin, and some pigments (such as anthocyanin, carotene, chlorophyll, and lutein) (33). The most striking difference between purple and green kale leaves is the pigment or the color of the leaves.

Leaf color not only shows the characteristics of the plant but is also related to the pigment content and potential of it, including its antioxidant properties. Purple kale leaves have a main pigment content, namely anthocyanin (purple pigment), reaching 1.73 mg/g (34). Green kale leaves have a main pigment content, namely chlorophyll (green pigment), up to 145 mg/g (35). Based on previous studies, it is known that various magenta, blue, and red fruits and vegetables that are rich in anthocyanins are included in the category of foods with high antioxidants with the potential to contribute more than 20% of the daily antioxidant intake needed, while green vegetables rich in chlorophyll are included in foods with low antioxidants (36). This may be one of the factors that causes the antioxidant activity of purple kale to be better than that of green kale.

The antioxidant activity of anthocyanins can be caused by two mechanisms. The first mechanism, anthocyanins, plays a role in Hydrogen Atom Transfer and single-electron transfer. In the HAT mechanism, free radicals remove hydrogen atoms from antioxidants. In this reaction, free radicals are converted into more stable products. In the SET mechanism, anthocyanins donate electrons to free radicals, which also form more stable products. These two mechanisms can occur simultaneously (37).

This research showed that the antioxidant activity of purple kale (strong) is better than that of green kale leaves extract (moderate) based on IC50 values in the DPPH method. This study has not yet explained the active compound content in purple and green kale, but the Rf value obtained from TLC may be a reference. However, the differences in antioxidant activity between purple and green kale leaves extract need to be explored further, such as the pigment or active metabolite implicated in antioxidant activity, in the next study.

Conclusion

DPPH method showed that the antioxidant activity (IC_{50}) of Vitamin C, purple kale, and green kale leaves extract successively are 3.19 µg/ml, 100.03 µg/ml, and 144,35 µg/ml. Ethanol extract of purple kale leaves has better antioxidant activity (strong antioxidant) compared to green kale leaves (moderate antioxidant).

Acknowledgment

The researcher would like to thank the Faculty of Health Sciences, Universitas Anwar Medika, for the facilities provided in the Organic Chemistry Laboratory and Instrumentation Laboratory.

Conflict of Interest

There is no conflict of interest

References

1. Arnanda QP, Nuwarda RF. Penggunaan Radiofarmaka Teknisium-99M Dari Senyawa Glutation dan Senyawa Flavonoid Sebagai Deteksi Dini Radikal Bebas Pemicu Kanker. Farmaka Suplemen [Internet]. 2019;14(1):1–15. Available from: https://doi.org/10.24198/jf.v17i2.22071.
2. Wijaya DP, Paendong JE, Abidjulu J. Skrining Fitokimia dan Uji Aktivitas Antioksidan dari Daun Nasi (Phrynium capitatum) dengan Metode DPPH (1,1-difenil-2-pikrilhidrazil). J MIPA. 2014;3(1):11. Available from: https://doi.org/10.35799/jm.3.1.2014.3899
3. WHO. World health statistics 2023: monitoring health for the sdgs, sustainable development goals. Milbank Mem Fund Q [Internet]. 2023;27(2):136. Available from: https://www.who.int/publications/book-orders.
4. Abidin J, Hasibuan FA. Pengaruh dampak pencemaran udara terhadap kesehatan

untuk menambah pemahaman masyarakat awam tentang bahaya dari polusi udara. Pros Semin Nas Fis Univ Riau IV. 2019;5(4):1–7. Available from: https://jurnal.peneliti.net/index.php/JIWP/article/view/9583
5. Nurkhasanah MA, Si A, Mochammad S, Bachri S, Si M, Si DS, et al. Antioksidan dan Stres Oksidatif. 2023. 1–3 p.
6. Saha SK, Lee S Bin, Won J, Choi HY, Kim K, Yang GM, et al. Correlation between oxidative stress, nutrition, and cancer initiation. Int J Mol Sci. 2017;18(7). Available from: doi: 10.3390/ijms18071544.
7. Caturano A, D'Angelo M, Mormone A, Russo V, Mollica MP, Salvatore T, et al. Oxidative Stress in Type 2 Diabetes: Impacts from Pathogenesis to Lifestyle Modifications. Curr Issues Mol Biol. 2023;45(8):6651–66. Available from: doi: 10.3390/cimb45080420.
8. Kibel A, Lukinac AM, Dambic V, Juric I, Relatic KS. Oxidative Stress in Ischemic Heart Disease. Oxid Med Cell Longev. 2020;2020. Available from: doi: 10.1155/2020/6627144
9. Wibawa JC, Wati LH, Arifin MZ. Mekanisme Vitamin C Menurunkan Stres Oksidatif Setelah Aktivitas Fisik. JOSSAE J Sport Sci Educ. 2020;5(1):57. Available from: http://dx.doi.org/10.26740/jossae.v5n1.p57-63
10. Salmiyah S. BA. 50 10,94. Hospital Majapahit. 2018;10(1):43–50.
11. Lučić D, Pavlović I, Brkljačić L, Bogdanović S, Farkaš V, Cedilak A, et al. Antioxidant and Antiproliferative Activities of Kale (Brassica oleracea L. Var. acephala DC.) and Wild Cabbage (Brassica incana Ten.) Polyphenolic Extracts. Molecules. 2023;28(4). Available from: doi: 10.3390/molecules28041840.
12. Papierska K, Ignatowicz E, Jodynis-Liebert J, Kujawska M, Biegańska-Marecik R. Effects of Long-Term Dietary Administration of Kale (Brassica oleracea L. var. acephala DC) Leaves on the Antioxidant Status and Blood Biochemical Markers in Rats. Polish J Food Nutr Sci. 2022;72(3):239–47. Available from: https://doi.org/10.31883/pjfns/152434
13. Diana Eka Handayani, Rini Yanuarti, S.Pi., M.Si., Apt. Febri Hidayat, S.Si., M.B.A. Uji Aktivitas Antioksidan Ekstrak Etanol 96% Dari Sayuran Kale (*Brassica olerace* L.) Pasar Dan Hidroponik Dengan Metode DPPH (1,1-diphenyl-2-picrylhydrazi). ISTA Online Technol J. 2023;4(1):01–12. Available from: https://doi.org/10.62702/ion.v4i1.68.
14. Hashem F, Motawea H, Shaker K, El-Shabrawy AR, El-Sherbini S, Farrag AR. Hepatoprotective activity of Brassica oleracea L. var . Italica. Egypt Pharm J. 2013;12(2):177. Available from: DOI: 10.1055/s-0033-1352132
15. Šamec D, Urlić B, Salopek-sondi B. superfood : Review of the scientific evidence behind the statement. Crit Rev Food Sci Nutr [Internet]. 2019;0(0):1–12. Available from: https://doi.org/10.1080/10408398.2018.1454400
16. Liu X, Zhang B, Wu J, Li Z, Han F, Fang Z, et al. Pigment variation and transcriptional response of the pigment synthesis pathway in the S2309 triple-color ornamental kale (Brassica oleracea L. var. acephala) line. Genomics [Internet]. 2020;112(3):2658–65. Available from: https://doi.org/10.1016/j.ygeno.2020.02.019
17. Harborne JB. Phytochemical Dictionary. A Handbook of Bioactive Compounds from Plants Third Edition. Biochem Syst Ecol. 1998;21(8):849.
18. Ali R, Sajjad N, Qureshi S, Hassan S, Ahmad M, Ganie SA, et al. Evaluation of Antioxidant and Immunomodulatory Potential of Methanolic Extract of Juniperus squamata Buch.-Ham. ex D.Don. Free Radicals Antioxidants. 2018;8(2):111–9. Available from: DOI: 10.5530/fra.2018.2.17.
19. Handayany GN, Umar I, Ismail I. Formulasi Dan Uji Efektivitas Antioksidan Krim Ekstrak Etanol Daun Botto'-Botto' (Chromolaena odorata L.) dengan METODE DPPH. J Kesehat.

2018;11(2):86. Available from: DOI : 10.24252/kesehatan.v11i2.5944.
20. Faustino H, Gil N, Baptista C, Duarte AP. Antioxidant activity of lignin phenolic compounds extracted from kraft and sulphite black liquors. Molecules. 2010;15(12):9308–22. Available from: doi: 10.3390/molecules15129308.
21. Kemenkes RI. Farmakope Indonesia Edisi VI. Departemen Kesehatan Republik Indonesia. 2020.
22. Dharma MA, Nocianitri KA, Yusasrini NLA. Pengaruh Metode Pengeringan Simplisia Terhadap Kapasitas Antioksidan Wedang Uwuh. J Ilmu dan Teknol Pangan. 2020;9(1):88.
23. Yulianti W, Ayuningtyas G, Martini R, Resmeiliana I. Pengaruh Metode Ekstraksi Dan Polaritas Pelarut Terhadap Kadar Fenolik Total Daun Kersen (*Muntingia Calabura L*). J Sains Terap. 2021;10(2):41–9. Available from: https://doi.org/10.29244/jstsv.10.2.41-49
24. Yolanda Simamora AC, Yusasrini NLA, Kencana Putra IN. Pengaruh Jenis Pelarut Terhadap Aktivitas Antioksidan Ekstrak Daun Tenggulun (*Protium javanicum* Burm. F) Menggunakan Metode Maserasi. J Ilmu dan Teknol Pangan. 2021;10(4):681.
25. Kedare SB, Singh RP. Genesis and development of DPPH method of antioxidant assay. J Food Sci Technol. 2011;48(4):412–22. Available from: doi: 10.1007/s13197-011-0251-1.
26. Piszcz P, Tomaszewska M, Głód BK. Estimation of the total antioxidant potential in the meat samples using thin-layer chromatography. Open Chem. 2020;18(1):50–7. Available from: DOI: 10.1515/chem-2020-0007
27. Christodoulou MC, Orellana Palacios JC, Hesami G, Jafarzadeh S, Lorenzo JM, Domínguez R, et al. Spectrophotometric Methods for Measurement of Antioxidant Activity in Food and Pharmaceuticals. Antioxidants. 2022;11(11). Available from: https://doi.org/10.3390/antiox11112213.
28. Traber MG, Stevens JF. Vitamins C and E: Beneficial effects from a mechanistic perspective. Free Radic Biol Med. 2011;51(5):1000–13. Available from: doi: 10.1016/j.freeradbiomed.2011.05.017
29. Akbari A, Jelodar G, Nazifi S, Sajedianfard J. An Overview of the Characteristics and Function of Vitamin C in Various Tissues: Relying on its Antioxidant Function. Zahedan J Res Med Sci. 2016;In Press(In Press). Available from: https://doi.org/10.17795/zjrms-4037
30. Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, et al. Oxidative Stress: Harms and Benefits for Human Health. Oxid Med Cell Longev. 2017;2017. Available from: doi: 10.1155/2017/8416763.
31. Agwu E, Ezihe C, Kaigama G. Antioxidant Roles/Functions of Ascorbic Acid (Vitamin C). Ascorbic Acid - Biochem Funct. 2023;(Vitamin C):1–17. Available from: DOI: 10.5772/intechopen.110589.
32. Korus A, Słupski J, Gebczyński P, Banaś A. Effect of preliminary processing and method of preservation on the content of glucosinolates in kale (*Brassica oleracea* L. var. acephala) leaves. Lwt. 2014;59(2P1):1003–8. Available from: https://doi.org/10.1016/j.lwt.2014.06.030.
33. Ortega-Hernández E, Antunes-Ricardo M, Jacobo-Velázquez DA. Improving the health-benefits of kales (Brassica oleracea l. var. acephala dc) through the application of controlled abiotic stresses: A review. Plants. 2021;10(12). Available from: https://doi.org/10.3390/plants10122629
34. Zhang B, Hu Z, Zhang Y, Li Y, Zhou S, Chen G. A putative functional MYB transcription factor induced by low temperature regulates anthocyanin biosynthesis in purple kale (Brassica Oleracea var. acephala f. tricolor). Plant Cell Rep. 2012;31(2):281–9. Available from: doi: 10.1007/s00299-011-1162-3
35. Korus, A and Kmiecik, W. Content of

Carotenoids and Chlorophyll Pigments in Kale *(Brassica oleracea* L. var. Acephala) Depending on the Cultivar and the Harvest Date. EJPAU Food Science and Technology. 2007;10 (1): 1–8.

36. Cömert ED, Mogol BA, Gökmen V. Relationship between color and antioxidant capacity of fruits and vegetables. Curr Res Food Sci. 2020;2:1–10. Available from: https://doi.org/10.1016/j.crfs.2019.11.001.
37. Sadowska-Bartosz I, Bartosz G. Antioxidant Activity of Anthocyanins and Anthocyanidins: A Critical Review. Int J Mol Sci. 2024;25(22). Available from: https://doi.org/10.3390/ijms252212001.