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FULL PAPER

Antiradical activity, total phenolic, and total flavonoids extract and fractions of pumpkin (*Cucurbita moshata* Duch) leaves

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Abstract

Pumpkin (*Cucurbita moshata* Duch) is one of the Cucurbitaceae plants, which traditionally used to treat skin diseases, measles, jaundice, insomnia, cancer and enhances endurance. Therefore, it was necessary to explore the potential of pumpkin leaves as antiradical. This research aim was to examine the antiradical activity and total phenolic and total flavonoids of pumpkin leaves extract and its fractions using the DPPH method and determined the phenolic and flavonoid contents. Pumpkin leave powder was extracted with methanol. Furthermore, water was added into methanol extract, and be partitioned using n-hexane and ethyl acetate to obtain n-hexane, ethyl acetate, and water fractions. The antiradical activities of pumpkin leave extract and fractions were determined using DPPH (2,2-diphenyl-1-picryhidrazyl) method. Ethyl acetate fraction obtained higher antiradical activities (IC_{50} 6.737±0.196 µg/mL). Correlation of total phenolic and flavonoid contents to inhibit DPPH radical showed that phenolic and flavonoid contents on pumpkin leaves could be inhibited DPPH radical R^2 = 0.8994 and R^2 = 0.9061, respectively. Extracts and fraction pumpkin leaves show strong antiradical activity with DPPH methods, so their potential as antiradical can developed and can be used as a functional food.

1. Introduction

Since ancient times, plants and their metabolites have been used as medicine. Natural source of antioxidant from Traditional medicine is widely used, which these antioxidant substances may help to develop new potential drug (Bag and Devi, 2015). Antioxidants are defined as a compound that delays or prevents oxidation of biomolecules such as lipid, protein, and DNA, by counteracting free radicals from these biomolecules which prevent cell damage (Proestos *et al*., 2013; Labiad *et al*., 2017). In the last several decades, many researchers have traced the antioxidant activity of plants. Where, it is believed that the use of natural antioxidants will reduce the chance of degenerative diseases, such as cardiovascular disease and cancer (Elkhamlichi *et al*., 2017).

Pumpkin (*Cucurbita moschata*) is a plant of the family Cucurbitaceae which is widely distributed in tropical, subtropical, temperate, and hot regions (Al-Qaisy and Rathi, 2020). Traditionally, pumpkin (*C. moshata*) is used to treat skin diseases, measles, jaundice, insomnia, cancer, and can help to enhance endurance. While pumpkin seed oil can be used in hypertension, arthritis, hypercholesterolemia, bladder disorders, and urethral pressure treatment (Suresh and Sisodia, 2018). Phytochemical screening shows flesh and pumpkin seeds containing flavonoids, terpenoids, saponins, and tannins (Marbun *et al*., 2018).

The objective of the research was to determine the potential antiradical and phenolic and flavonoids total of pumpkin extracts and fractions leaves

2. Materials and methods

2.1 Materials

Pumpkin leaves were obtained from Padala'a Village, Kepulauan Menui District, Moruali Regency, Central Sulawesi Province, DPPH (2,2-diphenyl-1-picryhidrazyl) (Sigma-Aldrich®, USA), Gallic Acid (Sigma-Aldrich®, USA), Quercetin (Sigma-Aldrich®, USA), methanol (E. Merck, Germany), ethyl acetate, n-hexane, and aquadest.

2.2 Extraction

Pumpkin leaves were dried in the sun and covered with black cloth. Then, the dried leaves were powdered. Pumpkin leaf powder (30 g) was macerated with

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methanol for 3×24 hrs, where every 24 hrs the mixture was filtered, and the solvent was replaced. The extract was evaporated to dryness using Rotary Evaporator. Water (1 L) was added into methanol extract (40 g), then the extract was fractionated with n-hexane and ethyl acetate to obtain the n-hexane, ethyl acetate, and water fraction, as shown in Figure 1.

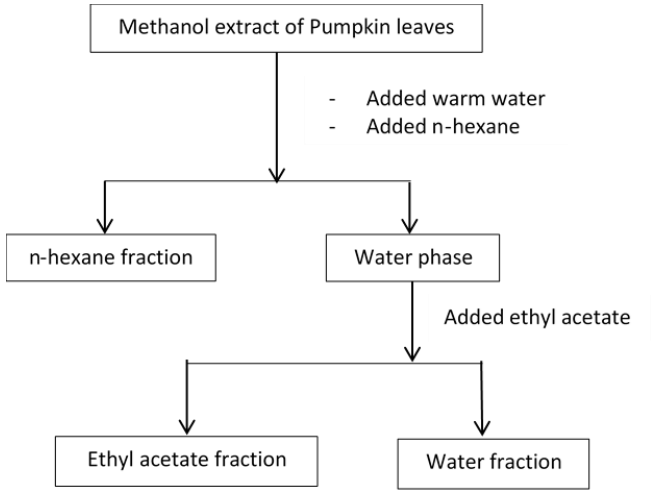

Figure 1. Schematic fractionation of pumpkin leaves methanol extract

2.2.1 Yield extract

The yield extract that has been obtained is calculating using Equation 1 (Nurmala and Novianti, 2020)

$$yield\ extract = \frac{weight\ of\ extract\ obtained}{weight\ of\ simplicia} \times 100\% \quad (1)$$

2.2.2 Yield fraction

The yield fraction that has been obtained is calculating using Equation 2

$$yield\ fraction = \frac{weight\ of\ fraction}{weight\ of\ extract} \times 100\% \quad (2)$$

2.3 Phytochemical screening

Phytochemical screening was conducted to determine the profile of secondary metabolites in langsat seeds extracts and fractions. Phytochemical screening methods based on (Yamin *et al*., 2020).

2.3.1 Alkaloids

The extract and fractions of *Lansium domesticum* Coor seeds were inserted separately into 1 mL test tubes and added with three drops of Dragendorff's reagent. The formation of brown precipitate indicated the presence of alkaloid.

2.3.2 Flavonoids

The extract and fractions of *Lansium domesticum* Coor seeds were inserted separately into test 1 mL tubes and added with 0.2 g of magnesium powder and 2 mL of concentrated HCl. The formation of red, orange and green solutions indicated the flavonoid presence.

2.3.3 Tannin

The extract and fractions of *Lansium domesticum* Coor seeds were inserted separately into 1 mL test tubes and added with 1 mL of 1% Fe (III) chloride solution. The formation of blue to black solution indicated the presence of tannin.

2.3.4 Terpenoid

The extract and fractions of *Lansium domesticum* Coor seeds were inserted separately into 1 mL test tubes and added with 0.5 mL of acetic acid anhydride and 2 mL of concentrated sulfuric acid. The formation of green, bluish, and brown solutions indicated the terpenoid presence.

2.3.5 Saponin

The extract and fractions of *Lansium domesticum* Coor seeds were inserted separately into 1 mL test tubes and added with 2 mL of hot water, then cooled and shaken for 10 s. It was declared positive for saponin if the fume generated stabilized in less than 10 mins.

2.4 Determination of antiradical activity with DPPH method

The determination of radical activity was done by following the Garcia method (Garcia *et al*., 2012) which modified the number of samples to use. Methanol p.a (3 mL) and 1 mL of DPPH radical (2,2-diphenyl-1-picryhidrazyl) was added to the sample (1 mL). The mixture was shaken until homogeneous, then incubated in a dark room for 30 mins. Then the absorbance of the sample was measured using a UV-Vis spectrophotometer at a wavelength of 513 nm. The antioxidant activity of extract and its fractions to inhibit DPPH radicals can be calculated using the following equation:

$$\%\ \text{inhibition} = \frac{A_c - A_s}{A_c} \times 100\% \quad (3)$$

Where % inhibition = percentage of DPPH radical inhibition, Ac = Absorbance of control and As = Concentration of sample

The value of antioxidant strength (IC_{50}) was calculated based on the linear regression equation between % inhibition with the concentration of sample or fractions, where the x-axis was the concentration while the y-axis was % inhibition. The regression equation y = bx + a. Then, the y value was replaced by 50. Where IC_{50} was defined as the concentration of the sample needed to

inhibit 50% of DPPH radicals (Rohman *et al*., 2017).

2.5 Determination of total phenolic content

Determination of total phenolic content of pumpkin leaves extracts and fractions using the modifying Folin-Ciocalteau method according to John *et al.* (2014). Each concentration series of the sample (1 mL, respectively) were taken and added 0.4 mL of the Folin-Ciocalteau reagent, then shaken. After 8 mins later, added 4 mL of 7% Na_2CO_3 and shaken until homogeneous then sufficient with up to 10 mL volume, allowed to stand for 30 mins and then absorbed by using UV-Vis spectrophotometry at a wavelength of 750 nm. The measurement of each sample concentration series was carried out three times for replication. Total phenolic levels are expressed as Gallic Acid Equivalents (GAE).

2.6 Determination of total flavonoid content

Measurement of total flavonoid levels was carried out using the colorimeter method (John *et al*., 2014; Vyas *et al*., 2015) by modifying, 10 mg of sample dissolved with methanol p.a. 10 mL. Then, 1 mL was taken with 3 mL of methanol p.a., then 0.2 mL of 10% aluminum chloride and 0.2 mL of potassium acetate 1 M were added and the volume of 10 mL was sufficient with distilled water. The mixture was incubated for 30 mins. Then the absorbance was measured using a UV-Vis spectrophotometer at 435 nm wavelength. Total flavonoid levels are expressed as Quercetin Equivalent (QE).

3. Results and discussion

In this study, the extraction method used was maceration, then followed by fractionation using solvents with different polarities. The purpose of using solvents with different polarities was to extract completely compounds whether non-polar, semipolar, and polar. The same compound can be found in different solvents, but the levels are different in each solvent. This depends on the distribution coefficient of the compound (Yamin *et al*., 2010). The results of pumpkin leave extraction and fractionation are presented in Table 1.

Table 1. Pumpkin leave extraction and fractionation results

No.	Solvents	Sample weight (g)	Extract/fractions weigh (g)	Yield (%)
1	Methanol	350	89.2	25.57
2	N-hexane	40	8.2	20.5
3	Ethyl acetate	40	16.4	40
4	Water	40	15.4	38.5

Based on the data in Table 1 shows that the crude extract yield is 25.57%. This result indicates that the pumpkin leaves can be utilized. Even after fractionation, the ethyl acetate fraction shows the highest yield of 40%, which means that the compounds in pumpkin leaves are mostly semi-polar. The compound will be easily attracted to a suitable solvent, according to the principle of "like dissolved like", where compounds will be attracted to solvents with the same polarity (Amaro *et al*., 2015).

Phytochemistry screening showed the extract and fraction of pumpkin leaves contained secondary metabolite alkaloids, flavonoids, tannin, saponin and terpenoids. The result of phytochemistry screening shown in Table 2.

Table 2. Phytochemical screening extract and fractions of pumpkin leaves

Testing	Sample			
	Methanol extract	n-hexane fraction	Ethyl acetate fraction	Water fraction
Flavonoid	+	+	+	+
Alkaloid	+	+	+	+
Tannin	+	+	+	+
Terpenoid	+	+	+	+
Saponin	+	+	+	+

Utilization radical DPPH (2,2-diphenyl-1-picryhidrazyl) in determining the strength of free radical caused by DPPH is a stable radical (Shekhar and Anju, 2014), which receives electrons or hydrogen radical to become a stable diamagnetic molecule (Arina and Rohman, 2013). Besides, DPPH radicals are also considered a standard colorimetric technique to measure the antioxidant activity of extracts and pure compounds from plants (Mishra *et al*., 2012). The reduction ability of DPPH free radicals is based on a decrease in wavelength at 513 nm after adding antioxidants. In this study, the standard used is vitamin C and solvent for dissolving DPPH using methanol. The use of methanol is based on its sensitivity compared to other solvents (Rohman *et al*., 2017).

The antiradical activity was determined using vitamin C as a positive control. The result in Table 3, shows the IC_{50} values of pumpkin leave extracts and fractions with vitamin C as positive controls. Pumpkin leave extract and fractions showed antiradical activity based on the IC_{50} value. The smaller IC_{50} value of the sample indicates that the stronger functions as an antiradical. Based on the data in Table 3 ethyl acetate fraction is the most powerful fraction as antiradical. This is in line with different research which the ethyl acetate fraction of the red fruit (*Panandus conoideus* L.) (Rohman *et al*., 2010), senggani leaves (*Melastoma candidum* D. Don) (Marjoni and Zulfisa, 2017),

rambutan peel (Rohman *et al*., 2017). Figure 2 shows the correlation of ethyl acetate fraction inhibition with the amount of DPPH radical, ethyl acetate fraction can be further separated to obtain an active isolate.

Table 3. IC_{50} values extract and fractions of pumpkin leaves

Sample	IC_{50} value (µg/mL)
Methanol extract	8.832±1.429
Ethyl acetate fraction	6.7371±0.1959
N-hexane fraction	9.6789±0.5427
Water fraction	11.8052±0.63
Vitamin C	4.2454±0.0293

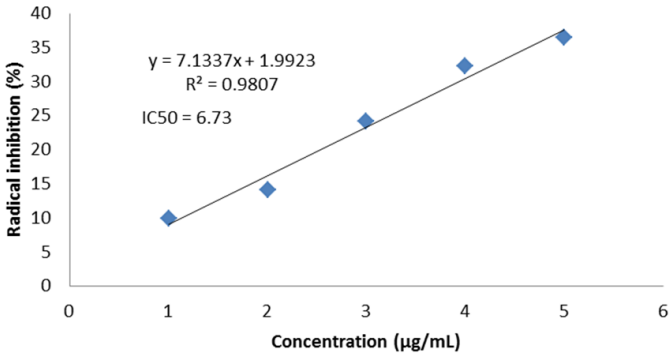

Figure 2. Correlation between the concentration of ethyl acetate fraction (µg/mL) with radical inhibition (%)

Phenolic and flavonoid compounds are known to have antiradical activity. These compounds can donate hydrogen radicals contained in the hydroxy group (-OH) to attached in radical DPPH, hence the DPPH radical becomes stable (Pietta, 2000; Heim *et al*., 2002; Agati *et al*., 2012). Therefore, some antioxidant activity of natural substances associated with phenolic compounds and flavonoids (Rohman *et al*., 2017)

Phenolic and flavonoids are the most abundant compounds in plants that are beneficial in human health (Wijaya *et al*., 2017). Total phenolic and flavonoid levels have a correlation with antioxidant power in extracts and fractions (Nur *et al*., 2019). Table 4 and Table 5 shows the total phenolic and flavonoid content of pumpkin leaf extracts and fractions. The ethyl acetate fraction obtained high phenolic and flavonoid levels compared to methanol extract, n-hexane, and water fractions. Phenolic and flavonoid levels of ethyl acetate fraction were 56.43 g GEA/100 g sample and 55.07 g QE/100 g sample, respectively. These results indicated that the value of antioxidant strength correlates with phenolic and flavonoid levels contained in the extract and fraction of pumpkin leaves.

The correlation between antiradical strength (IC_{50}) with phenolic and flavonoid levels of extracts and pumpkin leaf fraction is shown in Figure 3. The correlation coefficient (R^2) is used to determine the

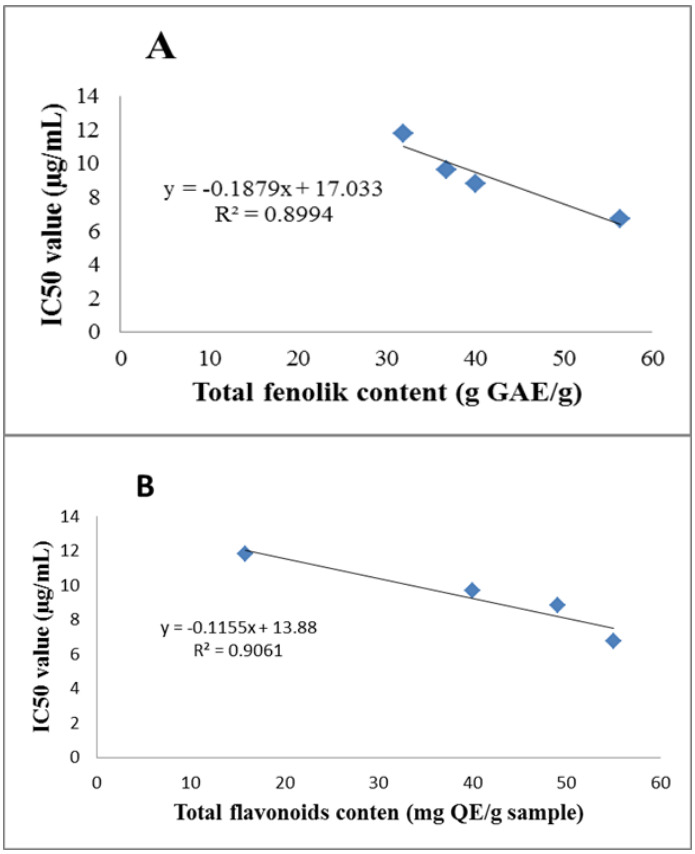

Figure 3. Correlation between total phenolic content (A) and total flavonoid content (B) (x-abscissa) with IC_{50} value (y-ordinate) of pumpkin leaves extract and fractions

Table 4. Total phenolic content in pumpkin leaves extract and fractions

Sample	Linear regression of Gallic Acid	Correlation coefficient	Average absorbance	Phenolic value (g GEA/100g sample)
Methanol extract			0.615	40.14
Ethyl acetate fraction	y = 0.007x + 0.334	R^2 = 0.9874	0.729	56.43
N-hexane fraction			0.592	36.86
Water fraction			0.558	32

Table 5. Total flavonoid content in pumpkin leaves extract and fractions

Sample	Linear regression of quercetin	Correlation coefficient	Average absorbance	Flavonoid value (g QE/100g sample)
Methanol extract			0.506	49.11
Ethyl acetate fraction	y = 0.0057x + 0.2261	R^2 = 0.9612	0.54	55.07
N-hexane fraction			0.454	39.98
Water fraction			0.316	15.77

effect of phenolic and flavonoid content on antiradical strength. Figure 3 shows the correlation between IC_{50} values with total phenolic content and flavonoids. Absis (x) is the IC_{50} value and ordinate (y) is the phenolic and flavonoid content of the methanol extract and its fractions. The R^2 value obtained is 0.8994 and 0.9061. This indicates that 89.94% of antiradical strength is influenced by phenolic content and 90.61% is influenced by flavonoid content contained in pumpkin leave extract and fraction.

4. Conclusion

Pumpkin leaves have antiradical abilities, which the ethyl acetate fraction exhibits the strongest antiradical. The correlation between total phenolic, flavonoid content, and radicals inhibition is very high. These show the compounds most responsible as antiradical are phenolic and flavonoid compounds. Thus, pumpkin leaves can be developed as a functional food.

Conflict of interest

The authors declare no conflict of interest.

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