International Food Research Journal 28(4): 839 - 847 (August 2021)

Journal homepage: *http://www.ifrj.upm.edu.my*

Antioxidant activity and total phenolic content of fresh and cured rhizomes of *Curcuma longa* and *Etlingera philippinensis*

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Article history

Received: 2 October 2019
Received in revised form: 4 November 2020
Accepted: 25 March 2021

Keywords

curcumin content,
Curcuma longa,
Etlingera philippinensis,
total antioxidant activity,
total phenolic content

Abstract

The search for effective natural antioxidants has been increasing nowadays due to human health concern. Further investigation of the Zingiberaceae plants such as *Etlingera philippinensis*, a Philippine endemic plant, and *Curcuma longa* (turmeric) is key towards alleviating this concern. The present work aimed to provide fundamental information on the total antioxidant activity (TAA), total phenolic content (TPC), and curcumin content of the ethanolic extracts of the fresh and cured rhizomes of *C. longa* and *E. philippinensis*. Healthy and fresh rhizomes of *E. philippinensis* were collected from Kibawe, Bukidnon, while *C. longa* were from Musuan, Bukidnon, Philippines. TAA determination was done using phosphomolybdenum method, while TPC and curcumin content using spectrophotometric method. Results showed that the TAA of fresh and cured rhizomes of C. longa were significantly higher than those of fresh and cured rhizomes of *E. philippinensis*. The TPC of fresh and cured rhizomes of *C. longa* were significantly higher than those of fresh and cured rhizomes of *E. philippinensis*. Significantly higher curcumin content was also observed in the rhizomes of *C. longa* as compared to that in the fresh and cured rhizomes of *E. philippinensis*. Generally, the ethanolic extracts of the cured rhizomes of *E. philippinensis* and *C. longa* exhibited higher TAA, TPC, and curcumin content than the fresh one. TAA, TPC, and curcumin were significantly correlated with each other. It can thus be concluded that *E. philippinensis* could also be a source of valuable curcumin.

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Introduction

Plants are well-known to possess medicinal properties that fight numerous diseases (Shahid-Ud-Daula *et al.,* 2015). These medicinal plants are rich in secondary metabolites such as alkaloids, glycosides, flavonoids, steroids, and other phenolic compounds that are important sources for new chemical substances with significant therapeutic effects (Omodamiro and Jimoh, 2014) such as antibacterial and antioxidant activities. The search for novel and effective antibacterial and antioxidant compounds of plant origin has been done due to their low toxicities and strong bioactivities (Shahid-Ud-Daula *et al.,* 2015).

Among the natural sources of bioactive compounds is the Zingiberaceae plant, commonly known as ginger, which is a perennial herb that produces aromatic rhizomes. The plant exists in about 50 genera and 1,300 species worldwide, and is mainly distributed in the South and Southeast Asia (Chen *et al.,* 2008). According to Chan *et al.* (2008), the leaves and rhizomes of ginger species are widely used as spice, condiment, and traditional medicine. It is generally believed that antioxidants produced by ginger plants are transported to the rhizomes where they are accumulated (Chan *et al.,* 2008). Several studies have shown that the rhizome contains higher phenolic content, antioxidant, and antibacterial activity than its leaves (Chan *et al.,* 2008; 2011).

In this present work, two genera from the family Zingiberaceae were investigated, namely *Curcuma longa* L. (Figure 1a and 1b) and *Etlingera philippinensis* (Ridl.) R.M.Sm. (Figure 1c and 1d). *C. longa* is also known as turmeric. It is traditionally produced and consumed as turmeric powder (Prasad and Aggarwal, 2011), and widely used as food additive in many Asian dishes, and also as traditional remedy to treat diseases such as diarrhoea, coryza, dermatosis, rheumatism, and household remedy for sprains and swellings (Ammon and Wahl, 1991). According to Suhaj (2006), the major functional compound present in *C. longa* is curcumin which has a pharmacological effect such as anti-inflammatory, antioxidant, antidiabetic, antibacterial, hepatoprotective, and anti-cancerous properties (Krup, 2013).

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DOI: https://doi.org/10.47836/ifrj.28.4.20

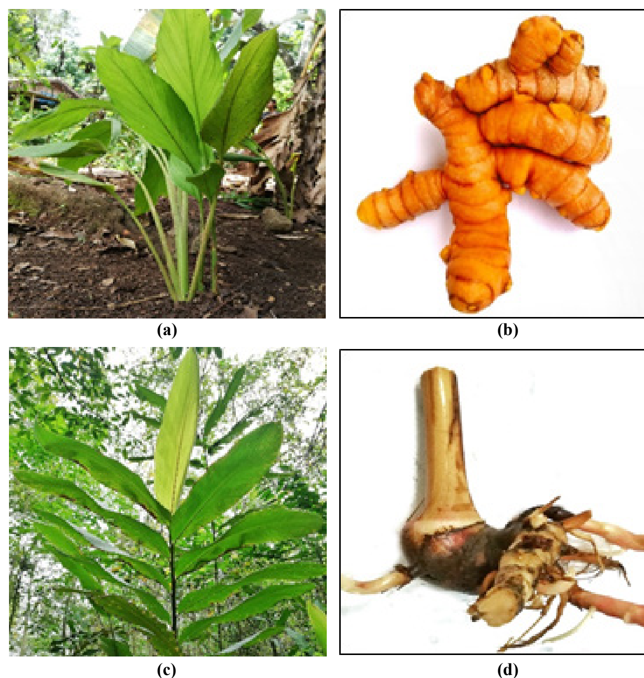

Figure 1. Photographs of the leaves and rhizome of *Curcuma longa* [(a) and (b)] and *Etlingera philippinensis* [(c) and (d)], respectively.

E. philippinensis, locally known as *"tagbak"* in Bisaya, is an endemic species of the Philippines (Elmer, 1915; Acma, 2010). According to Chan *et al.* (2007), *Etlingera* species exhibited antibacterial properties. Phytochemical screening in the leaves and rhizome of *E. philippinensis* revealed the presence of alkaloids, tannins, saponins, flavonoids, and steroids (Barbosa *et al.,* 2016). High levels of chlorogenic acid have also been reported in the leaves of *E. philippinensis* (Barbosa *et al.,* 2017).

Moreover, ethanol and water extracts of *E. philippinensis* leaves and rhizomes were found to possess 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (Barbosa *et al.,* 2016). Methanolic extracts of the leaves and rhizomes of *E. philippinensis* also exhibited antioxidant activity (Mabini and Barbosa, 2018). Gounder and Lingamallu (2012) have reported that the cured rhizomes of *C. longa* have higher yield of volatile oils than those of the fresh and dried. Additionally, the dried rhizome exhibited higher antioxidant activity than those of the fresh and cured.

The present work was conducted to investigate the effect of curing process on the antioxidant activity and level of phenolic compounds such as curcumin in fresh rhizomes. In the present work, the ethanolic extracts of the fresh and cured rhizomes of *C. longa* and E. philippinensis were evaluated with respect to their total antioxidant, total phenolic content, and curcumin content. Curing process was done by boiling fresh and clean rhizome of the *E. philippinensis* and *C. longa* separately in distilled water for 40 min prior to cutting into thin pieces and sun-drying.

Materials and methods

Chemicals

The chemicals and reagents used in the present work were liquid nitrogen, analytical grade of absolute ethanol, gallic acid, ascorbic acid, curcumin, Folin-Ciocalteu reagent, sodium carbonate, sodium phosphate, ammonium molybdate, and sulphuric acid.

Sample collection

Fresh rhizomes of *C. longa* and *E. philippinensis* were collected from Musuan, Maramag, Bukidnon (7° 46' 49" North, 125° 03' 29" East, and 1,086 ft. elevation) and Kibawe, Bukidnon (7° 29' 15" North, 125° 03' 38" East, and 1,202 ft. elevation), respectively. The collected samples were placed in a clean net bag, and transported to the Laboratory of Chemistry, Department of Chemistry, College of Arts and Sciences, Central Mindanao University for sample preparation. The plant samples were identified by Dr. Fulgent P. Coritico of the Centre of Biodiversity Research and Extension, CMU.

Sample preparation and solvent extraction

Fresh rhizome

Sample preparation and solvent extraction of the fresh rhizomes were performed using the method

described by Chan *et al.* (2007). The fresh and clean rhizomes of *E. philippinensis* and *C. longa* were powdered using mortar and pestle under liquid nitrogen. One gram of the powdered rhizomes was placed in 50 mL centrifuge tube, and then extracted with 50 mL absolute ethanol with continuous mixing at 850 rpm for 1 h at room temperature. Extracts were filtered using Whatman No. 1 filter paper, and stored at -20°C for further analyses. Five separate extractions were done per plant species. The extract obtained after filtration was considered as the fresh rhizome sample.

Cured rhizome

Sample preparation and solvent extraction of cured rhizomes were performed using the method described by Prasad and Aggarwal (2011) and Chan *et al.* (2007) with slight modification on the grinding process. The fresh and clean rhizomes of *E. philippinensis* and *C. longa* were boiled separately in distilled water for 40 min. The rhizomes were removed from the water, sliced thinly, and immediately sun-dried until the loss of weight was more than 80%. The sun-dried samples were grounded to a fine powder using a blender (Oster; 10-speed). One gram of the powdered rhizome was placed in 50 mL centrifuge tube and then extracted with 50 mL absolute ethanol with continuous mixing at 850 rpm for 1 h at room temperature. The extracts were filtered using Whatman No.1 filter paper, and stored at -20°C for further analyses. Five separate extractions were done per plant species. The extract obtained after filtration was considered as the cured rhizome sample.

Determination of total antioxidant activity

Preparation of standards and sample solutions

Four hundred mg/L stock solution of ascorbic acid was prepared by dissolving 0.0100 g of ascorbic acid in ethanol, and diluting the resulting solution to 25 mL. Various concentrations (0, 1, 20, 40, 60, 80, 100, 120, 140, and 160 mg/L) were prepared as working standards for the calibration curve. From the sample stock solution (20 g/L) of fresh and cured rhizomes of *C. longa*, 5 µL of sample was diluted to 400 µL to give 0.25 g/L test solution. For the fresh and cured rhizomes of *E. philippinensis*, 20 g/L sample stock solution was used as test solution in the assay.

Assay

The total antioxidant activity (TAA) of the sample extracts was determined by adapting the method previously described by Prieto *et al.* (1999) with several modifications such as the use of Eppendorf tubes as the reaction vessel and centrifugation after the reaction. In an Eppendorf tube containing 400 µL of the test solution, 1,200 µL of a reagent solution (prepared by mixing equal amount of 0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) were added. The Eppendorf tubes were then covered tightly with aluminium foil, and incubated at 95°C in an oven for 90 min. Following incubation, the mixture was cooled to room temperature. The mixture was centrifuged at 5,000 rpm for 3 min. Two hundred microliters of the supernatant liquid were transferred into a 96-well plate. The absorbance of the mixture was measured at 695 nm using a microplate reader. The same procedure was done on the ascorbic acid standards for the calibration curve and blank (ethanol). The TAA, expressed in milligram ascorbic acid equivalents per gram sample (mg AAE/g sample), was calculated using Eq. 1:

$$\text{Total antioxidant activity, } \left(\frac{mg\ AAE}{g\ sample}\right) = \frac{A}{B} \quad \text{(Eq. 1)}$$

where, A = ascorbic acid concentration of the sample solution determined from the calibration curve (mg AAE/L), and B = concentration of the sample test solution (g/L).

Determination of total phenolic content

Preparation of the standards and sample solutions

Six hundred mg/L stock solution of gallic acid was prepared by dissolving 0.0300 g of gallic acid in ethanol, and diluting the resulting solution to 50 mL. Various concentrations (0, 6, 12, 18, 24, 36, 48, 60, and 72 mg/L) were prepared as working standards for the calibration curve. From the sample stock solution (20 g/L) of the fresh and cured rhizomes of *C. longa*, 9 µL of sample was diluted to 300 µL to give 0.6 g/L sample test solution. For the fresh and cured rhizomes of *E. philippinensis*, 20 g/L sample stock solution was used as test solution in the assay.

Assay

The determination of total phenolic content (TPC) of the sample extracts was determined following the method previously described by Ainsworth and Gillespie (2007) with modifications using Eppendorf tubes and centrifugation after reaction. In an Eppendorf tube containing 300 µL of the test solution, 300 µL of 10% Folin-Ciocalteu reagent was added. After 5 min, 1,200 µL of 10% sodium carbonate was added. The tubes were incubated at room temperature for 90 min. After centrifugation at 5,000 rpm for 3 min, 200 µL of the

supernatant was transferred into a 96-well plate. The absorbance was determined at 750 nm using a microplate reader. The same procedure was done for the working standards of the calibration curve and blank (ethanol). The TPC, expressed as milligram gallic acid equivalent per gram sample (mg GAE/g sample), was calculated using Eq. 2:

$$\text{Total phenolic content, } \left(\frac{mg\ GAE}{g\ sample}\right) = \frac{A}{B} \quad \text{(Eq. 2)}$$

where, A = gallic acid concentration of the sample solution determined from the calibration curve (mg GAE/L), and B = concentration of sample test solution (g/L).

Determination of curcumin content

Preparation of standards and sample solutions

The determination of the curcumin content was done using the method previously described by Sharma *et al.* (2012) with some modifications using Eppendorf tubes and microplate. The standard stock solution of curcumin with the concentration of 100 mg/L was prepared by dissolving 10 mg of curcumin in ethanol, and diluting the solution to 100 mL. Various concentrations (0, 1, 2, 3, 4, 5, 6, and 7 mg/L) were prepared as working standards for the calibration curve. From the sample stock solution (20 g/L) of the fresh and cured rhizomes of *C. longa*, 10 µL of sample was diluted to 2,000 µL to give 0.10 g/L sample test solution. For the fresh and cured rhizomes of *E. philippinensis*, 20 g /L sample stock solution was used as test solution in the assay.

Assay

Two hundred microliters of the test solution were transferred into a 96-well plate. The absorbance of the working standard and sample extract was determined at 421 nm using a microplate reader. The same procedure was done for the working standards and blank (ethanol). The curcumin content, expressed as milligram curcumin per gram sample (mg curcumin/g sample), was calculated using Eq. 3:

$$\text{Curcumin content, } \left(\frac{mg\ curcumin}{g\ sample}\right) = \frac{A}{B} \quad \text{(Eq. 3)}$$

where, A = curcumin concentration of the sample solution determined from the calibration curve (mg curcumin/L), and B = concentration of sample test solution (g/L).

Statistical analysis

Five replicates and five trials per replicate were done for the determination of the TAA, TPC, and curcumin content. The obtained data were subjected to statistical analysis using analysis of variance (ANOVA) at 0.05 level of significance. Correlations between TAA and TPC, TAA and curcumin content, and TPC and curcumin content were determined using Pearson Correlation Analysis. Grubb's Test was used for outliers.

Results and discussion

Total antioxidant activity

The quantification of TAA of the fresh and cured rhizomes of *E. philippinensis* and *C. longa* was determined using phosphomolybdenum method. The results of the determination of the TAA, expressed as milligram ascorbic acid equivalents (AAE) per gram sample of the fresh and cured rhizome of *E. philippinensis* and *C. longa*, are summarised in Table 1. The results of ANOVA showed that there was a significant difference in the TAA between *E. philippinensis* and *C. longa* for both the fresh and cured rhizomes. There was also a significant difference in the TAA between the fresh and cured rhizomes of *C. longa*. However, there was no significant difference in the TAA between the fresh and cured rhizomes of *E. philippinensis*. Generally, the TAA of the cured rhizomes for both ginger species were higher than that of the fresh rhizomes. In addition, the TAA of *C. longa* was significantly

Table 1. Mean of total antioxidant activity of the fresh and cured rhizomes of *Etlingera philippinensis* and *Curcuma longa*.

Plant	Total antioxidant activity, mg AAE/g sample (% RSD)	
	Fresh	**Cured**
Etlingera philippinensis	0.53^{bc} (1.98)	1.17^{bc} (4.11)
Curcuma longa	28.18^{ad} (6.01)	37.66^{ac} (2.57)

Means (n = 25) with the same superscript letter in a column (^{a,b}) and in a row (^{c,d}) are not significantly different at $p > 0.05$.

higher than that of *E. philippinensis*. These findings indicate that the TAA of ginger rhizomes depends on the preparation process as well as the ginger species.

In phosphomolybdenum method, molybdenum (VI) is reduced to molybdenum (V) in the presence of a reducing agent (antioxidant) with subsequent formation of a green phosphomolybdate (V) complex which has the maximum absorption at 695 nm (Kumaran and Karunakaran, 2007). The higher antioxidant activity of *C. longa* rhizome may be attributed to its major phenolic compounds such as curcumin and demethoxycurcumin (Zaeoung *et al.,* 2005; Jayaprakasha *et al.,* 2006). Masuda *et al.* (1999) have also reported strong antioxidant activity of curcuminoids from turmeric. The TAA has been accounted for the presence of oleoresin and essential oil such as aromatic turmerone and alpha-turmerone in the rhizome of *C. longa* (Singh *et al.,* 2010). Chan *et al.* (2008) have also reported that ascorbic acid equivalent antioxidant capacity (AEAC) of methanolic extracts of the rhizomes of *C. longa* (390 ± 127 AA/100 g) was significantly higher than the rhizomes of *Etlingera* species such as *E. elatior* (295 ± 96 AA/100 g) and *E. maingayi* (122 ± 53 AA/100 g).

Curing process has the advantage of sterilising the rhizomes before drying (Plotto, 2004). Curing of the rhizomes reduces drying time, lowers the moisture content, as well as improves curcuminoids extractability; but, has no effect on the total volatile oil content (Buescher and Yang, 2000). It has been previously reported that volatile oils from the cured *C. longa* rhizomes exhibited higher antioxidant activity than that of the fresh (Gounder and Lingamallu, 2012). The volatile oil is present in the oil cells and ducts located in the meristematic region of the rhizome (Ravindra *et al.,* 2007). In the case of the cured rhizomes, oil cells are not damaged, and the loss during drying process is avoided (Gounder and Lingamallu, 2012). The retention of the volatile oils and enhancement of the curcuminoids known as antioxidants by curing process may explain higher TAA in the cured than fresh rhizomes observed in the present work.

Total phenolic content

The quantification of TPC in the fresh and cured rhizomes of *E. philippinensis* and *C. longa* was determined by employing Folin-Ciocalteu method with gallic acid as standard. The results of the assay, expressed as milligram gallic acid equivalents (GAE) per gram sample of the fresh and cured rhizome of *E. philippinensis* and *C. longa*, are summarised in Table 2. The results implies that there were more phenolic compounds in the rhizomes of *C. longa* such as curcuminoids and sesquiterpenoids (Kumar *et al.,* 2016) than the rhizomes of *E. philippinensis*. The results of ANOVA showed that there was significant difference in TPC between *E. philippinensis* and *C. longa* for both the fresh and cured rhizomes. There was also significant difference in TPC between the fresh and cured rhizomes of *C. longa*. However, there was no significant difference in TPC between the fresh and cured rhizomes of *E. philippinensis*.

Plants are known for containing rich sources of phenolic compounds (an -OH group attached to a benzene ring). These phenolic compounds are known for its antioxidant activity because of its high redox potentials which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers (Tsao and Deng, 2004). Thus, phenolic compounds can scavenge reactive oxygen intermediates without promoting further oxidative reactions (Smirnoff, 2005). Moreover, Folin-Ciocalteu method was used to determine the TPC. The mechanism involved in this method is electron transfer in alkaline medium from phenolic compounds to phosphomolybdic/phosphotungstic acid complexes to form blue complexes that are determined spectroscopically (Singleton *et al.,* 1999). The phenolate ions present in the sample had likely destroyed the yellow Folin-Ciocalteu phenol reagent and reduced the reagent, thereby producing the characteristic blue colour of the resulting solution (Craft *et al.,* 2012). Similar results have been noted in the study of Chen *et al.* (2008)

Table 2. Mean of total phenolic content of the fresh and cured rhizomes of *Etlingera philippinensis* and *Curcuma longa*.

Plant	Total phenolic content, mg GAE/g sample (% RSD)	
	Fresh	**Cured**
Etlingera philippinensis	0.21^{bc} (7.62)	0.88^{bc} (2.46)
Curcuma longa	7.11^{ad} (7.38)	32.36^{ac} (4.52)

Means (n = 25) with the same superscript letter in a column (^{a,b}) and in a row (^{c,d}) are not significantly different at $p > 0.05$.

Table 3. Mean curcumin content of the fresh and cured rhizomes of *Etlingera philippinensis* and *Curcuma longa*.

Plant	Curcumin content, mg curcumin/g sample (% RSD)	
	Fresh	Cured
Etlingera philippinensis	0.01[bc] (1.76)	0.02[bc] (4.37)
Curcuma longa	3.00[ad] (3.59)	14.32[ac] (4.17)

Means (n = 25) with the same superscript letter in a column ([a,b]) and in a row ([c,d]) are not significantly different at $p > 0.05$.

which showed significantly higher TPC in the rhizomes of *C. longa* than some of the *Etlingera* species such as *E. elatior* and *E. maingayi*.

Curcumin content

Curcumin content, expressed as milligram curcumin per gram sample of the fresh and cured rhizome of *E. philippinensis* and *C. longa*, are summarised in Table 3. The results of ANOVA indicate that there were significant differences in curcumin content between *E. philippinensis* and *C. longa* for both the fresh and cured rhizomes. There was also a significant difference in curcumin content between the fresh and cured rhizomes of *C. longa*. However, there was no significant difference in curcumin content between the fresh and cured rhizomes of *E. philippinensis*.

The quantification of curcumin content in the fresh and cured rhizomes of *E. philippinensis* and *C. longa* was determined using a micro- spectrophotometer with maximum absorption peak around 421 nm (Hazra *et al.,* 2015). Curcumin, in an analogy to other β-diketones, is expected to exist in the enol configuration based on the results of the unirradiated curcumin and curcuminoids through nuclear magnetic resonance. Conjugation of the π-electrons may result in the coupling of the two feruloyl chromophores into one extended π-electron system if the two ends of the chromophore communicate via resonance structures in the enol forms (Bong, 2000).

Several studies have reported that curcuminoids in the rhizomes of *C. longa* contain curcumin and derivatives such as demethoxycurcumin, bis-demethoxycurcumin, 5'-methoxycurcumin, dihydrocurcumin, and cyclocurcumin (Revathy *et al.,* 2011; Sambhav *et al.,* 2014). Habsah *et al.* (2005) reported that *E. elatior* was found to have curcumin derivatives such as 1,7-bis(4-hydroxyphenyl)-2,4,6-heptatrienone, demethoxycurcumin, and 1,7- bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one.

Correlation between TAA, TPC, and curcumin content

Pearson correlation analysis was used to evaluate the relationship between TAA and TPC, TAA and curcumin, and TPC and curcumin content in all samples. As shown in Table 4, there was a significantly strong positive correlation between TAA and TPC, TAA and curcumin, and TPC and curcumin content observed in all samples as evident in the p-values which were less than 0.01 at 0.01 level of significance. This implies that the levels of TPC, TAA, and curcumin content were significantly related to each other. This further suggests that the TAA, TPC, and curcumin content significantly affected each other.

The present findings imply that antioxidant activities can be attributed to the presence of phenolic compounds in the fresh and cured rhizomes of *E. philippinensis* and *C. longa*. The higher antioxidant

Table 4. Pearson correlation coefficient (r) between TAA, TPC, and curcumin content.

	Correlation coefficient (r)	p-value*
TAA vs TPC	0.851	0.000
TAA vs curcumin content	0.854	0.000
TPC vs curcumin content	0.998	0.000

*p-value < 0.01 is considered statistically significant.

activity of *C. longa* could be due to the presence of phenolic compounds including curcumin which is very well known for its antioxidant activity (Miquel *et al.,* 2002). Toda *et al.* (1985) have reported that the antioxidant activities of curcumin, demethoxycurcumin, and bisdemethoxycurcumin were stronger than that of alpha-tocopherol. This strong activity of curcuminoids suggests the antioxidant potentials of *C. longa* extracts.

Mabini and Barbosa (2018) have reported on the contribution of phenolic compounds in the methanolic extracts of the leaves and rhizomes of *E. philippinensis* to TAA, and the significant correlation between TAA and TPC. A strong correlation has also been reported previously between TAA and TPC in the methanolic extracts of the leaves and rhizomes of *H. coronarium* (Redondo and Barbosa, 2018). A study of Kasangana *et al.* (2015) also proved that there was a strong correlation between TAA and classes of phenolic compounds such as hydroxycinnamic acids.

Conclusion

The TAA, TPC, and curcumin content of the cured rhizomes of *C. longa* and *E. philippinensis* were significantly higher as compared to those in the fresh rhizomes. The TAA, TPC, and curcumin content of *C. longa* rhizomes were also significantly higher than those of *E. philippinensis*. Based on the correlation analysis, a significant positive correlation was observed between TAA and TPC, TAA and curcumin, and TPC and curcumin content in all the analysed samples.

Acknowledgement

The authors are grateful to Gloria Jesusa D. Baltazar and Melania M. Enot for the improvement of the manuscript. Heartfelt thanks are also given to Rainear A. Mendez of the Natural Science Research Centre, CMU and to the staff of Tuklas Lunas Development Centre, CMU for the help in the chemical analyses. Special thanks also go to the Department of Chemistry, College of Arts and Sciences, CMU.

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