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Phytochemical compounds and antioxidant activity of *Coleus tuberosus* flesh and peel on different solvent

[1]Nugraheni, M., [2]Santoso, U. and [3]Windarwati

[1]*Department of Culinary Art Education, Engineering Faculty, Yogyakarta State University, Indonesia*
[2]*Department of Food Science, Faculty of Agricultural Technology, Gadjah Mada University, Indonesia*
[3]*Dr. Sardjito General Hospital, Yogyakarta, Health Ministry of Republic Indonesia*

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Abstract

Three different solvents were used to extract the phytochemical compounds in the peel and flesh of *Coleus tuberosus*. The results of extraction with different solvents were compared based on the levels of phytochemicals compounds and its antioxidant activity. Extraction of bioactive compounds was done by the maceration method using methanol, ethyl acetate, and chloroform for seven days at a ratio of 1:5 (w/v). Determination of total phenolic contents and flavonoids was conducted via spectrophotometric method. Antioxidant activity was evaluated based on DPPH, peroxide value and TBA value. The results showed that the methanol solvent extracted higher total phenolic and flavonoids compounds than the ethyl acetate and chloroform. The antioxidant activity of methanol extracts was higher compared to ethyl acetate and chloroform extracts for both flesh and peel of *C. tuberosus*. The extract of the peel of *C. tuberosus* had antioxidant activity higher compared to the flesh extract with the use of methanol, ethyl acetate or chloroform as solvent. There was a positive and significant correlation between total phenolic and flavonoids content against DPPH and a negative and significant correlation with peroxide and TBA value.

1. Introduction

Coleus tuberosus include local tubers that belong to Lamiaceae family, sub nepetoideae family, tribe Ocimeae and has an essential role in food security that contains some types of bioactive compounds known to function as antioxidants and anti-cancer. The bioactive compounds identified from *C. tuberosus* were triterpenic acid including ursolic acid and oleanolic acid, total phenol and flavonoid (Mathe *et al.,* 2007; Nugraheni *et al.,* 2011). Some research showed that bioactive compounds in fruits and vegetables namely total phenolic content, flavonoid, and triterpenic acid have the ability as an antioxidant (Fu *et al.*, 2017; Shiraishi *et al.,* 2018). The potential of an antioxidant is associated with the ability to free scavenging radicals, donates hydrogen atoms or electrons and chelates metal to decrease the risk of degenerative disease such as cancer and diabetes mellitus (Wang *et al.,* 2018; Arif *et al.,* 2018).

The effort to obtain natural antioxidants from plants, fruits and vegetables can be performed by several methods of solvent extraction, maceration, supercritical fluid extraction and so on. However, the number of bioactive compounds and antioxidant activity does not only depend on the extraction process but, it is also influenced by the type of solvent used. The extraction process aims to get certain parts of ingredients that contain bioactive compounds (Azmir *et al.,* 2013; Resende *et al.,* 2017). The extraction method used by many is distillation and solvent extraction. Solvent extraction can be done in the two ways: aqueous phase and organic phase. Extraction of the aqueous phase is done using water as solvent, while the organic phase uses organic solvent (Bodoira *et al.,* 2017; Chen *et al.,* 2017).

Based on the polarity, the type of solvent and the method may affect their ability to prevent oxidation. If the solvent is polar, it is able to extract the alkaloid compounds, total phenolic, flavonoids, carotenoids, tannin, sugar, amino acids, and glycosides content. The objectives of this research were to identify the phytochemical compounds and to evaluate the antioxidant activity of *C. tuberosus* flesh and peel using different solvents.

*Corresponding author.
Email: *mutiara_nugraheni@uny.ac.id*

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2. Materials and methods

2.1. Chemicals and reagents

The chemical materials used were methanol, ethyl acetate, distilled water, Folin-Ciocalteu reagent, $NaCO_3$, and gallic acid, $NaNO_2$, $AlCl_3.6H_2O$, NaOH, BHT, 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma Aldrich) linoleic acid, chloroform (HPLC grade), quercetin (Sigma Aldrich) as standard.

2.2. Sample preparation

C. tuberosus peel was separated from the flesh using a peeler. The flesh was then thinly sliced with a thickness of 1-1.5 mm. The flesh and peel were examined as a subject separately. The flesh and peel of *C. tuberosus* were dried with a cabinet drier at a temperature of 40°C for 24 hrs. After drying, the dried peel and flesh was milled into flour and sifted using a Tyler sieve 80 mesh before storing at -20°C for further analysis.

2.3. Extraction process

The peel and flesh flour were subjected to extraction by maceration method using solvents methanol, ethyl acetate, and chloroform. The ratio of flour to solvent was 1:5 (w/v). The process of maceration was carried out for seven days. After seven days, the solution was filtered with Whatman No. 1 filter paper and evaporated using a rotavapor to remove the solvent. The extracts were then stored at -22°C.

2.4. Determination of total phenolic content

The total phenolic content of the different solvents, i.e., methanol, ethyl acetate and chloroform, extract of *C. tuberosus* flour were determined using spectrophotometric method (Singleton *et al.,* 1999). At a concentration of 100 mg/L, 0.2 mL of the extract were added to 2.5 mL of 10% Folin-Ciocalteu reagent and 2 mL of 7.5% Na_2CO_3.The mixture was incubated for 15 mins at 45°C. The absorbance of the solution was measured using a spectrophotometer at a wavelength of 765 nm. The content of total phenolic was expressed as milligrams (mg) of gallic acid equivalent per gram of the dried extract (mg of GAE/g extract). Each sample was analyzed in triplicate to obtain an average value.

2.5. Determination of total flavonoids content

The analysis of the levels of flavonoids was done with spectrophotometric method (Quettier-Deleu *et al.,* 2000). At a concentration of 1000 mg/L, 1 mL of the aqueous solution extract was added to 1 mL 2% $AlCl_3$ dissolved with ethanol 50%. At the twentieth minute of incubation, the mixture was homogenized using the vortex and the absorbance was measured at 415 nm. The analysis was done in triplicate measurements, and the content of flavonoids was expressed as quercetin equivalents (mg of quercetin/g of extract).

2.6. Antioxidant activity evaluation: DPPH method

Evaluation of antioxidant activity was performed with the DPPH method (Singh *et al.,* 2009). The extracts were aliquoted (40 µg/mL) and mixed with 2 mL of DPPH (0.1 mM in methanol solution). The solution was homogenized with vortex, which was stand for 30 mins at room temperature, protected from light. The absorbance of the solution was measured at a wavelength of 517 nm. The butylated hydroxytoluene (BHT) concentration at 40 µg/ml was used as the comparative compound. The radical scavenging activity was calculated using the formula:

$$\frac{A_0 - A_1}{A_0} \times 100\%$$

Where A_0 is the absorbance of the control (without the extract), and A_1 is the absorbance of BHT or the extract.

2.7. Antioxidant activity evaluation: peroxide value

The peroxide value was determined using the ferric thiocyanate (FTC) method (Zahin *et al.,* 2009) for antioxidant activity. The extracts (40 µg/mL) were mixed with BHT (40 µg/mL) in absolute ethanol, 4.1 mL of 2.5% linoleic acid in absolute ethanol, 8.0 mL of 0.05 M phosphate buffer (pH 7.0) and 3.9 mL of water was placed in a screw cap vial and incubated at 40°C in dark. To 0.1 mL of this solution was added 9.7 mL of 75% ethanol and 0.1 mL of 30% ammonium thiocyanate. Precisely after 3 mins, 0.1 mL of 0.02 M ferrous chloride in 3.5% HCl was added to the reaction mixture and the absorbance of was measured at 500 nm at 24 hrs interval until five days. All measurements were carried out in triplicate. The peroxide value was expressed as milliequivalent per 1000 g of sample.

2.8. Antioxidant activity evaluation: thiobarbituric acid (TBA)

The antioxidant activity evaluation with thiobarbituric acid (TBA) was referred to Zahin *et al.* (2009). The sample solution (1 mL) was added with 2 mL of 0.67% 2-thiobarbituric acid and 2 mL of 20% trichloroacetic acid, as prepared in the FTC method. The mixture was placed in a boiling water bath. After cooling, was centrifuged at 3000 rpm for 20 mins. The absorbance of the supernatant was measured at 552 nm. The antioxidant activity was based on the absorbance on the final day of the FTC method. Malonaldehyde concentration calculated with standard 1,1,3,3-tetraethoxypropane (TEP). The standard curve used malonaldehyde 1,1,3,3-tetraethoxypropane solution

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(TEP). TBA value stated was expressed as mg MDA/1000 g of the sample.

2.9. Statistical analysis

The correlation between the treatment (the relationship between total phenolics content components with the level of total and total antioxidant activity and phenolic content relationship with antioxidant activity) tested with Pearson correlation bivariate using Statistical Product and Service Solution (SPSS) 12.0 for windows.

3. Results and discussion

3.1. Levels of phytochemicals

Polarity differences can give different results in the extract of phytochemical compounds in vegetables and fruits. The necessary efforts to the selection of the type of solvent compound to obtain optimum results on the type of different bioactive compounds are required. The extraction process of total flavonoids and phenolic content were done using three solvents namely methanol, ethyl acetate, and chloroform. The use of different solvents was to find out the type of solvent that can extract the optimum of total phenols and flavonoids level. The extraction was done by the maceration method for seven days.

Table 1. The levels of total phenols and total flavonoids on *C. tuberosus* flesh and peel in different solvent

Solvent	TPC (mg GAE/g)	TFC (mgQCE/g)
Peel		
Methanol extract	7.73±0.08fC	8.55±0.07fC
Ethyl acetate extract	6.20±0.11cB	5.52±0.19eB
Chloroform extract	1.78±0.03aA	1.61±0.09cA
Flesh		
Methanol extract	7.24±0.10eB	2.31±0.13dC
Ethyl acetate extract	7.14±0.12dB	1.45±0.17bB
Chloroform extract	1.84±0.08bA	0.27±0.03aA

Different letters (a-f) within the column indicate significant differences in flesh and peel of *C. tuberosus* on different solvent at $P < 0.05$.
Different capital letters (A-C) within the column in *C. tuberosus* flesh or peel indicate significant differences in different solvents at $P < 0.05$.

The levels of total phenolic and flavonoids of the extracts of *C. tuberosus* peel and flesh with different solvents are shown in Table 1. The peel extract contents of total phenolic and flavonoids were higher than the flesh extract of *C. tuberosus*. The results were in agreement with the research conducted by Ahmadi-Alfazi *et al.* (2015) and Saidani *et al.* (2017). Total phenolics compounds acres fills up in higher amounts extracted with a more polar solvent. Based on this result, it was shown that the number of total phenolic and flavonoids extracted using methanol was higher than the extraction using ethyl acetate and chloroform. The higher number of total phenolic and flavonoids extracted using methanol indicates that the total phenolic content and flavonoids have polar non-polar bonds than a tendency. Many research also showed that the extraction of total phenolic and flavonoids using polar solvents can provide significant results than using a non-polar solvent (Do *et al.,* 2014; Pintac *et al.,* 2018).

3.2. Antioxidant activity evaluation via DPPH method

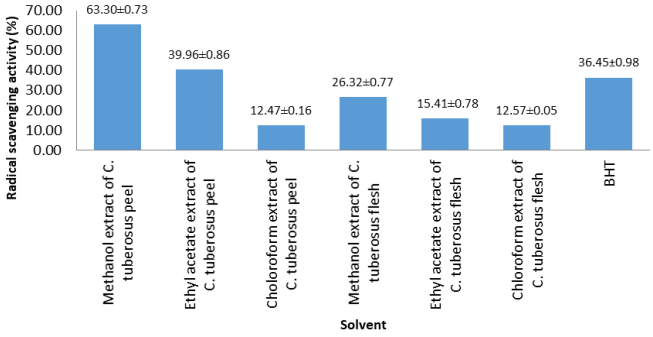

Figure 1. Antioxidant activity of *C. tuberosus* peel and flesh with different solvent based on DPPH method

DPPH method is used to evaluate the activity of antioxidant a substance. It was evaluated by measuring scavenging free radicals using a synthetic radical which is 1, 1, 2, 2- diphenyl picrylhydrazin (DPPH). The DPPH changes are detected by the decrease of the DPPH absorbance in the solution when added with antioxidant compounds. Figure 1 shows the antioxidant activity value measured using the DPPH method. The significant decrease in absorbance indicates a higher antioxidant activity of the solution. The methanol extract which contains higher total phenolic and flavonoids extract than that of ethyl acetate and chloroform extract demonstrated greater antioxidant activity when compared to the antioxidant activity of the extract of ethyl acetate and chloroform (Figure 1).

Figure 1 also shows the tendency of peel had a higher antioxidant activity than the flesh of *C. tuberosus*. The different antioxidant activity relates to the difference between the content of total phenolics and flavonoids in the flesh and peel (Table 1). Antioxidant activity differences on the flesh and peel can be related to the differences in the bioactive compounds (Wang *et al.,* 2012; Raudone *et al.,* 2017). Medini *et al.* (2014) stated that total phenolics and flavonoids have an excellent free radical scavenging ability and can be used as a radical scavenger, acting possibly as a primary antioxidant.

This research proves that the percentage of radical scavenging activity on the peel of *C. tuberosus* is higher than the flesh. The peel contained more bioactive compounds which have the greater ability to transfer

hydrogen atoms to DPPH free radicals to form a stable compound diphenyl picrylhydrazyl stable. The higher diphenyl picrylhydrazyl that formed shows higher antioxidant capability, especially on the scavenging of free radicals. Antioxidant activity differences were allegedly caused by bioactive compounds that are in the peel and flesh of *C. tuberosus*, suppose maslinic acid and phytosterol (stigmasterol, beta-sitosterol, campesterol) (Fu *et al.,* 2014; Prasad *et al.,* 2016).

3.3. Antioxidant analysis with peroxide value method

Antioxidant activity with peroxide value method was used to find out the capabilities of the peel and flesh extract of *C. tuberosus* in different solvents in inhibiting the lipids peroxidation. Peroxide is formed as a result of the process of oxidation that occurs in the oil during the incubation period (Halvorsen *et al.,* 2011). Table 2 shows that the bioactive compounds found in extract peel and flesh of methanol, ethyl acetate, and chloroform can reduce the radical hydroxyl, radical peroxide, and super peroxide on the system of an emulsion to inhibit oxidation. Antioxidants neutralize free radicals by giving one of its electrons to the free radicals and transforms into non-radical forms. The addition of antioxidants can inhibit the rate of increase in the number of peroxides.

Peroxide value in the linoleic system increased with increasing incubation time of the peel or flesh of methanol, ethyl acetate, chloroform extract of *C. tuberosus*, and BHT. Peroxide number in the system that given methanol, ethyl acetate, chloroform extract of *C. tuberosus* peel or flesh and BHT were lower than controls. This research proved that the extract of methanol, ethyl acetate, chloroform of *C. tuberosus* extract and BHT could suppress the formation of linoleic acid hydroperoxide.

The methanol extract of *C. tuberosus* flesh or peel can suppress peroxide value higher than chloroform and ethyl acetate extract. The ability to suppress peroxide value was shown by the lowest of a number of meq peroxide on the fifth day with methanol extracts compared to ethyl acetate and chloroform extracts. The methanol, ethyl acetate and chloroform extract of *C. tuberosus* peel inhibit lipid peroxidation greater than the methanol, ethyl acetate and chloroform extract from *C. tuberosus* flesh.

Methanol extract, ethyl acetate extract and chloroform extract of flesh and peel of *C. tuberosus*, and BHT added to the system can inhibit lipid peroxidation, hence ammonium thiocyanate is formed less. The oxidation of linoleic acid can create free radicals as a result of the incubation process at 40°C. Free radicals cause the fatty acid to change to lipid peroxide and oxidizes Fe^{2+} to $Fe^{3+.}$ cations that increase the number of oxidation will react specifically with ammonium thiocyanate, forming ammonium hexothiocyanotoferrate (III) $(NH_4)_3[Fe(SCN)_6]$ and the color red. The ability of the antioxidant to inhibit oxidation is shown with the least amount of Fe^{2+} oxidized by peroxide linoleic acid to Fe^{3+}, which is indicated by the decrease in redness intensity. Lipid autooxidation is a chain reaction of free radicals that might be blocked by methanol, ethyl acetate and chloroform extract of flesh and peel of *C. tuberosus*, therefore, stopping the reaction at the termination stage.

Methanol, ethyl acetate, and chloroform extract of *C. tuberosus* peel can inhibit lipid peroxidation greater than the methanol, ethyl acetate and chloroform extract of *C. tuberosus* flesh. The higher inhibition of the peel extract can be related to the content of the total phenolic content and total flavonoid that was higher than the flesh extract (see Table 1). The difference in the ability of antioxidant activity can also be related to the different content of total phenol and flavonoids in the flesh and peel (Aalolam *et al.,* 2016). Many research has proven that phenol showed antioxidant activity based on FTC method (Gharibi *et al.,* 2013; Yu *et al*., 2013).

Table 2. The peroxide value on different solvent of the flesh and peel of *C. tuberosus* , BHT and control; incubation on 40°C, for 5 days (meq peroxide/1000g of sample)

Solvent	Zero time	1 days	2 days	3 days	4 days	5 days
			Peel			
Methanol	0.07±0.01^{a}	0.09±0.01^{a}	0.42±0.03^{b}	0.52±0.02^{a}	0.62±0.02^{a}	0.56±0.06^{a}
Ethyl acetate	0.07±0.01^{a}	0.51±0.04^{d}	0.73±0.05^{d}	1.83±0.06^{e}	1.13±0.08^{c}	0.78±0.08^{c}
Chloroform	0.07±0.01^{a}	0.32±0.02^{c}	0.52±0.04^{c}	1.51±0.07^{d}	1.26±0.06^{d}	1.73±0.06^{d}
			Flesh			
Methanol	0.07±0.01^{a}	0.15±0.01^{b}	0.29±0.00^{ba}	0.91±0.05^{b}	0.79±0.03^{b}	0.69±0.02^{ab}
Ethyl acetate	0.07±0.01^{a}	0.41±0.04^{c}	0.77±0.06^{d}	1.25±0.05^{c}	0.87±0.10^{b}	0.79±0.08^{bc}
Chloroform	0.08±0.01^{a}	0.52±0.02^{e}	1.18±0.07^{e}	1.62±0.06^{f}	1.52±0.04^{e}	1.87±0.08^{e}
BHT	0.07±0.01^{a}	0.33±0.04^{c}	0.48±0.03^{bc}	0.58±0.06^{a}	0.64±0.05^{a}	0.72±0.04^{c}
Control	0.07±0.01^{a}	1.08±0.05^{e}	1.15±0.08^{e}	2.54±0.07^{f}	2.89±0.08^{e}	2.50±0.07^{e}

Different letters (a-f) within the column indicate significant differences at $P < 0.05$.

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Table 3. TBA value on different solvent of the peel and flesh of *C. tuberosus* , BHT and control (as mg MDA/1000 g of sample)

Solvent	Zero time	1 days	2 days	3 dyas	4 days	5 days
			Peel			
Methanol	1.39±0.03[a]	2.37±0.06[c]	2.94±0.06[b]	5.31±0.50[a]	5.89±0.04[a]	6.08±0.02[a]
Ethyl acetate	1.32±0.04[a]	1.65±0.07[b]	2.45±0.23[a]	8.38±0.08[c]	8.24±0.05[c]	7.47±0.02[c]
Chloroform	1.30±0.6[a]	3.55±0.08[e]	3.96±0.07[c]	9.39±0.20[e]	10.20±0.34[c]	8.39±0.34[e]
			Flesh			
Methanol	1.36±0.05[a]	2.41±0.07[c]	3.11±0.07[b]	7.14±0.11[b]	8.13±0.06[b]	6.92±0.03[b]
Ethyl acetate	1.35±0.08[a]	1.69±0.03[b]	2.24±0.08[a]	8.41±0.09[c]	8.23±0.04[c]	8.08±0.01[d]
Chloroform	1.36±0.05[a]	1.35±0.09[a]	4.33±0.20[d]	9.83±0.09[f]	11.78±0.15[d]	9.52±0.12[f]
BHT	1.30±0.05[a]	2.72±0.04[d]	3.80±0.05[c]	8.08±0.07[d]	9.71±0.10[e]	7.84±0.09[d]
Control	1.32±0.05[a]	4.96±0.13[f]	5.56±0.16[e]	8.05±0.20[g]	11.87±0.13[f]	11.47±0.13[g]

Different letters (a-f) within the column indicate significant differences at $P < 0.05$.

3.4. Antioxidant activity evaluation with TBA

Antioxidant activity of methanol extract, ethyl acetate extract and chloroform extract from the flesh and peel of *C. tuberosus* and BHT were evaluated using the TBA method. The malondialdehyde of linoleic acid incubated with methanol extract, ethyl acetate extract and chloroform extract from the flesh and the peel, BHT, increased after five days of observation. Table 3 shows that the bioactive compounds extract from methanol solvent for both peel and flesh extract have higher antioxidative capabilities compared to the other solvents.

This research suggests that the antioxidant activity of methanol extract of *C. tuberosus* flesh or peel have the highest antioxidant activity compared ethyl acetate and chloroform extract. However, on the fifth day, the methanol extract of *C. tuberosus* peel and flesh had the lowest mg MDA compared to ethyl acetate and chloroform extract of *C. tuberosus* peel and flesh. The sequence of the inhibitory ability of MDA-TBA is methanol extract > ethyl acetate extract > chloroform extract. The ability to inhibit the formation of the complex MDA-TBA on methanolic extract, ethyl acetate extract and chloroform extract of *C. tuberosus* can be related to the total phenolic and flavonoid content (Table 1). These results were consistent with other research that indicated the content of phenols and flavonoids in the sample could suppress the MDA on the evaluation of antioxidants with TBA (Adebiyiab *et al.,* 2017).

Some research showed that the extract of *C. tuberosus* also contains bioactive compounds such as ursolic acid, oleanolic acid (Nugraheni *et al.,* 2011), maslinic acid, and phytosterol compounds may inhibit peroxidation lipids by suppressing the formation of MDA so that the condensation reaction between the MDA and TBA that form the complex of the MDA-TBA can be hindered (Khennouf *et al.,* 2010). The antioxidant activity difference is related to the difference in the content of bioactive compounds that are located on the peel or the flesh and thus, influence the ability of the formation of the complex MDA-TBA (Jung *et al.,* 2011).

3.5. A correlation between phytochemical compounds with antioxidant activity three methods (DPPH antioxidant, FTC, and TBA)

Table 4. Correlation of bioactive compounds with antioxidant activity

Parameter	DPPH	TBA value	Peroxide value
Total phenolic	0.639*	-0.990*	-0.706*
Total Flavonoids	0.983*	-0.604*	-0.785*
DPPH	1	-0.829*	-0.634*
Peroxide value	-0.634*	0.692*	1
TBA value	-0.829*	1	0.692*

* Significant correlation at 0.05 level

There is a correlation between the number of total phenolic and flavonoids on the extracts of *C. tuberosus* peel and flesh with antioxidant activity. Table 4 shows that the total phenolic and flavonoids have a positive and significant correlation with the DPPH method. This shows the high content of total phenols and flavonoids can increase antioxidant activity with the DPPH method.

The total phenolic content and total flavonoid with TBA and Peroxide value showed a negative and significant correlation. It indicates that higher levels of total phenolic and flavonoids have an impact on the low level of peroxide and TBA value. The low level of peroxide and the TBA value shows that the extract has antioxidant activity. Phenolic compounds have the ability to scavenge free radicals; it is proven by the existence of the strong correlation between the total phenolic content compounds and RSA (Pandey *et al.,* 2017; Shao *et al.,* 2018).

A correlation between the method of determination of antioxidant activity shows that the DPPH have a negative correlation with TBA and peroxide value. A negative correlation indicates that the evaluation of antioxidant activity with the DPPH method is inversely

proportional to the number and peroxide TBA. A high percentage of DPPH method showed high antioxidant activity. While low peroxide value and TBA value low indicate a high antioxidant activity, related to its ability to inhibit the lipids peroxidation.

4. Conclusion

Methanol is shown to extract higher content of total phenolic content, and flavonoid. The methanolic extract has greater antioxidant activity than ethyl acetate extract and chloroform extract on the antioxidant analysis with the DPPH method, peroxide value, and TBA value. The peel of *C. tuberosus* extracted with methanol, ethyl acetate and chloroform have higher total phenolic content, and flavonoid than the flesh of *C. tuberosus*. The peel extract of *C. tuberosus* had higher antioxidant activity compared to the flesh extract based on DPPH, peroxide value and TBA value.

Conflict of interest

The authors declare no conflict of interest

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