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Determination of free radical scavenging activity, phenolic and flavonoid content of seven corn cultivars from the Southwest Maluku District, Indonesia

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Abstract. *Sinay H, Mahulette F, Yáñez JA. 2022. Determination of free radical scavenging activity, phenolic and flavonoid content of seven corn cultivars from the Southwest Maluku District, Indonesia. Biodiversitas 23: 5974-5981.* The variation of corn (*Zea mays* L.) kernel might be considered as a surrogate indicator of the phytochemical and bioactive content. This study aimed to determine the phenolics, flavonoids, and free radical scavenging activity of seven local corn cultivars from Kisar Island, Southwest Maluku District in Indonesia. Sample extraction was done by the maceration method. Flavonoid content was determined using the Dowd method, and total phenolic content was performed using the Folin-Ciocalteu method. Free radical scavenging activity was determined using the DPPH radical scavenging method. One-way ANOVA was performed with Duncan multiple range tests to assess the differences between corn seed cultivars, and $p < 0.05$ were regarded as statistically significant. The results show that the total flavonoid content varied from 8.463 mg QE/mg (*Putih* cultivar) to 40.075 mg QE/mg (*Kuning Dalam* cultivar). Total polyphenol content also varied between 105.63 mg GAE/mg (*Merah Delima Tongkol Putih* cultivar) and 136.41 mg GAE/mg (*Pulut* cultivar). The antioxidant activity by DPPH assay determined that the seven local corn cultivars had IC_{50} values that were similar to quercetin (used as control), some were even lower. The IC_{50} value of the seven local corn cultivars ranged from 5.56 to 16.96 µg/mL, while quercetin had an IC_{50} value of 11.97 µg/mL. It was observed that the *Merah Delima Tongkol Cokelat, Merah Delima Tongkol Putih,* and *Putih* cultivars had comparable antioxidant activity to the control quercetin. The *Merah Darah, Kuning Genjah,* and *Kuning Dalam* cultivars exhibited a statistically significant higher antioxidant capacity than quercetin. These results can contribute to the selection of corn cultivars to harvest for commercial purposes and potential nutraceutical applications.

Keywords: Corn, cultivar, DPPH, Indonesia, total flavonoid, total phenolic

INTRODUCTION

Indonesia presents several local food resources, but due to the lack of nutrient content, antioxidant capacity, bioactive compounds characterization, and the stigma of being inferior foods, some of these local food resources are underutilized (Fetriyuna et al. 2021). This is contradictory to Indonesia's high prevalence of undernutrition and nutrition insecurity (Durst and Bayasgalanbat 2014; Fatmaningrum et al. 2016; Fetriyuna et al. 2021). One of the staple foods that Indonesia produces is corn, which is widely cultivated across Indonesia, mainly in Kisar Island in the Southwest Maluku district, and grows every season (Alfons et al. 2003; Phalan et al. 2013; Sinay et al. 2016a,b; Sinay and Karuwal 2017; Sinay and Arumingtyas 2018; Sinay and Karuwal 2014, 2018; Sinay and Suripatty 2019; Sinay and Tanrobak 2020).

Seven local corn cultivars with distinct color have been identified in Indonesia, namely: *Merah Delima Tongkol Cokelat*, *Merah Delima Tongkol Putih*, *Merah Darah*, *Kuning Genjah*, *Kuning Dalam*, *Pulut*, and *Putih* (Alfons et al. 2003; Kamaludin et al. 2021). Because of this diversity of corn cultivars, it has been successfully used as ready-to-use supplementary food (RUSF) aimed at improving the nutrition of moderate acute malnutrition (MAM) in children under five years old in Indonesia (Fetriyuna et al. 2021). Also, the agricultural waste from corn has been used in the country to increase productivity and carcass characteristics of lambs (Purbowati et al. 2021) and cattle (Tahuk et al. 2020) fed with this fibrous agricultural waste as a substitute for regular grass. Furthermore, the use of corn to produce biodiesel for the United States market has caused changes in land use for cultivating corn in Malaysia and Indonesia, compared to other vegetables like soy (Taheripour and Tyner 2020).

A poor diet can be a contributing factor to the development of chronic diseases such as cardiovascular disease and cancer (Andrews et al. 2006; Kyle and Duthie 2006; Remsberg et al. 2006; Yáñez et al. 2006; Ramos-Escudero et al. 2009, 2010; Xiong et al. 2009; Yáñez et al. 2012b). It can also cause comorbidities that can be detrimental to the recovery from recent diseases such as COVID-19 (Alvarez-Risco et al. 2021a,b). In addition, the modern polluted environment contributes to the development of free radical compounds, which can interact with macromolecules such as protein, fat, carbohydrates, and nucleic acid in the human body (Birben et al. 2012; Wijayanti et al. 2018; Alkadi 2020). Excessive reactions between free radicals and these macromolecules induce the formation of Reactive Oxygen Species (ROS). In humans, the formation of ROS can cause an oxidant/antioxidant imbalance that can damage the organs of the body and trigger the initiation and progress of premature aging, as well as many degenerative diseases such as diabetes, coronary heart disease, kidney disorders, and cancer (Zhang et al. 2017).

On the other hand, adequate consumption of cereals, grains, vegetables, fruits, and herbs contributes to beneficial effects on health and in the prevention of various ailments (Shahidi and Naczk 2003; Naczk and Shahidi 2006; Mejia-Meza et al. 2008; Ramos-Escudero et al. 2008; Ramos-Escudero et al. 2009, 2010; Bonin et al. 2010; Mejia-Meza et al. 2010; Davies and Yáñez 2012; Ramos-Escudero et al. 2012b; Yáñez et al. 2012a; Alarcón et al. 2017; Alvarez-Risco et al. 2018; Delgado-Zegarra et al. 2018). This protective effect has been attributed to the natural presence of bioactive compounds such as polyphenols, which include flavonols, flavones, catechins, flavanones, and anthocyani(di)ns, either as aglycones and glycosides, which are present in plants (Roupe et al. 2005; Yáñez and Davies 2005; Yáñez et al. 2007b, 2011, 2012a,b,c; Takemoto et al. 2008; Vega-Villa et al. 2008, 2009, 2011; Bermudez-Aguirre et al. 2010; Bonin et al. 2010; Ramos-Escudero et al. 2010a,b; Serve et al. 2010; Davies and Yáñez 2012; Chemuturi and Yáñez 2013; Mejia-Meza et al. 2013; Alrushaid et al. 2017; Villena-Tejada et al. 2021). In addition, polyphenols have an antioxidant capacity that can reduce the activity of free radicals by donating one of their electrons to free radicals, and this can inhibit their reaction with macromolecules and cells and the formation of ROS (Esmaeili et al. 2015; Remsberg et al. 2007; Birben et al. 2012). The antioxidant activity of phenolic compounds is caused by the presence of the hydroxyl group in their chemical structure that can donate their hydrogen atom to free radicals, causing the free radicals to be neutralized and stabilized (Shetty 2004; Rent 2007; Ranilla et al. 2009; Lee et al. 2010).

Corn (*Zea mays* L.) has been reported to be an important source of polyphenols distributed throughout the plant (Pedreschi and Cisneros-Zevallos 2006, 2007). For instance, phenolic acids such as vanillic acid, protocathetic acid, ferulic acid, p-coumaric acid, p-hydroxybenzoic acid, p-hydroxyphenyl acetic acid, syringic acid, and caffeic acid have been identified in corn (Sosulski et al. 1982; Pedreschi and Cisneros-Zevallos 2007; Gonzalez-Manzano et al. 2008). In addition, flavonoids such as quercetin, hesperetin, and kaempferol have also been reported (Yáñez et al. 2005; Pedreschi and Cisneros-Zevallos 2007; Ranilla et al. 2009). In Indonesia, the *Pulut* (waxy) corn variety (Mohamed et al. 2017) and the *Manado Kuning* variety (Budiarso 2017) have been recognized as sources of antioxidants. However, the antioxidant capacity, flavonoid, and phenolic compounds content of local corn cultivars from Indonesia have not been reported. Therefore, this study aimed to determine the total flavonoid, phenolic, and free radical scavenging activity of local corn seeds from Kisar Island (Southwest Maluku District) in Indonesia.

MATERIALS AND METHODS

Plant materials

The genetic material of corn (*Z. mays*) seeds was acquired from local farmers on Kisar Island, Southwest Maluku District in Indonesia. Figure 1 shows the seven corn cultivars collected (Figure 1). For the processing of the samples, they were air-dried at room temperature for seven days until the cornhusk was yellow and dry. Then, corn kernels were removed from the cobs and collected to perform the extraction.

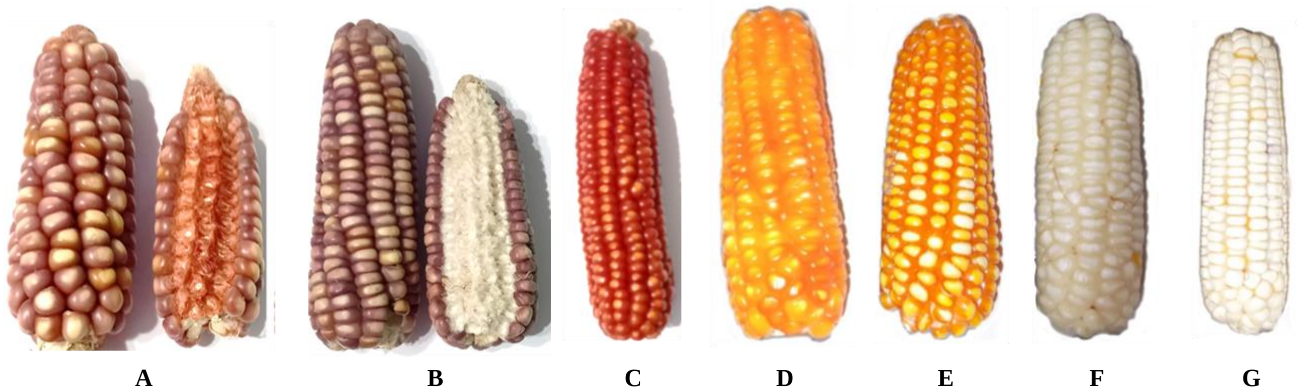

Figure 1. Seven local corn cultivars from Kisar Island, Southwest Maluku, Indonesia. A. Merah Delima Tongkol Cokelat, B. Merah Delima Tongkol Putih, C. Merah Darah, D. Kuning Genjah, E. Kuning Dalam F. Pulut, and G. Putih

Chemicals

Methanol, quercetin, gallic acid, Folin-Ciocalteu reagent, aluminum chloride, calcium acetate, sodium carbonate and *DPPH* (2,2-difenil-1-pikrilhidrazil) were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA).

Sample preparation

The corn kernels were ground using a mortar and pestle and filtered through a mesh with a pore diameter of 400 µm. The extraction was performed using a previously reported method (Mohsen and Ammar 2009) with slight modifications. At room temperature, approximately 20 g of corn powder was macerated with 500 mL 75% of aqueous methanol (v/v) for 24 hours. Next, the supernatant was decanted into a beaker glass. The residue was macerated for a second time using the same procedure. Finally, both supernatants were combined and filtered through No.1 Whatman filter paper and concentrated under a rotary vacuum evaporator (HS-2005 S/NV-0027, Jisico. Co. Ltd, Seoul, South Korea) at 45°C for 72 hours.

Content of total flavonoids

The content of flavonoids was determined according to the Dowd method as previously described (Meda et al. 2005) with slight modifications. Briefly, 0.5 mg of concentrated extract was weighed and diluted with 2 mL of 96% methanol in a test tube, and 2 mL of 2% aluminum chloride was added. The absorbance was measured immediately using a UV-Vis spectrophotometer (UV-Vis 752B, Nanyang Chemical Technology Indonesia) with a wavelength of 415 nm. As a standard, quercetin was used, and the total flavonoid content was expressed as milligrams of quercetin equivalent (QE) per gram dry weight (DW) of a sample.

Content of total polyphenols

Total polyphenols were determined according to the Folin-Ciocalteu Method (Singleton et al. 1999), using gallic acid as standard. First, the 1 mg of concentrated extract was weighed and suspended with 1 mL of ethanol. Then 0.2 mL of the solution was put into a test tube, and 15.8 mL of distilled water and 0.1 mL of 50% of Folin-Ciocalteu reagent were added. The mixture was shaken and left to react for 8 minutes. After that, 3 mL of 2% sodium carbonate solution was added and shaken for 3 minutes. The mixture was incubated in the dark for 2 hours at room temperature. The absorbance was measured at a wavelength of 765 nm with a UV-Vis spectrophotometer (UV-Vis 752B, Nanyang Chemical Technology Indonesia). As a standard, gallic acid was used, and the total phenolic content was expressed as milligrams of gallic acid equivalent (GAE) per gram dry weight (DW) sample.

Measurement of antioxidant activity by DPPH assay

DPPH radical scavenging was determined according to a previously reported method (Burda and Oleszek 2001). A total of 0.5 mL of extract was added with 2 mL of 93 µM 1,1-diphenyl-2-picrylhydrazyl (DPPH) solution. After vigorous shaking, the mixture was placed in the dark for 30 min at room temperature. The degree of fading of the solution color from purple to yellow indicates the efficiency of free radical scavenging. Immediately after the color change occurred, the absorbance value was determined using a UV-Vis spectrophotometer (UV-Vis 752B, Nanyang Chemical Technology Indonesia) at 517 nm. Quercetin was used as a control since it is a flavonoid with well-characterized antioxidant activity (Torres et al. 2005). A standard curve was constructed using the following quercetin concentrations: 2.5, 5.0, 7.55, 10, and 12.5 ppm. The inhibition percentage (I%) was calculated using the equation:

$$I\% = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

Where; $A_{control}$ represents the absorbance of control and A_{sample} represents the absorbance of sample.

Fifty percent inhibitory concentration (IC_{50}) of each sample represents the concentration of sample in which 50% DPPH was inhibited. IC_{50} was calculated by constructing curves of the percentage of DPPH inhibition as Y axis, and extract concentration as X axis. The obtained linear regression equation ($y = 8.999x + 57.678$) was used to calculate the IC_{50} value by replacing Y with 50. The results were expressed as µg of sample/mL. The IC_{50} were categorized into four categories: IC_{50}<50 µg/mL regarded very strong, IC_{50} = 50-100 µg/mL regarded strong, IC_{50} = 101-150 µg/mL regarded medium, and IC_{50} >150 µg/mL regarded as weak antioxidant activity (Molyneux 2004).

Statistical analysis

The results were reported as the mean ± standard deviation (SD) of three replications of each sample. The data were analyzed by using the one-way analysis of variance (ANOVA) and followed by Duncan's multiple range test. The values of $p < 0.05$ were regarded as statistically significant. All statistical analysis was performed by using SPSS statistical package version 22.0 (SPSS Inc. Chicago, IL, USA).

RESULTS AND DISCUSSION

Total flavonoid and polyphenols content

The content of total flavonoids and polyphenols of the seven local corn cultivars is presented in Table 1. The total flavonoid content of local corn seeds from Kisar Island, Southwest Maluku District, varied from 8.463 mg QE/mg (*Putih* cultivar) to 40.075 mgQE/mg (*Kuning Dalam* cultivar). Total polyphenol content also varied between 105.63 mg GAE/mg (*Merah Delima Tongkol Putih* cultivar) and 136.41 mg GAE/mg (*Pulut* cultivar).

One-way ANOVA was used to determine the presence of significant differences among the mean of total flavonoids and polyphenol content of local corn cultivars. The results of ANOVA showed that the corn cultivars had a significant difference ($p < 0.05$) in the total flavonoids and phenolics content. Although corn cultivars had a significant effect on flavonoids and phenolics contents, there were no significant differences between cultivars as seen in *Merah Delima Tongkol Cokelat*, *Merah Delima Tongkol Putih*, *Pulut,* and *Putih*, and also between *Merah Darah* and *Kuning Genjah* for flavonoids content respectively. In terms of flavonoid content, *Kuning Dalam* cultivars were significantly different from all cultivars, while for the phenolics content, there were no significance differences between *Merah Delima Tongkol Putih*, Merah Darah, Kuning Genjah, and Puti Cultivar, respectively. *Kuning Dalam* cultivars were significant differences from *Merah Delima Tongkol Putih*, *Merah Darah*, *Kuning Genjah*, *Pulut*, and *Putih* but not significant differences from *Merah Delima Tongkol Cokelat*. Likewise, *Pulut* cultivars were significant differences from *Merah Delima Tongkol Putih*, *Merah Darah*, *Kuning Genjah*, *Kuning Dalam,* and *Pulut* but not significant differences from *Merah Delima Tongkol Cokelat.*

Measurement of antioxidant activity by DPPH assay

The seven local corn cultivars had IC_{50} values similar to quercetin (used as a control), some were even lower. The IC50 value of the seven local corn cultivars ranged from 5.56 to 16.96 µg/mL, while quercetin had an IC_{50} value of 11.97 µg/mL. The IC_{50} of extract of corn from Kisar Island with quercetin as standard is shown in Table 2.

All cultivars' free radical scavenging activity was very strong, similar to quercetin. This finding shows that local corn cultivars from Kisar Island have the potential to be developed into nutraceutical formulations. It has been known for a long time that antioxidant compounds such as flavonoids and phenolics can inhibit free radicals because these compounds can donate their hydrogen atoms to free radicals, thereby inhibiting the further activity of free radicals. Antioxidants can prevent oxidative propagation by stabilizing the generated radicals in the human body and helping reduce oxidative damage (Shalaby 2019). There are two main types of antioxidants, the primary antioxidants that can break the chain reaction or free radical scavengers and the secondary or preventive ones (Santos-Sánchez et al. 2019). The secondary antioxidant mechanisms may include metal deactivation, lipid hydroperoxides inhibition by interrupting the undesirable volatile production, primary antioxidants regeneration, and singlet oxygen elimination (Santos-Sánchez et al. 2019). Therefore, antioxidants can be defined as those substances that can prevent or greatly slow the oxidation of easily oxidizable materials in low amounts (Santos-Sánchez et al. 2019).

Flavonoids are a type of polyphenols and are widely recognized polyphenols and are abundant in the pericarp and aleuron of corn grains (Ramos-Escudero et al. 2012a; Bae et al. 2021). In our study, the content of polyphenols and flavonoids was determined from whole grain flour, which could be a reason for the strong antioxidant capacity obtained from the tested samples. However, with the different colors in corn kernels, such as purple, red and yellow, it can be assumed that its antioxidant activity is not only due to the presence of flavonoids but may also be due to the presence of other compounds such as anthocyanins and carotenoids which were not evaluated in this study.

Table 1. Total flavonoid and polyphenol content of seven local corn cultivars from Kisar Island, Southwest Maluku District in Indonesia

Name of local corn cultivars	Total flavonoid content (mg QE/mg DW)	Total polyphenol content (mg GAE/mg DW)
Merah Delima Tongkol Cokelat	10.78 ± 7.08^{c}	129.63 ± 1.86^{ab}
Merah Delima Tongkol Putih	8.68 ± 0.19^{c}	105.63 ± 0.88^{c}
Merah Darah	22.30 ± 0.66^{b}	112.63 ± 1.33^{c}
Kuning Genjah	18.17 ± 0.53^{b}	112.08 ± 8.60^{c}
Kuning Dalam	40.08 ± 0.99^{a}	123.86 ± 6.77^{b}
Pulut	8.46 ± 0.70^{c}	136.41 ± 2.91^{a}
Putih	5.99 ± 0.74^{c}	113.30 ± 2.08^{c}

Note: Values with different letters in each column are significantly different ($p<0.05$) as assessed by Duncan's multiple range test

Table 2. DPPH IC_{50} of seven local corn cultivars from Kisar Island, Southwest Maluku District in Indonesia

Name of local corn cultivar	IC_{50} (mg/mL) (mean ± SD)	Category of antioxidant activity[1]
Merah Delima Tongkol Cokelat	11.60 ± 0.48^{b}	Very strong
Merah Delima Tongkol Putih	12.52 ± 1.48^{b}	Very strong
Merah Darah	5.94 ± 0.34^{a}	Very strong
Kuning Genjah	5.71 ± 3.96^{a}	Very strong
Kuning Dalam	5.56 ± 0.33^{a}	Very strong
Pulut	16.96 ± 1.25^{c}	Very strong
Putih	15.01 ± 3.03^{b}	Very strong
Quercetin	11.97 ± 0.09^{b}	Very strong

Note: Values with different letters in each column are significantly different ($p<0.05$) as assessed by Duncan's multiple range test. 1IC_{50}<50 µg/mL is regarded as very strong, IC_{50} = 50-100 µg/mL is regarded as strong, IC_{50} = 101-150 µg/mL is regarded as a medium, and IC_{50} >150 µg/mL is regarded as having a weak antioxidant activity (Molyneux 2004)

Likewise, for the flavonoids and phenolics, One-way ANOVA (Table 2) was conducted on the free radical scavenging activity. The results show a significant effect of corn cultivars on the free radical scavenging activity. It was observed that the *Merah Delima Tongkol Cokelat, Merah Delima Tongkol Putih* and *Putih* cultivars had comparable antioxidant activity to the control quercetin. The *Merah Darah, Kuning Genjah* and *Kuning Dalam* cultivars exhibited a statistically significant higher antioxidant capacity than quercetin. As observed in Figure 2, the *Pulut* cultivar had the highest phenolic content, the third lowest in flavonoids, and a very strong antioxidant activity but was lower than quercetin. The *Kuning Dalam* cultivar was the highest in flavonoids but the second in phenolic and had a strong free radical scavenging activity higher than quercetin. The *Merah Delima Tongkol Cokelat* cultivar is the fourth in flavonoids but the second in phenolic and very strong in antioxidant activity comparable to quercetin.

Pearson correlation coefficients

Correlation coefficients for the antioxidant activity (DPPH) concerning the total content of bioactive compounds (flavonoids and polyphenols) in the different corn cultivars were calculated by the Pearson coefficient (Asuero et al. 2006; Gonzalez et al. 2006; Ramos-Escudero et al. 2012a). As shown in Table 3, there are differences in the correlation between antioxidant activity and total polyphenol and total flavonoid content. It was observed that the correlation coefficient (r=-0.784) between the DPPH and total flavonoid was significantly different (p-value <0.05) and that this type of bioactive compound contributes the most to the antioxidant activity. This result correlates with previous reports on guava (Thaipong et al. 2006), purple corn (Ramos-Escudero et al. 2012a), plums, peaches, and nectarines (Gil et al. 2002). However, the correlation coefficient between DPPH and total polyphenol was not statistically significant. This could mean that there is the potential for other bioactive compounds or antinutrients to have an antagonistic effect on the antioxidant activity. This has been reported with binary and ternary mixtures of gallic, ferulic, and caffeic acid that reported antagonistic antioxidant effects (Olszowy et al. 2019).

Differences in flavonoids, phenolics, and free radical scavenging activity may be due to differences in pigmentation in corn kernels (Figure 1). It is well-recognized that plants with intense colors such as red, purple, or yellow contain pigments such as anthocyanins or carotenoids (Ramos-Escudero et al. 2012b). The *Merah Delima Tongkol Putih* has a darker color, which could mean it contains the highest anthocyanin content, and based on our results, it presents a total polyphenol content of 129.63 ±1.86 mg GAE/mg DW, which was not statistically different from the *Pulut* cultivar (136.41 ± 2.91 mg GAE/mg DW) that presents a white-yellow color. The content of anthocyanins or carotenoids was not tested in this study, but because of their nature could contribute to the free radical scavenging activity of corn cultivars. This could contribute to our results, where cultivars with high flavonoids and phenolics do not necessarily have a strong free radical scavenging activity. It is also important to note that multiple sub-families of polyphenols do not contribute color to fruits and vegetables (Yáñez et al. 2007a).

Furthermore, various factors can affect the content of flavonoids and phenolics. It has been reported that biosynthesis, accumulation and/or transport of bioactive compounds such as total phenolic and flavonoids in corn kernels depend on multiple factors, such as environmental, cultivation practices, soil fertility, fertilizations, genotype, epigenetic factors and genotype-environment interactions (Martínez-Martínez et al. 2019). Therefore, the observed difference in the content of flavonoids, phenolics and free radical inhibitory activity could be attributed to genetic differences among the corn cultivars, which would affect the content of bioactive compounds. Another important consideration is that the bioactive compounds (flavonoids and phenolics) present in the corn kernels are in their glycoside form, which has been reported to have weak free radical scavenging activity (Molyneux 2004). Furthermore, the purity of the extract can also affect the antioxidant capacity since the presence of other compounds can weaken the free radical scavenging activity, as previously reported (Sami and Rahimah 2016).

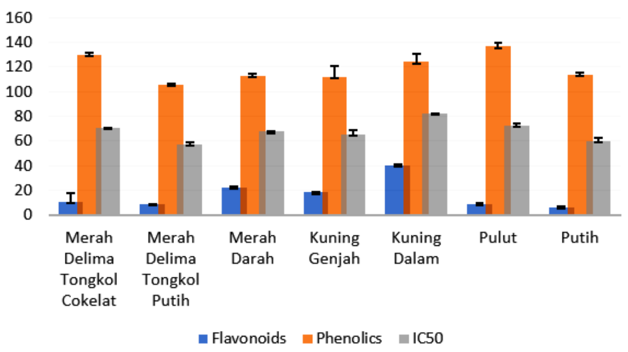

Figure 2. Comparison of flavonoids, phenolics, and IC_{50} of seven local corn cultivars from Kisar Island, Southwest Maluku District, Indonesia

Table 3. The correlation coefficient of antioxidant activity (DPPH IC_{50}) concerning total content of polyphenols and flavonoids of seven local corn cultivars from Kisar Island, Southwest Maluku District, Indonesia

Characteristic	N	DPPH IC_{50}			
		T statistic	Degrees of freedom (DF)	p-value	Pearson coefficient (r)
Total flavonoid	7	2.824	5	0.037	-0.784*
Total polyphenol	7	0.853	5	0.433	0.356

Note: * Significant at the p-value <0.05

In conclusion, Based on the result and discussion, it can be concluded that the *Pulut* cultivar exhibited the highest total polyphenol content, while the *Kuning Dalam* cultivar reported the third highest total polyphenol content, the highest total flavonoid content and one of the strongest antioxidant activity. These results can contribute to the selection of corn cultivars to harvest for commercial purposes and potential nutraceutical applications.

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