Non-Specific Parameters Standardization and Antioxidant Activity of Red Paprika (Capsicum annuum L.) Fruit

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Abstract. Red paprika (Capsicum annuum L.) contains various of carotenoids, β-carotene, polyphenols, and vitamine as an antioxidant. This research aims to determine non-specific parameters standardization of red paprika fruit methanolic extract and antioxidant activity of red paprika fruit ether fraction. Red paprika fruit was extracted by maceration using methanol. The crude extract extract was further fractionated using ether. The red paprika fruit methanol extract was analyzed non-specific parameters such as water content, ash content, acid insoluble ash content and its organoleptic. The red paprika fruit ether fraction was analyzed an antioxidant activity by 2,2–diphenyl–1–picrylhydrazyl (DPPH) free radical scavenging assay. This method was based on measurement of absorbance from DPPH residue using a UV-Vis spectrophotometer at wavelength of 517 nm. The antioxidant activity was expressed as IC_{50} value. The results of non-specific parameter standardization from red paprika methanolic extract included the water content, an ash content and an acid-insoluble ash content were obtained at 4.32 ± 0.58 % v/w, 26.73 ± 0.10 % w/w, and 11.91 ± 0.37 % w/w, respectively. An organoleptics of the red paprika methanolic extract were found viscous and red-brown color. The antioxidant activity of red paprika fruit ether fraction and β-carotene were 48.50 ± 2.19 and 40.22 ± 2.00 µg/mL, respectively. The antioxidant activity power of the red paprika (Capsicum annuum L.) fruit ether fraction was significantly more potent (IC_{50} 16.53 ± 0.16 µg/mL) than β-carotene (IC_{50} 20.32 ± 0.63 µg/mL).

Key words: Antioxidant, red paprika, Capsicum annuum, DPPH, ether fraction

INTRODUCTION

Free radical namely reactive oxygen species (ROS) can arise normally during metabolism. ROS such as superoxide ($O_2^{\bullet}$), hydroxyl (OH) and hydorgen peroxide (H_2O_2) can damage the essential protein, DNA and lipid. These effect cause various human deseases, include atherosclerosis, liver injury, cancer, cardiovascular deseases, rheumatism and neurodegenerative disorders (Selvakumar *et al*., 2011). Antioxidant is a substant that inhibits reaction promoted by ROS (Huang *et al*., 2005). Antioxidants can stabilize or deactivate free radicals before attact cells of the body (Joseph *et al*., 2009).

There was an interested to development of natural antioxidant from plant material. Red paprika (*Capsicum annuum L*.) fruit which derived from Solanaceae is a source of natural antioxidant. Red paprika (*Capsicum annuum L*.) (Figure 1) is known as sweet bell pepper (Hassan *et al.*, 2019). Red paprika has many chemical compounds that can be useful for the survival of living things. The chemical compounds contained in the red paprika such as poliphenol: kaempferol O-pentosyldihexoside and feruloyl glucoside (Park *et al.*, 2012). One of the most important flavonoids in pepper is myricetin, which is confirmed by the role of structure in determining antioxidant potential (Imran *et al.*, 2018). Red pepper contains oxygenated carotenoids that impart red color, such as capsanthin (Figure 2A), capsorubin (Figure 2B) and cryptocapsin (Figure 2C), which have potent antioxidant properties (Imran *et al.*, 2018). β-carotene (Figure 2D), β-cryptoxanthin, violaxanthin and zeaxanthin contained in red paprika are the sources of the yellow-orange color (Arimboor *et al.*, 2015). The presence of these compounds might assist to reduce the risk of oxidative stress-related disorders (Kim *et al.*, 2016). The red paprika contains high water and carbohydrate content, as well as low protein and fat content. This fruit also have a sufficient amount of dietary fiber to be classified as high-fiber foods, which has significant effects on consumers' nutrition and health. Furthermore, red paprika provide several essential nutrients, including minerals (calcium, magnesium, potassium, sodium, and phosphorus) also rich in vitamins (B, A, D, C, E, and K) (Anaya-Esparza *et al.*, 2021).

Figure 1. Fruit of red paprika (*Capsicum annum L.*)

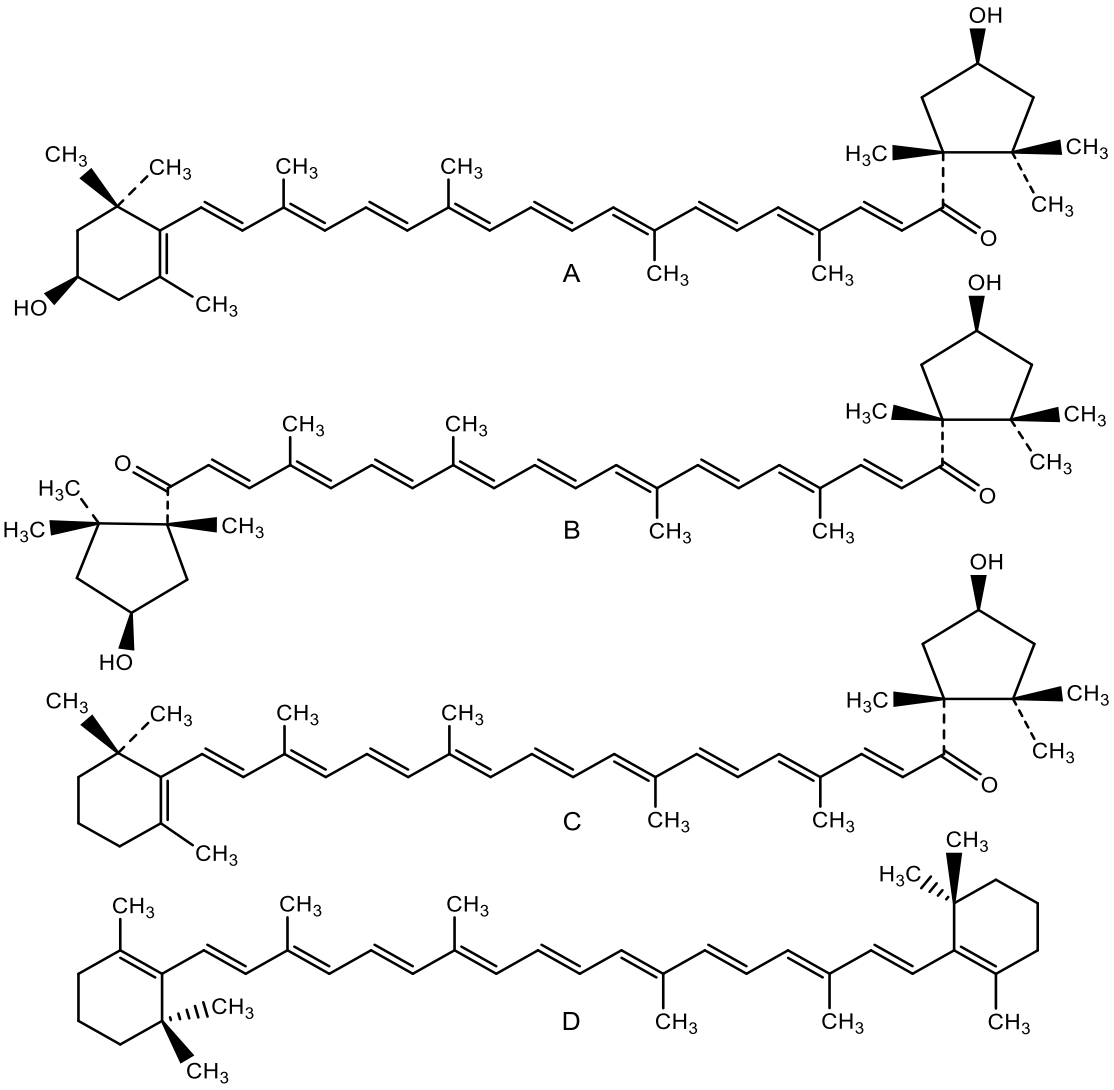

Figure 2. Structure of carotenoids in red paprika: capxanthin [A], capsorubin [B], cryptocapsin[C], and β-carotene [D] (Arimboor *et al.*, 2015; Hassan *et al.*, 2019)

Antioxidant activity of red paprika (Capsicum annuum L.) fruit methanol extract has been studied previously by Warsi and Guntarti (2016). Further research need to be explored on the extract to obtanied extract with optimal activity. It has been known that the main content of red paprika is carotenoids (Brezeanu *et al.*, 2022). Extraction or fractionation of carotenoids from red paprika usually using organic solvent such ether (Kultys & Kurek, 2022). Fractionation of red paprika extract using organic solvent include ether have a specific impact on the effective extraction of substituents such as carotenoids (Li *et al.*, 2021). Ether is a non-polar solvent. Therefore the fractionation using ether can extract non-polar

compounds, such as non-polar carotenoids (Kultys & Kurek, 2022). This study aimed to analyze the antioxidant activity of ether fraction from red paprika methanol extract by 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity. The current study also aimed to evaluate non-specific parameters standardization of red paprika fruit methanol extract, inlcuded water content, ash content, acid insoluble ash content and organoleptic.

METHODS

1. Extraction and Fractionation

The red paprika fruit was extracted according the procedure described by Medina-Juárez *et al.* (2012) with modification. The fresh red parika fruit was weighed 500 g and macerated using methanol (1:1). The sample was stirred for 3 hours, then allowed to stand for 24 hours in the dark. The macerate was filtered by a Büncher funnel. The sample was remacerated for 2 times. The filtrate was evaporated using rotary evaporatory to remove the solvent at 64°C until viscous.

The fractionation process of the extract was described by (Mandey *et al.*, 2019) with modifications. The viscous extract was fractionated using ether. The extract was dissolved in 20 mL of water free oxygenated and ether (1:1), then stirred for 15 minutes. The mixture was stand for a few minute until formed two layers, then separated. The ether phase was separated. The insoluble fraction was refractionated. The filtrate was evaporated on the waterbath until viscous. This process was repeated for three times.

2. Determination of Non-Specific Parameters Standardization

2.1. Procedures to Determine of Non-Specific Parameters Standardization

The procedures for assessing the non-specific parameters standardization of red paprika fruit methanol extract were carried out according to the Indonesian Herbal Pharmacopoeia (Anonyme, 2009), include water content, ash content, acid insoluble ash content and its organoleptic. All the tests were run in three times. The data were expressed as mean ± SD.

2.2. The calculation of Non-Specific Parameters Standardization

Water content was calculated according to Equation 1, whereas ash content and acid insoluble ash content were determined based on Equation 2.

$$\text{Water Content } (\%) = \frac{\text{Final Volume (mL)} - \text{Initial Volume (mL)}}{\text{Mass of Sample (g)}} \text{ X } 100 \qquad (1)$$

$$\text{Ash Content } (\%) \text{ or Acid Insoluble Ash content } (\%) = \frac{\text{Mass of Ash (g)}}{\text{Mass of Sample (g)}} \text{ X } 100 \qquad (2)$$

3. Analysis of Antioxidant Activity

3.1. Preparation of Sample Solution

A 50.0 mg of the red paprika fruit ether fraction was dissolved in methanol up to exactly 50.0 mL (1 mg/mL). This stock solution was diluted with methanol until obtained final concentration of 5; 20, 30, 50, 60 and 80 µg/mL.

3.2. Preparation of β-Carotene Solution

A 5.0 mg of β-carotene was dissolved in methanol up to 5.0 mL (1 mg/mL). The stock solution was diluted with methanol to obtain fractions with different final concentrations inlcuded 7.5; 17.5; 27.5; 37.5; 47.5 and 57.5 µg/mL.

3.3. Preparation of DPPH 0.15 mM

A 9.86 mg of DPPH was mixed with methanol in a 25-mL volumetric flask, to provide a stock solution for the 0.15 mM DPPH solution. As the working solution, the stock solution was pipetted 15.0 mL and then diluted with methanol to 100.0 mL.

3.4. The Measurement of Absorbance of The Sample

The antioxidant activity analysis according to procedur that described by Selvakumar et al.

(2011). A 1.0 mL of test sample was added with 1.0 mL of DPPH 0.15 mM solution and 1.0 mL of methanol. The mixtures were homogenized by sonicator, then kept in the dark at room temperature for 60 minutes. The absorbance was measured using a UV-Vis spectrophotometer at 517 nm againts the blank. The blank was 2.0 mL of methanol and 1.0 mL for each concentration series of the sample. The tests were run in three times. The data was expressed as mean ± SD.

3.5. The Measurement of Negative Control

A volume of 1.0 mL DPPH 0.15 mM was mixed with 1.0 mL of methanol. The mixture was kept in the dark at room temperature for 60 minutes. Then, the absorbance was measured using a UV-Vis spectrophotometer at 517 nm againts the blank (2.0 ml of methanol).

3.6. The Measurement of β-Carotene

A volume of 1.0 mL β-carotene solution for each concentration was addition with 1.0. mL of DPPH 0.15 mM solution. The mixtures were homogenized by sonikator, then kept in the dark at room temperature for 60 minutes. The absorbances were measured using a UV-Vis spectrophotometer at 517 nm againts the blank. The blank is a mixtute of 2.0 mL methanol and 1.0 mL of each the concentration series from β-carotene. The tests were run in three times. The data was expressed as mean ± SD.

3.7. The calculation of Antioxidant Activity

The percentage of inhibition of DPPH was calculated using the Equation 3. Where A = absorbance of control and B = absorbance of sample.

$$\text{DPPH Inhibition (\%)} = A - \frac{B}{A} \text{ X } 100 \qquad (3)$$

The percent inhibition of DPPH was used to create a linear regression equation relationship between the concentration of the sample and % DPPH inhibition. The EC_{50} value was obtained by entering 50 to y of the equation (Equation 4). The smaller EC_{50} mean that the sample has a high antioxidant activity.

$$y = bx + a \qquad (4)$$

4. The Data Analysis

The EC50 value of the sample was analyzed statistically with SPSS at confidence level of 95%. The tests were inlcuded normality and homogenity. The normality analysis was performed by Kolmogorov-Smirnov test to know the distribution of data. The homogenity analysis was performed by Levene test to know the variant of homogeneous data. If the results of the analysis show that the data was normally distributed and homogeneous, then the analysis followed by one way anova. The data was to be normally distributed and homogeneous if each test result has a significance value greater than 0.05. The data between two samples were different if significance value less than 0.05.

RESULTS AND DISCUSSION

The red paprika (*Capsicum annuum* L.) fruit was extracted by maceration. Maceration is an extraction process wherein a container is filled with roughly powdered drug material (leaves, fruit, stem bark, or root bark). Menstruum is added on top of the drug material until completely covered. The container is sealed and left for a minimum of three days. The material is periodically shaken and stirred if it is placed inside a bottle. Filtration is used to separate the micelle from materials at the end of extraction. The menstruum is subsequently isolated from the micelle by evaporation. This method is a highly applicable approach that works well with thermolabile plant material, such as carotenoids (Abubakar & Haque, 2017). However, extraction using maceration method can also extract other substants, include polyphenols such as flavonoids in low yield (Hidayat & Wulandari, 2021).

The extraction using maceration method was influenced by the solvent. For solvent extraction, the decision of solvent is essential. Selectivity, solubility, cost, and safety should all be taken into consideration when choosing a solvent. Solvents having a polarity value close to the polarity of the solute are likely to perform better and vice versa, like dissolves like. Alcohols (etanol and metanol) are

often used as solvents in extraction processes related to phytochemical research (Zhang *et al.,* 2018). Methanol was used as solvent in this study, referring to previous research (Warsi and Guntarti, 2016). Based on previous research reported that mthanol was more effective to extract bioactive metabolites from Acanthophora spicifera compared with ethanol. This study mentionated that methanol extracts have been shown to have more anticancer activity cause by the efficacy of extraction techniques (Prasedya *et al.*, 2019).

In this study, the fresh red paprika fruit was mashed with a blender. This process aims to minimize the size the particles of the sample. It can improve solute difusion and solvent penetration, the tiny particle size will improve the extraction efficiency. However, too small of a particle size will result in an excessive amount of solute absorption in the solid and difficulty in the subsequent filtration (Zhang *et al*., 2018). The function of stirring in this process was to accelerate of isolation of the active substance in the sample. The macerate was stand for 24 hours. This process was arranged to diffuse of the active substances and dissolve in methanol. The maceration was carried out in a closed container, to prevent oxidation by light. The solvent replacement process on extraction was carried out to avoid saturation of methanol.

Table I. The yield of methanol extract and fraction of red paprika fruit.

Samples	Mass of Dried Plant	Mass of Extract/ Fraction (g)	Yield (%)	Image of Extract/ Fraction
Red paprika fruit methanol extract	500.00	34.38	6.88	
Red paprika fruit ether fraction	5.00	0.89	17.8	

Furthermore, red paprika methanol extract was fractionated using ether. Ether is a nonpolar solvent that can be used to extract substances including fatty acids, terpenoids, alkaloids, and coumarins. The solvent has a low boiling point, tasteless, and soluble with water. Additionally, it is a very stable chemical that does not react with metals, acids, or bases (Abubakar & Haque, 2017). The yield of methanolic extract and ether fraction of red paprika fruit is seen in Table I.

The red paprika fruit methanol extract was evaluated non-specific parameters standardization to know its quality. The data are shown in Table II. The water content was analyzed to know water residue in the extract. The water residue can reduse the quality of the extract. High water level in the extract can increase contamination. It is known that water is a medium growth of mold and mushroom. Result of this study, water content of the red paprika methanol extract was in line with requirement Indonesian Herbal Pharmacopoeia < 10% v/w (Anonyme, 2009). The extract also be analyzed of ash content and acid insoluble ash content. It were intended to know the remaining combustion of minerals and inorganic content, respectively. The required ash content and acid insoluble content in extract is less than 6.7 % and 1.9 %, respectively, compared with Piper retrofractum (Anonyme, 2009). The result of ash content analysis was showed that the red paprika methanol extact contains many minerals components with high level.

Table 2. The results of non-specifc parameters of standardization of red paprika fruit methanol extract

The Non-spesific Parameters	The Results of Non-spesific Parameters ± SD (%)	CV (%)
Water content	4.32 ± 0.58 % v/b	13.34
Ash content	26.73 ± 0.10 % g/g	0.39
Acid insoluble ash content	11.91 ± 0.37 % g/g	3.11
Organoleptis	Viscous, red-brown color	-

The antioxidant activity of the red paprika ether fraction was analyzed by the DPPH free radical scavenging assay. The DPPH method was chosen because simple, easy, fast, and requires only a small sample, but obtained a sensitive and accurate results (Marinova and Batchvarov, 2011). This assay was based on measurement of the absorbance of the DPPH residue that not react with an antioxidant in the sample. The compounds containing in the extract that act as an antioxidant donate an electron to the DPPH in order to neutralise the DPPH radical. After the reaction, the DPPH• radical generates the reduced form of DPPH (hydrazine form). The reaction causes the color of the DPPH to shift from purple to pale yellow (Bedlovicová *et al*., 2020). The reaction between antioxidant (feruloyl glicoside) and the DPPH free radical is displayed in Figure 3. The mecanism reaction is explained follow the reaction pattern that described by Gulcin & Alwasel (2023). In this reaction, feruloyl glicoside after donating an electron to DPPH free radikal produced feruloyloxy glicoside radical. The radical undergoes electron delocalization to form a quinoid structure that is a neutral structure.

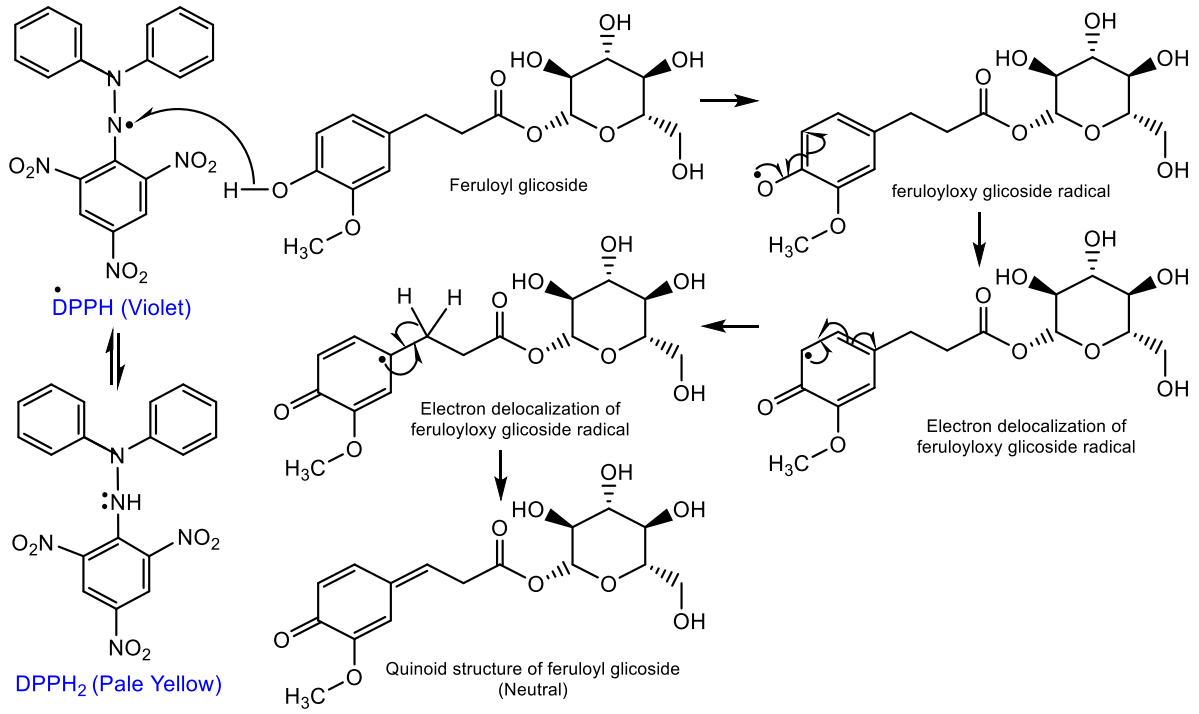

Figure 3. The reaction between antioxidant and the DPPH free radical (Gulcin & Alwasel, 2023)

The antioxidant activity of the samples in different concentrations were expressed in % DPPH inhibition. The graph of the samples relationship between concentration and percentage of inhibition to calculate IC_{50} value were displayed in Figure 4. The antioxidant activity was also expressed in IC_{50} value (Table III). IC_{50} is defined as the inhibition concentration of the antioxidant required to lower the initial DPPH concentration by 50 % (Munteanu & Apetrei, 2021). The smaller IC_{50} value indicates the greater of the antioxidant potential of the sample (Tamta et al., 2005). According to Sukandar et al. (2017) antioxidant of samples with IC_{50} value of < 50 µg/mL are classified as very powerful, 50-100 µg/mL as strong, 101-150 µg/mL as medium, and IC50 > 150 µg/mL as weak. The IC_{50} value of red paprika fruit ether fraction showed a very strong potential as DPPH free radical scavenging. The antioxidant power of this fraction (IC50 16.53 µg/mL) was more potent than red paprika methanol extract (IC_{50} 299.20 µg/mL), that reported by previous research (Warsi and Guntarti, 2016).

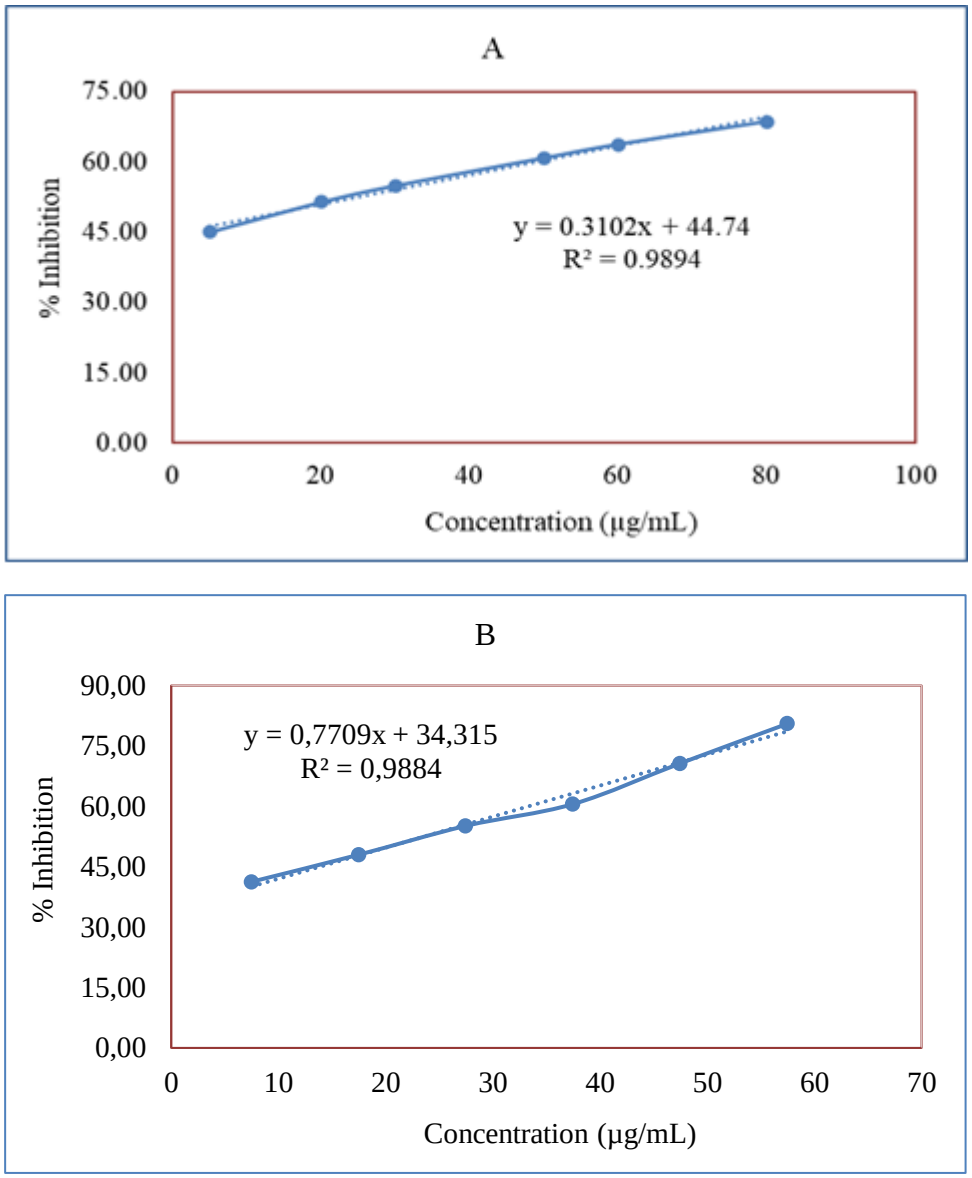

Figure 4. The Graph of % DPPH inhibition average: red paprika fruit ether fraction (A) and β- carotene (B)

Table 3. The results of antioxidant assay with DPPH

The samples	IC_{50} ± SD (µg/mL)	CV (%)	Antioxidant Power
Red paprika fruit ether fraction	16.53 ± 0.16[a]	0.98	Very strong
β-carotene	20.32 ± 0.63[b]	3.20	Very strong

Significant differences were indicated by different superscript letters in the same column

The result of statistically analysis with Kolmogorov-Smirnov test from IC50 obtained the significance value was 0.573 > 0.05. It can be concluded that the data is normally distributed. The significance value of Levene test was 0.669 that greater than 0.05. It can be concluded that the data homogeneous. Then, the analysis continued with parametric method is one way anova. One way anova is used to see the difference in significance between two samples. The result of one way anova analysis show that the ether fraction and standard of β-carotene were significantly different ($p = 0.006 < 0.05$). The antioxidant power of the sample was also expressed in EC50 value. The smaller of IC50 value was indicated the greater of antioxidant power. Antioxidant power of ether fraction from red paprika fruit was greater than β-carotene. However, the antioxidant power of both are very strong. This suggests that the antioxidant potential of ether fraction from red paprika is as high as β-carotene.

CONCLUSION

The results of non-parametric parameters standardization analyses of red paprika (*Capsicum annuum L.*) fruit methanol extract was obtained that water content fulfills the required standards. For its ash content and acid insoluble ash contents were not meet the required standards. Its organoleptic were viscous and red-brown color. The antioxidant activity power of the red paprika (*Capsicum annuum L.*) fruit ether fraction that measured using DPPH assay was significantly more potent (IC50 16.53 ± 0.16 μg/mL) than β-carotene (IC50 20.32 ± 0.63 μg/mL). The antioxidant power of both were classified as very strong.

REFERENCES

Abubakar, A. R., & Haque, M. (2017). Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes. Journal of Pharmacy and Bioallied Sciences, 12(1), 1–10. https://doi.org/10.4103/jpbs.JPBS

Anaya-Esparza, L. M., de la Mora, Z. V., Vázquez-Paulino, O., Ascencio, F., & Villarruel-López, A. (2021). Bell Peppers (Capsicum annum l.) Losses and Wastes: Source for Food and Pharmaceutical Applications. Molecules, 26(17), 5341. https://doi.org/10.3390/molecules26175341

Anonyme, 2009, Farmakope Herbal Indonesia Ith, 165-171, Departemen Kesehatan Republik Indonesia, Jakarta.

Arimboor, R., Natarajan, R. B., Menon, K. R., Chandrasekhar, L. P., & Moorkoth, V. (2015). Red Pepper (Capsicum annuum) Carotenoids as A Source of Natural Food Colors: Analysis and Stability-A Review. Journal of Food Science and Technology, 52(3), 1258–1271. https://doi.org/10.1007/s13197-014-1260-7

Bedlovicová, Z., Strapác, I., Baláž, M., & Salayová, A. (2020). A Brief Overview on Antioxidant Activity Determination of Silver Nanoparticles. Molecules, 25(14), 3191. https://doi.org/ttps://doi.org/10.3390/molecules25143191

Brezeanu, C., Brezeanu, P. M., Stoleru, V., Irimia, L. M., Lipșa, F. D., Teliban, G. C., Ciobanu, M. M., Murariu, F., Puiu, I., Branca, F., & Murariu, O. C. (2022). Nutritional Value of New Sweet Pepper Genotypes Grown in Organic System. Agriculture (Switzerland), 12(11), 1863. https://doi.org/10.3390/agriculture12111863

Gulcin, İ., & Alwasel, S. H. (2023). DPPH Radical Scavenging Assay. Processes, 11(8), 2248. https://doi.org/10.3390/pr11082248

Hassan, N. M., Yusof, N. A., Yahaya, A. F., Rozali, N. N. M., & Othman, R. (2019). Carotenoids of Capsicum Fruits: Pigment Profile and Health-Promoting Functional Attributes. Antioxidants, 8(469), 1–25. https://doi.org/10.3390/antiox8100469

Hidayat, R., & Wulandari, P. (2021). Eureka Herba Indonesia Methods of Extraction : Maceration , Percolation and Decoction. Eureka Herba Indonesia, 2(1), 68–74.

Huang, D., Ou, B. and Prior, R.L., 2005, The Chemistry Behind Antioxidant Capacity Assays, Journal of Agricultural and Food Chemistry, 53, 1841-1856.

Imran, M., Butt, M. S., & Suleria, H. A. R. (2018). Capsicum annuum Bioactive Compounds: Health Promotion Perspectives (J.-M. Merillon & K. G. Ramawat (eds.); Bioactive). Springer International Publishing AG. https://doi.org/10.1007/978-3-319-78030-6

Joseph, N.M., Monika, S., Alok, S., Alok, M. and Shruti, R., 2010, In-Vitro and In-Vivo Models for Antioxidant Activity Evaluation: A Review, International Journal of Pharmaceutical Sciences and Research, 1(1), 1-11.

Kim, J.-S., Lee, W.-M., Rhee, H. C., & Kim, S. (2016). Red Paprika (Capsicum annuum L .) and Its Main Carotenoids, Capsanthin and β-carotene, Prevent Hydrogen Peroxide-Induced Inhibition of Gap-Junction Intercellular Communication. Chemico-Biological Interactions, 254, 146–155. https://doi.org/10.1016/j.cbi.2016.05.004

Kultys, E., & Kurek, M. A. (2022). Green Extraction of Carotenoids from Fruit and Vegetable Byproducts: A Review. Molecules, 27(2), 518. https://doi.org/10.3390/molecules27020518

Li, N., Li, J., Ding, D., Xie, J., Zhang, J., Li, W., Ma, Y., Gao, F., Niu, T., Wang, C., & Bakpa, E. P. (2021). Optimum Parameters for Extracting Three Kinds of Carotenoids from Pepper Leaves by Response Surface Methodology. Separations, 8(9), 134. https://doi.org/10.3390/separations8090134

Mandey, F., Handayani, E., Nanda, W. E., & Noor, A. (2019). Extraction, Fractionation, And Antioxidant Examination Of Polyfloral Honey Originated From Bone Prefecture South Sulawesi Province. Indonesian Journal of Chemical Research, 7(1), 86–91. https://doi.org/10.30598//ijcr.2020.7-fmd

Marinova, G., Batchvarov, V., 2011, Evaluation of The Methods for Determination of The Free Radical Scavengings Activity By DPPH, Bulgarian journal of agricultural science, 17(1), 11-24.

Medina-Juárez, L. Á., Molina-Quijada, D. M. A., Del Toro-Sánchez, C. L., González-Aguilar, G. A., & Gámez-Meza, N. (2012). Antioxidant Activity of Peppers (Capsicum annuum L.) Extracts and Characterization of Their Phenolic Constituents. Interciencia, 37(8), 588–593.

Munteanu, I. G., & Apetrei, C. (2021). Analytical methods used in determining antioxidant activity: A review. International Journal of Molecular Sciences, 22(7), 3380. https://doi.org/10.3390/ijms22073380

Park, S., Jeong, W. Y., Lee, J. H., Kim, Y. H., Jeong, S. W., Kim, G. S., Bae, D. W., Lim, C. S., Jin, J. S., Lee, S. J., & Shin, S. C. (2012). Determination of Polyphenol Levels Variation in Capsicum annuum L. cv. Chelsea (Yellow Bell Pepper) Infected by Anthracnose (Colletotrichum gloeosporioides) using Liquid Chromatography-Tandem Mass Spectrometry. Food Chemistry, 130, 981–985. https://doi.org/10.1016/j.foodchem.2011.08.026

Prasedya, E. S., Martyasari, N. W. R., Hamdin, C. D., Miyake, M., Kobayashi, D., Widyastuti, S., Hazama, A., & Sunarpi, H. (2019). Effectiveness of Methanol Solvent Extraction for Red Macroalgae Acanthophora spicifera Antitumoric Activity. Pharmacognosy Journal, 11(3), 450–454. https://doi.org/10.5530/pj.2019.11.70

Selvakumar, K, Madhan, R., Srinivasan, G. and Baskar, V., 2011, Antioxidant Assays in Pharmacological Research, Review Article, Asian Journal of Pharmacy and Technology, 1(4), 99-103.

Sukandar, D., Nurbayti, S., Rudiana, T., & Husna, T. W. (2017). Isolation and Structure Determination of Antioxidants Active Compounds from Ethyl Acetate Extract of Heartwood Namnam (Cynometra cauliflora L.). Indonesian Journal of Applied Chemistry, 19(1), 11–17. https://doi.org/10.14203/jkti.v19i1.325