PHENOLIC COMPOUNDS AND ANTIOXIDANT ACTIVITIES OF MACERATED *ALPINIA GALANGA* STEMS AND LEAVES

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Received Date: July 12, 2019; Revised Date: October 22, 2019; Acceptance Date: December 20, 2019

Abstract

Alpinia galanga (*A. galanga*) is a popular culinary ingredient as well as medicinal herb commonly found in Southeast Asia. Compared to the rhizome of *A. galanga*, the leaves and stems are always being discarded as waste. In this research, active compounds from the stem and leaves of this plant were extracted using maceration technique with ethanol as solvent at a liquid-solid ratio of 20 ml/g. The influence of maceration time and maceration temperature on total phenolic content (TPC) and antioxidant activities of extract were studied using Folin-Ciocalteu assay and DPPH radical scavenging assay, respectively. The maceration time was from 1 to 5 hr and the maceration temperature was examined from 40 ºC to 70 ºC. Crude extract yield for stems and leaves obtained for all parameters of the maceration process was >11%. The highest TPC for leaves was obtained at 1 hr (0.038± 0.02 mg GAE/mg DW) and stems at 4 hr (0.024 ± 0.01 mg GAE/mg DW). Similar trend was also observed for antioxidant activity where the highest antioxidant activity obtained for leaves was at 1 hr (IC_{50}=0.136 ± 0.01 mg/ml) and for stems was at 4 hr (IC_{50}=0.320 ± 0.07 mg/ml). Maceration time however, has no significant influence on TPC (p>0.09) and antioxidant activity (p>0.16) of *A. galanga*. In terms of temperature, the highest TPC was recorded at 70 ºC for both leaves (0.034 ± 0.003 mg GAE/mg DW) and stems (0.019 ± 0.005 mg GAE/mg DW), while the highest antioxidant activity was from sample extracted at 40 ºC for both leaves (IC_{50} = 0.076 ± 0.05 mg/ml) and stems (IC_{50} = 0.454 ± 0.16 mg/ml). The best maceration conditions, considering from the antioxidant activity, for both leaves and stems of *A. galanga* were concluded at 1 hr and 40 ºC in the viewpoint of both extract quality, energy and cost efficiency.

Keywords: *Alpinia galanga*, Antioxidant activities, Maceration, Phenolic compound

Introduction

The World Health Organization (WHO) states that medicinal plants are being used as remedies by up to 95% of the populations in developing countries [1]. Furthermore, the global herbal drug market forecasted to reach US $5 trillion by the year 2050 [2], hence research effort regarding herbal drugs needs to be intensified considering its great future prospect.

Phenolic compounds from plants could be extracted using conventional techniques such as maceration and Soxhlet extraction or by using modern ones like ultrasound-assisted extraction, microwave assisted extraction, and supercritical fluid extraction. The choice of extraction techniques typically depends on the feasibility of the technique, availability of extraction equipment and convenience. Although modern extraction techniques offer high extraction yield and reduction in extraction time, conventional techniques are still relevant due to the ease of use and cheaper experimental setup. In the extraction technique used in this study, maceration time and maceration temperature are among the two factors that influence the extraction of phenolic compounds from plants. This is because sufficient time is

required for the compounds to migrate from plant sample to extraction solvent while an increment of temperature during extraction increase mass transfer rate and diffusion coefficient between plant compounds and solvent.

Of many edible native plants in Malaysia, considerable attention is given to *Alpinia galanga* (*A. galanga*), known locally as lengkuas. It is a perennial herb found commonly throughout India as well as in countries like Thailand, Indonesia, and China [3]. This plant not only grown in commercial scale but also could be easily found in kitchen gardens as it is one of the common ingredients of Southeast Asian cooking. Belonging to the Zingiberaceae family, *A. galanga* exhibits a huge range of biological activities including antitumor, antibacterial and anti-ulcer [4, 5]. Furthermore, this plant was also reported to contain antioxidant, the substance that is able to suppress radical chain reactions [6]. Its flower has three-fold higher total phenols content than rhizome and contains 1'-acetoxyeugenol acetate as the major compound [7]. Chouni et al. [8] states that the leaves and flowers of *A. galanga* showed the highest chelating and β-carotene bleaching ability, thus suspecting potential dietary source of natural antioxidant from flower and leaves.

Compared to the rhizomes which are prevalently used as spice in cooking, the *A. galanga* leaves and stems often being discarded as waste. Thus, both *A. galanga* leaves and stems were examined in this study with varying maceration time and temperature to evaluate whether considerable amount of phenolic compounds are present in them, hence recognizing their medicinal value, which would turn these plant's parts into valuable products.

Materials and Methods

Preparation of Plant Sample

Fresh discarded *A. galanga* leaves and stems were collected from Parit Nibong, Kedah, Malaysia. Plant samples were washed under running tap water to remove dirt and contaminants. Initial weight of the leaves and stems were measured separately before drying in an oven at 80 ºC until the weight of samples remained constant at 5% moisture content. This was followed by grinding of dried sample into powder and sieving through stainless steel mesh sieve with 710 µm opening to obtain samples of < 710 µm. The raw powdered samples were stored at 4 ºC in air-tight containers until further use.

Extraction of *Alpinia Galanga*

The influence of maceration time (1, 2, 3, 4, and 5 hr) on crude extract yield and extract quality was studied. Temperature and liquid-solid ratio of samples at respective extraction time were kept constant at 60 ºC and 20 ml/g. Powdered leaves was macerated in ethanol in a 200 ml beaker at the aforementioned parameters accompanied with stirring at 125 rpm using magnetic stirrer.

After completion of extraction, powdered leaves were separated from solvent using a muslin cloth followed by filtering by filter paper (Whatman no.1). Rotary evaporator was then used to concentrate the extract at vacuum pressure of 170 mbar, rotating speed of 60 rpm, and water bath temperature 60 ºC. The concentrated extract was then removed from the evaporation flask wall using a spatula and the crude extract yield (%) was calculated as the weight (g) of crude extracts obtained from weight (g) of raw materials. Crude extract was then kept in a sample bottle at 4 ºC for further analysis. The extract quality was then investigated and the best extraction time (1 hr) was applied for the subsequent study with varying maceration temperature (40, 50, 60 and 70 ºC), while maintaining the liquid-solid ratio of samples at 20 ml/g. The extraction process was then repeated using powdered stem samples.

Determination of Extract's Phenolic Content

The total phenolic content (TPC) of *A. galanga* extract was examined using Folin-Ciocalteu reagent (FCR) according to the method of Dolatabadi et al. [9] with slight modifications. Initially, FCR solution (the reference solution), sodium carbonate (Na_2CO_3) solution at 7.5% (w/v), standard gallic acid solution (0.0078125, 0.015625, 0.03125, 0.0625 and 0.125 mg/ml) and blank solution were prepared prior to the test. The reference solution was prepared by diluting FCR with distilled water at ten times dilution factor. The blank solution was prepared by mixing 5 ml of FCR solution, 1 ml of ethanol and 4 ml of 7.5% (w/v) Na_2CO_3 solution in a test tube. The extract of *A. galanga* was diluted in ethanol to give a concentration of 1 mg/ml. The diluted plant extract (1 ml) and standard gallic acid solutions of different concentrations were then pipetted into respective test tubes, followed by addition of 5 ml FCR solution and 5 ml Na_2CO_3 solution (7.5% (w/v)). The mixtures were incubated for 20 minutes at room temperature before absorbance of the mixtures was measured using UV-Vis spectrophotometer (Cary 60 UV-Vis, Agilent Technologies) at 760 nm. Total phenolic content was expressed as milligram of gallic acid per gram plant extract and determined using Equation (1):

$$A = \frac{c \times V}{m} \tag{1}$$

Where, A is the total phenolic content (mg GAE/mg plant extract), c is the concentration determined from standard gallic acid calibration curve (mg/ml), V is the volume of extract solution (ml), and m is the mass of extract used (g).

Measurement of Antioxidant Activity

In this study, 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging assay was used to determine antioxidant activity of crude extract [9]. The control solution (0.1 mM DPPH solution) was prepared by adding 4 mg DPPH in 100 ml ethanol. The standard solution was prepared by adding 1 mg ascorbic acid in 10 ml distilled water, rendering it a 0.1 mg/ml concentration. This aqueous ascorbic acid was then diluted to 0.0125, 0.00625, 0.003125 and 0.0015625 mg/ml. Blank solution contained merely ethanol. Then, the different concentrations of standard ascorbic acid solution (2 ml) were pipetted into respective test tubes. This was followed by the addition of 3 ml of DPPH solution and incubation of mixtures in the dark for 30 minutes. The absorbance of the mixtures was measured using UV-Vis spectrophotometer at 517 nm against blank solution. These steps were repeated by replacing ascorbic acid with crude extract of *A. galanga* leaves and stems, in which the extract solutions were prepared in serial concentrations for leaves (0.4, 0.2, 0.1, 0.05 and 0.025, 0.00625 mg/ml) and for stem (1.0, 0.5, 0.25, 0.125, 0.0625 mg/ml) on the basis that 1 mg/ml concentration of stock solution consists 1 mg plant extract and 1 ml ethanol. Inhibition activity (I%) was calculated using Equation 2 and a graph of I% against concentration of either crude plant extract or standard ascorbic acid was plotted to determine IC_{50}.

$$I\% = \frac{A_C - A_E}{A_C} \times 100\% \tag{2}$$

Here, A_C is the absorbance of control and A_E is the absorbance of extract or standard solutions.

Statistical Analysis

The results are expressed as mean ± standard deviation of triplicate assays and analysed by Microsoft Excel 2010. Student's *t*-test was used to determine statistically different values at a significance level of $p \leq 0.05$. Pearson correlations between variables were established using MINITAB software (version 19).

Results and Discussion

Effect of Extraction Time and Temperature on Yield of Crude Extract

The influences of time and temperature on the yield of crude extract are shown in Figure 1. With respect to maceration time, the highest yield of crude leave's extract (13.62%) was obtained at 4 hr whereas for crude stem's extract, the highest yield (13.22%) was obtained at 2 hr. In the case of maceration temperature, the highest yield for leave's extract (15.49%) and stem's extract (13.44%) were obtained at 60 °C and 70 °C, respectively. Both parameters were found to have no significant influence ($p>0.09$) on the crude extract yield of stems and leaves.

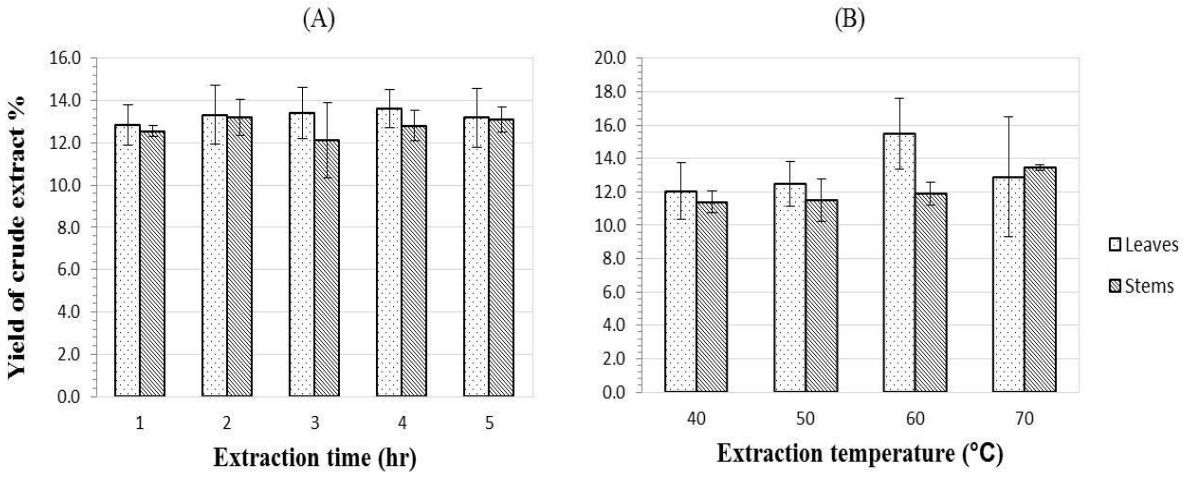

Figure 1. Influence of maceration time (A) and temperature (B) on the extraction yield (%)

Similar findings on non-significant influence of maceration time and temperature on the extraction yield has been reported by Andriyani et al. [10] on the extraction of *Zingiber officinale* at various time (30, 60 and 90 minutes) and temperature (50, 70, 90 °C). This shows that in this study, final equilibrium nearly attained between the active compound concentrations in the solid matrix and bulk solvent [11] after 1 hr extraction time even at elevated temperature.

Total Phenolic Content of Leaves and Stems Extracts

The TPC of each sample was obtained through interpolation from standard gallic acid curve. Although the TPC of leaves and stems were the highest for sample extracted for 1 hr (0.038 ± 0.03 mg GAE/mg DW) and 4 hr (0.024 ± 0.01 mg GAE/mg DW), respectively, the results in Figure 2 indicate that the maceration time has little or no influence on the TPC of leaves' extracts ($p>0.99$) and stem's extracts ($p>0.20$).

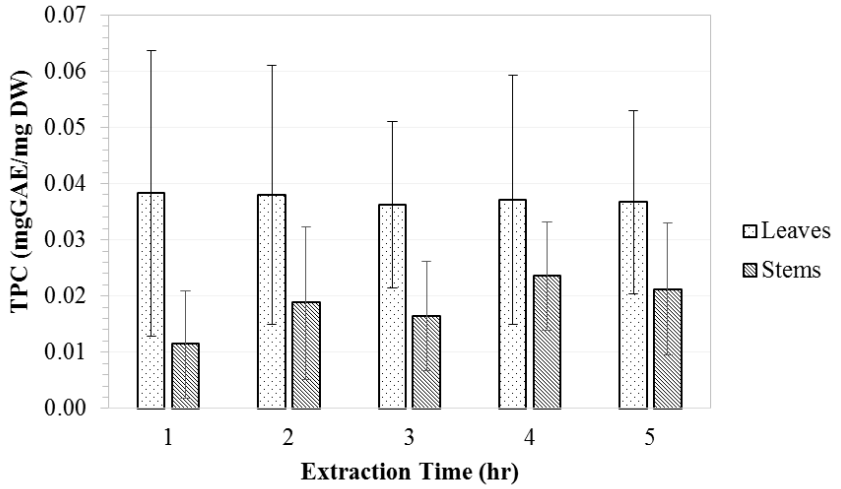

Figure 2. Influence of maceration time on phenolic content of *A. galanga* extracts

Typically, TPC tends to increase parallel to maceration time until a certain peak because solubility of compound in samples was proportional to the time spent to reach saturation point [12]. Nevertheless, prolonged extraction time could have deteriorating effect on the phenolic compounds due to oxidation. Experimental work from Juntachote et al. [13] showed that effect of extraction time was insignificant towards TPC of lemon grass, galangal and rosemary. This result was in line with findings from Kwon et al. [14] where TPC of ginseng extract was not influenced by the time required for extraction.

The maceration temperature was found to have little influence on the TPC of extracts (Figure 3). Although there was an increasing trend of TPC with temperature increment, the differences in TPC with increasing temperature was insignificant for both leaves (p>0.16) and stems (p>0.09). Since phenolic compounds are strongly bound to cell wall hemicellulose, alteration to the cell wall polysaccharides through heating at temperature higher than 70 °C or pre-treating *A. galanga* samples to degrade the cell wall prior to maceration may lead to increased extractability of phenolic compounds.

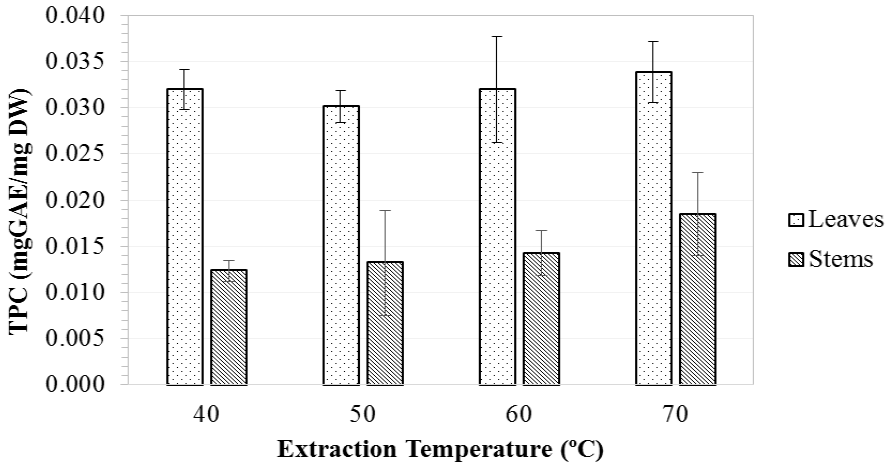

Figure 3. Effect of maceration temperature on TPC of *A. galanga* leaves and stems

Radical Scavenging Activity of Leaves and Stems Extracts

Antioxidant activity of *A. galanga* was studied based on the IC_{50} value where a lower IC_{50} indicates a higher antioxidant activity of the compound and vice versa [15]. The effect of maceration time on leaves and stems of *A. galanga* antioxidant activity are shown in Figure 4. Based on the result, the lowest IC_{50} value in leaves, which indicates the best antioxidant capability, was recorded at 1 hr (0.136 ± 0.01 mg/ml), followed by 3 hr (0.144 ± 0.03 mg/ml) and 5 hr (0.154 ± 0.05 mg/ml). No significant trend was observed from the results and it can be seen that maceration time has little effect on antioxidant activity of leave's extracts (p>0.16). A non-significant effect of maceration time on antioxidant activity was also observed for *A. galanga* stem (p>0.26). The lowest IC_{50} value recorded for stem's extract was at the maceration time of 4 hr while the highest IC_{50} value was observed at 1 hr (0.813 ± 0.74 mg/ml).

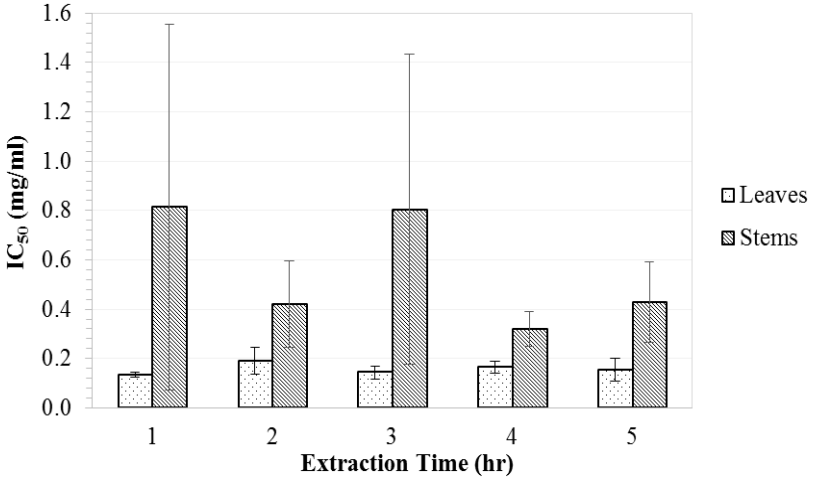

Figure 4. Influence of maceration time on *A. galanga* extract's antioxidant activity

Linear relationship between antioxidant activity and extraction time has been reported for the maceration of *Sargassum* sp. in ethanol for 6 to 24 hr, whereby the antioxidant activity was the highest for extract of sample macerated for 24 hr [12]. However, prolonged extraction time might also compromise the capability of antioxidant, as observed by Chew et al. [16] where the antioxidant activity of extracts of *Orthosiphon stamineus* (*O. stamineus*) macerated in ethanol decreased significantly when the extraction time exceeded 4 hr due to possible oxidation of the extract.

There are also studies reported that extraction time has no significant effect on antioxidant activity, which coincide with findings from results shown in Figure 4. Hashim et al. [17] found that radical scavenging activity, which indicates the antioxidant activity, has no significant increase when comparing extracts of *O. stamineus* macerated for 4 hr and 8 hr. Moreover, similar result was observed when Herrero et al. [18] conducted experiment on rosemary to study the influence of extraction time on plant's antioxidant activity using pressurized liquid extraction technique in various solvents. The experiment results showed that extraction time had minimal influence on the antioxidant activity of rosemary.

It can be seen from Figure 5 that IC_{50} value of extract from leaves was the lowest at 40 ºC (0.076 ± 0.05 mg/ml) and the highest at 70 ºC (0.146 ± 0.02 mg/ml). However, the antioxidant activity of *A. galanga* stems exhibited different trend from the leaves. At similar

extraction temperature range, stem extract's antioxidant activity first decreased when maceration temperature was raised from 40 ºC (IC_{50} = 0.454 ± 0.16 mg/ml) to 60 ºC (IC_{50} = 0.734 ± 0.28 mg/ml). The antioxidant activity then increased upon further heating of sample at 70 ºC (IC_{50} = 0.502 ± 0.13 mg/ml).

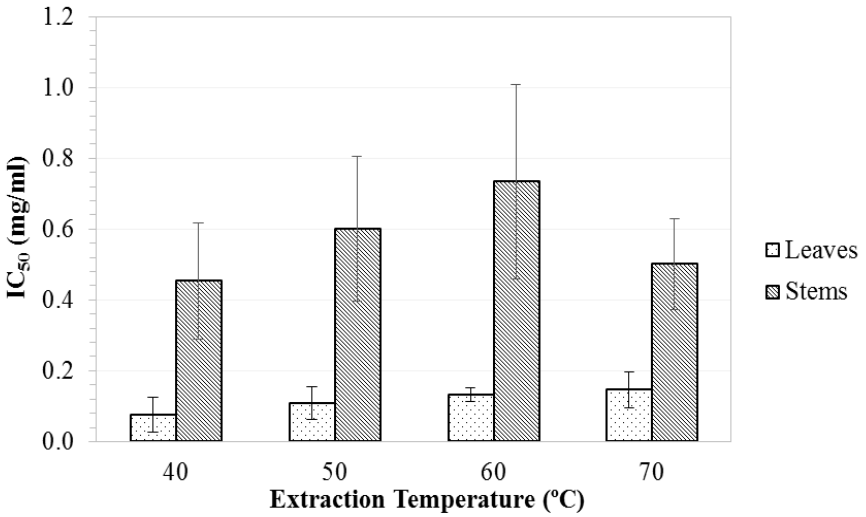

Figure 5. Influence of maceration temperature on antioxidant activity of *A. galanga* extracts

The result obtained by *A. galanga* leaves is aligned with study conducted by Bahrin et al. [19] on *Ficus carica* leaves in which temperature higher than 50 ºC will cause thermal degradation of phenolic compounds which leads to decreased in antioxidant activity as phenolic compounds were oxidized and hydrolysed at high temperature. However, it is important to take note that a slightly higher extraction temperature could improve mass transfer rate and solubility between compound in plant extract and solvent. Moreover, different polyphenol antioxidants are being extracted at both low and high temperature. The result of antioxidant activity of *A. galanga* stems points out that the stem of this plant contains high proportions of thermo labile and thermo stable components. Similar findings was reported on the extraction of *Guiera senegalensis* [20], where the best antioxidant activity was achieved from sample extracted at low temperature (40 ºC) and high temperature (70 to 85 ºC), whereas samples extracted in between these temperature points have lower antioxidant activities.

To further understand the interrelationship between antioxidant capacity and the TPC, the correlations under different extraction conditions were analysed and presented in Table 1. Under the influence of maceration time, a strong significant (p<0.05) negative correlation was observed between TPC and DPPH (r = - 0.869) for leave's extract. On the other hand, a medium non-significant negative correlation was observed for stem's extract. As for the influence of extraction temperature, small non-significant and strong non-significant negative correlations between TPC and DPPH were observed for leave's extract and stem's extract, respectively. This shows that the antioxidant activity in *A. galanga* extract is not exclusively governed by phenolic compound and could also be contributed by proteins and minerals that exist in this plant [21]. Thus, although high TPC was obtained at example 60 °C, the other compounds that contributed to the antioxidant activity might have degraded when extracted at that parameter.

Table 1. Correlation Coefficient (*r*) between Activities of Antioxidant and TPC under the Influence of Plant's Component, Maceration Time and Maceration Temperature

Source of Extract	Leaves	Stems
Extraction Parameter		
Time	-0.869*	-0.33
Temperature	-0.166	-0.557

*$p<0.05$

Conclusions

In the current study, the crude extract yield, TPC and antioxidant activity of extracts obtained at 1 hr and at 40 ºC were not significantly different from the ones obtained at longer maceration time or at higher maceration temperature. From economic point of view, the best maceration conditions determined based on antioxidant capability are at maceration time of 1 hr and maceration temperature of 40 ºC for both *A. galanga* leaves and stems, with IC_{50} at 0.076 ± 0.05 mg/ml and 0.454 ± 0.16 mg/ml, respectively. The negative correlations between the antioxidant activity and TPC of extracts indicate that phenolic compounds are not the sole contributor to the antioxidant capacity. The results from the current study are important to determine the range of factors that has a significant effect on the recovery of active compounds. The main challenge faced in the current study was obtaining standardized results due to the heterogeneity of the plant samples in terms of plant age or plant development. Thus, screening of raw materials is a necessary step when exploiting agriculture waste in order to obtain high quality standardized extracts.

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