Antioxidant activity, total phenolic and flavonoid contents and analyses of active compounds in *Stemona collinsae* Craib crude extracts

Boonjira Rutnakornpituk[1,2]*, Chatchai Boonthip[1], Jittikan Kerdpan[1], Nutchaya Fakfeang[1] and Wanatee Kholam[1]

[1]Department of Chemistry, Faculty of Science, Naresuan University, Phitsanulok, 65000 Thailand
[2]Center of Excellence in Biomaterials, Faculty of Science, Naresuan University, Phitsanulok, 65000 Thailand
*Corresponding author. E-mail: boonjirab@nu.ac.th

Abstract

In this work, the analysis of total phenolic contents (TPC), total flavonoid contents (TFC), antioxidant activities and active compounds in *Stemona collinsae* Craib crude extracts–from roots, stems and leaves were reported. All crude samples were extracted from dried samples preparing from an oven-drying method at 70°C for three days. TPC was determined via a Folin-Ciocalteu method, TFC was analyzed via an aluminum chloride colorimetric method and antioxidant activity were assessed using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method. The crude extracts from roots showed higher total phenolic and flavonoid contents and better antioxidant activity than those from stems and leaves. The analysis of active compounds such as phenolic and flavonoid compounds in the crude extracts were carried out *via* a high performance liquid chromatography (HPLC) technique. Three active compounds including p-coumaric acid, feruilc acid and p-hydroxybenzoic acid were found in all crude extracts. The p-hydroxybenzoic acid was a major phenolic compound in all crude extracts. In addition, the types and amounts of active compounds in the crude extract from roots were higher than those from stems and leaves.

Keywords: *phenolic content, flavonoid content, antioxidant activity*

Introduction

Nowadays, the medicinal plants are potential sources of natural antioxidants that might serve as the lead in the development of new drugs. It has been known that many medicinal plants are excellent sources of phytochemicals such as phenolic and polyphenolic compounds (e.g. phenolic acids, tannins and flavonoids), many of which have potent antioxidant activities and are often exploited in food products and in various medicinal treatments [Vinutha B. *et. al.*, 2007; Bag G.C. *et.al.*, 2015]. At present, much attention has been paid on sources of free radicals because they are the main cause of human illness. Free radicals can form in human body via various means such as from metabolism process or even from stress. When the number of free radicals is high, various diseases like heart disease, brain disease or cancer may occur [Halliwell B., 2009; Jirum J. *et.al.*, 2011]. Generally, human bodies have their own substances that can help to get rid of free radicals. These substances can be divided into two main groups; 1) substances which control or prevent the formation of free radicals, including enzymes like superoxide dismutase, glutathione peroxidase, catalase and peroxidase and, 2) antioxidants which can destroy the chain reaction of the formation of reactive

oxygen species (ROS), a major substance which can produce free radicals [Velioglu Y. S. *et.al.*, 2009, Chanwitheesuk A. *et.al.*, 2005]. However, these free radicals may cause damages to cells and issues, which essentially affect human wellbeing. Thus, it is crucial to have essential sources containing additional antioxidant. However, some synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have been reported to be toxic to human. Therefore, natural antioxidants have gained increasing interest because epidemiological studies signified that frequent consumption of natural antioxidants is associated with a lower risk of cardiovascular disease and cancer. In addition, natural antioxidants have become the target of a great number of research studies in finding the sources of potentially safe, effective and cheap antioxidants [Temple N.J., 2000; Thaiponga K. *et.al.*, 2006; Caia Y. *et.al.*, 2004]. Many researchers reported the new antioxidants in many types of plants because plant extraction is a facile and inexpensive means compared to modern medicines [Halliwell B., 2009; Breinholt V., 1999]. It has been discovered that different types of plants contain different types of antioxidants that can inhibit free radicals. Among the variety of phenolic compounds, phenolic acids have attracted considerable interest in the past few years due to their many potential health benefits. Phenolic acids are efficient antioxidants and have been reported to show different biological activities such as antibacterial, anticarcinogenic, anti- inflammatory, anti- viral, anti- allergic, estrogenic, and immune-stimulating agents [Breinholt V., 1999; Duthie G.G. *et.al.*, 2000]. In addition, the studies in recent years have shown that polyphenols in plants scavenge active oxygen species and effectively prevent oxidative cell damage [Wang L. *et.al.*, 2013; Mattila P. *et.al.*, 2007; Chirinos R. *et.al.*, 2007]. At present, many researches have studied the amounts of overall phenolic compounds in medicinal plants and have high amounts of phenolic compounds, and possess different degrees of antioxidants [Nooman A. K. *et.al.*, 2008].

Stemona collinsae Craib is a medicinal plant in Stemona species and known as Non Tai Yak. The Stemona species have long been used as a traditional Thai medicine with a wide range of various medicinal and biological properties such as liver cancer protection, skin infections and anti-parasitic agent [Khorphueng P. *et.al.*, 2006; Seger C. *et.al.*, 2004]. According to previous researches, there have already been some studies on chemical constituents and biological activities from the crude extracts of plants in Stemona species. For example, the previous chemical investigations of plants in the Stemona species revealed the presence of alkaloids as the main group of chemical constituents [Kaltenegger E. *et.al.*, 2003; Greger H., 2006; Schinnerl J. *et.al.*, 2007]. In addition, some of isolated alkaloids have been studied for biological activities [Shinozaki H. *et.al.*, 1985]. For instance, tuberostemonine, a prototype *Stemona* alkaloid, exhibited a comparable depressing effect on glutamate-induced response to those of glutamate inhibitor. Furthermore, several Stemona alkaloids have been found to exert insecticidal effects [Goetz M. *et.al.*, 1973; Sakata K. *et.al.*, 1987; Brem B. *et.al.*, 2002]. Zhao et al reported that the ethanol extract of Stemona collinsae Craib. inhibited the growth of many kinds of bacteria and fungi [Zhao W.M. *et.al.*, 1995]. In addition, Yang et al. [Yang X.Z. *et.al.*, 2006] described that several stilbenoids from roots of *Stemona sessilifolia* showed antibacterial activities against Staphylococcus aureus and Staphylococcus epidermidis. Xu et.al.[Xu Y.T. *et.al.*, 2006] presented that the root extracts of *Stemona tuberosa* Lour., *Stemona japonica* (Bl.) Miq. and *Stemona. sessilifolia* (Miq.) Miq. have long been used in traditional medicinal plant for the treatment of respiratory diseases and to prevent human and cattle parasites. In addition,

Khorphueng et al. [Khorphueng P., *et al.*, 2006] reported the isolation and X-ray crystal structure of 6-deoxyclitoriacetal from *Stemona collinsae*, and described its potent cytotoxic activity against human breast carcinoma (BT479), lung carcinoma (CHAGO), hepato-carcinoma (Hep-G2), gastric carcinoma (KATO3) and colon carcinoma (SW620). Moreover, some research reported that the antitumoral activity of the crude extracts from *Stemona tuberosa* and *Stemona collinsiae* was evaluated against eight cell lines of the medullary thyroid carcinoma (MTC).

Moreover, plants in Stemona species are also used as an ingredient in anticancer and chronic anti-inflammation drug formula [Pilli R.A. *et.al.*, 2005]. As can be seen from the previous research mentioned above, many studies have reported biological activities in plants in the Stemona species crude extracts. However, only a few bioassays have been evaluated the bioactivities and active compounds from *Stemona collinsae* Craib. crude extract, particularly from leaves, stem and roots of *Stemona collinsae* Craib. Therefore, the main objective of this work was to investigate the total phenolic contents, total flavonoid contents, antioxidant activities and types of some active compounds in crude extracts from leaves, stem and roots of *Stemona collinsae* Craib. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging agent was used to investigate antioxidant activities of the extracts. The aluminium trichloride and the Folin-Ciocalteu colorimetric methods were employed to determine total flavonoid and total phenolic contents, respectively. Because of all of these methods provide a facile and rapid means to evaluate by spectrophotometry, so it can be useful to assess various products at a time [Mathew S. et.al., 2006; Garcia E.J. *et.al.*, 2012]. Also, quantitative analyses of active compounds in all of crude extracts were performed via a high performance liquid chromatography (HPLC) technique.

2. Experimental

Plant materials and apparatus

Plant materials: *Stemona collinsae* Craib from root, leave and stem used in this study was obtained from Uttaradit province, Thailand, in November 2015. The plants were kept at -4 °C until used.

Chemicals and reagents: The standard reagents of phenolic acids were obtained from two manufacturers. Caffeic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid, ferulic acid, quercetin and kaempferol were purchased from Sigma Chemical Co., and catechin, ellagic acid, epicatechin, gallic acid, luteolin and cinnamic acid were obtained from Fluka. Methanol (AR grade), Abs. ethanol, ethyl acetate (AR grade), diethyl ether (AR grade) and acetonitrile (HPLC grade) were obtained from Labscan. DPPH was purchased from Fluka, and the Folin-Ciocalteu's reagent was obtained from Merck.

Apparatus: HPLC analyses were performed on Agilent 1100 Series HPLC with a diode array detector operating at wavelengths between 200 and 600 nm. The column was VertiSep UPS C18 HPLC column, 4.6 mm×250 mm. Spectrophotometric determinations were performed on a Biotek a Synergy HT Microplate Reader.

Preparation of dried plant materials

The collected plant materials were cut into small pieces and dried in an oven at 70 °C for three days. All dried samples were ground and sifted through the sieve in 100 mesh to obtain powder and kept at -20 °C until used.

Preparation of the crude extracts

In the extraction process, 5 g aliquot of dried powders of root, leave and stem from *Stemona collinsae* Craib were extracted with 10 ml of 1% of acetic acid in methanol under mechanical stirring at room temperature for 1 h. The extracts were shaken for 15 min, centrifuged (3,000 rpm) for 15 min at room temperature and then the organic layer was collected. The extraction of remained powder was repeated twice. Then, the extracts from all the three washes were pooled and concentrated under vacuum at 60 ^{o}C to obtain a dried crude extract. The crude extracts were stored at 4^{o}C for further use.

Measurement of total phenolic contents

Total phenolic content was determined using the Folin-Ciocalteu colorimetric method using gallic acid as a standard phenolic compound [Serea C. *et.al.*, 2011; Prior R.L. *et.al.*, 2005; Rebaya A. *et.al.*, 2014]. Briefly, 0.5 ml of each crude extract (1 mg/mL) was made up to 1 mL with methanol, mixed thoroughly with 1.5 ml of the Folin–Ciocalteu reagent, followed by an addition of 2 mL of 20% (w/v)) sodium carbonate. The mixture was left for 30 min at room temperature for color development. The absorbance of the resulting blue-colored solutions was measured at 765 nm. Total phenolic content was performed, based on a standard calibration curve of gallic acid in methanol. The mean (+SD) results of triplicate analyses were expressed as gallic acid equivalents (GAE) in milligrams per gram of dry-material.

Measurement of total flavonoid content

The flavonoid content was determined by an aluminium trichloride method using quercetin as reference compound according to the method described by Zhishen et.al. [Rebaya A. *et.al.*, 2014, Zhishen J. et.al., 1999]. Briefly, 125 µL of each crude extract was added to 75 µL of a 5% NaNO2 solution. After 5 min, 150 µL of aluminium chloride (10%) was added to the mixture, which was then allowed to stand for another 5 min. Then, 750 µL of NaOH (1 M) was added to the mixture, followed by the addition of 275 µL of deionized water. After 15 min of incubation, the mixture turned to pink and the absorbance was measured at 510 nm wavelength. The measurement was done in triplicate and the total flavonoids content was expressed as gram of quercetin equivalents (QE) per 100 g of dried extract. For calibration, the standard curve of quercetin (20 to 120 mg/mL) was used.

Determination of antioxidant activity

The antioxidant activity of the crude extracts was determined by the DPPH assay, according to the method of Nooman and Marxen [Nooman A. K., et.al., 2008; Marxen K. et. al., 2007] with slight modification. Briefly, 1.5 mL of various concentrations of extract solutions was mixed with 1.5 mL DPPH solution (3×10^{-4} M). The mixtures were placed in the dark and left for 30 min at room temperature for incubation. Absorbance of the solution was measured at 517 nm wavelength having ethanol as a blank and BHT as a positive control. The following formula was used to calculate the percentage inhibition of DPPH scavenging activities of crude extracts. All test analyses were repeated in triplicate.

$$\text{Inhibition percentage} = (1\text{-}A)/A_0) \times 100$$

where A_0 is the absorbance of the control reaction (containing all reagents except the extract), and A is the absorbance of the extract.

Sample extraction and hydrolysis for analysis of active compounds

Extraction was carried out as previously described by Mattila [Mattila P., 2007]. Briefly, 0.5 g of dried samples were extracted in 7 mL of the mixture of methanol, containing 2 g/L of butylatedhydroxyanisole (BHA) and 10% acetic acid (85:15). The mixture was stirred for 1 h at room temperature and adjusted to a volume of 10 mL with distilled water and mixed well. Then, the mixture was added with 12 mL of distilled water having 1% ascorbic acid and 0.415% ethylenediaminetetraacetic acid tetrasodium salt dehydrate (EDTA), followed by addition of 5 ml of 10% w/v NaOH as alkaline hydrolysis to cleave phenolic acids bound. The reaction was left for 16 h at room temperature. After the alkaline hydrolysis, the mixture was acidified to pH 2 with *conc*.HCl, followed by extraction with diethyl ether (DE) and ethyl acetate (EA, 1:1 v/v). The organic layer was separated and stored as the first portion. Then, 2.5 ml of *conc*.HCl as acid hydrolysis was added into the residue and incubated at 60 ^{o}C for 1 h. After the acid hydrolysis, the extracts were again extracted similarly to above procedure. The second portion of the organic layer was combined with the first part, followed by evaporation to dryness. The extracts were then dissolved into 2 mL of methanol and the mixture was filtered and analyzed for total phenolic acids via HPLC.

Quantitative analyses of active compounds using an HPLC technique

All active compounds were analyzed on an Agilent 1100 Series HPLC with a diode array detector and detected at 280, 320 and 360 nm. The sample (20 µl) was injected with the flow rate 1 mL/min into the column and the signals were recorded between 200 and 600 nm wavelengths. The column was VertiSep UPS C18 HPLC column, 4.6 mm×250 mm, 5 µm operated at 25^{o}C. A gradient elution program was utilized and the elution solvents were acetonitrile (A) and 0.2% acetic acid in water (B). The samples were eluted according to a linear gradient: 0-25 min, 15-25%A; 25-30 min, 25-40%A; 30-42 min, 40%A; 42-45 min, 40-15%A and 45-50 min, 15%A isocratic and then washing and reconditioning of the column. Identification and quantification of all active compounds in the samples were performed by comparison with chromatographic retention times and areas of standard compounds. The calibration curves of phenolic acid standards were established using the known concentrations of the standard compounds.

Statistical analysis

The statistical analyses were carried out by ANOVA using the general linear model of SPSS 14.0 for Windows. All analyses was carried out in triplicate and expressed as mean ± SD to show variations in the various experimental data. P values less than 0.05 were considered statistically significant.

Results and discussion

Determination of total phenolic and flavonoid contents in Stemona collinsae Craib crude extracts

The Folin- Ciocalteu colorimetric method was used to investigate the total phenolic contents because this technique is simple, sensitive, and precise [Serea C. *et.al.*, 2011, Prior R. L. *et.al.*, 2005]. Gallic acid (GA) was used as a standard reagent (y= 0.0042x +0.004; R^2=0.9996). Total phenolic content was expressed as gallic acid equivalents (GAE) in mg/g crude extract. The total flavonoid content among the crude extracts was determined using a spectrophotometric method with an aluminum chloride colorimetric method. The content of flavonoids was expressed in term of quercetin equivalent (mg of QU/g of extract) using the standard curve (y = 0.0049x +0.0633, R^2 = 0.9990). The choice of gallic acid and quercetin as standard reagents is based on the availability of the stable and pure substances. In addition, the response of gallic acid and quercetin has been shown to be equivalent to most other phenolic compounds and flavonoids in vegetables on a mass basis [Prior R. L. et. al., 2005, Rebaya A., *et. al.*, 2014]. The total phenolic and flavonoid contents in all crude extracts of *Stemona collinsae* Craib are shown in Table 1. The comparison of total phenolic and flavonoid contents in each crude extract indicated that the crude extracts from root of *Stemona collinsae* Craib contains the highest phenolic and flavonoid concentration, followed by stem and leave, respectively.

Table 1. Total phenolic and flavonoid contents in all crude extracts of *Stemona collinsae* Craib.[a]

Crude extracts	Total phenolic contents (mg GAE/ g crude extract)	Total flavonoid contents (mg QU/ g crude extract)
Leave	6.21±0.15	5.92±0.75
Stem	8.75±0.33	13.26±0.45
Root	15.58±0.67	22.12±0.89

[a] The reported numbers are the average of triplicates ± SD ($p < 0.05$).

According to the information in Table 1, the highest content of total phenolics and flavonoids was detected in crude extract from root with 15.58 mg GAE and 22.12 mg QU, respectively. The lowest total phenolic and flavonoid contents were obtained in crude extract from leave. These results indicated that each part of *Stemona collinsae* Craib contained phenolic and flavonoid compounds at different levels. From previously reported works, the extracted compounds from root of *Stemona collinsae* Craib had various biological properties related to antioxidant mechanisms and showed the more than those obtained from leave and stem [Khorphueng P. *et.al.*, 2006; Chanmahasathien W. *et.al.*, 2011; Brem B. *et.al.*, 2004; Schinnerl J. *et.al.*, 2007). These could support the finding that the highest content of total phenolics and flavonoids in crude extracts of *Stemona collinsae* Craib was in the root.

Investigation of antioxidant activities of crude extracts

There are many different experimental methods by which the free radical scavenging activity can be determined. In this report, the crude extracts were subjected for the evaluation of antioxidant activity using a DPPH radical scavenging method. When DPPH reacts with an antioxidant compound, which can donate hydrogen, the DPPH is decolorized. The changing of colour can be quantitatively measured from

absorbance (Abs) at 517 nm wavelength. The use of DPPH provides a facile and rapid means to evaluate antioxidant activity [Nooman A.K. *et.al.*, 2008; Marxen K. *et.al.*, 2007]. In addition, it can incorporate many samples in a short time span and is sensitive enough to distinguish active ingredients at low concentrations [Esmaeili A.K. *et.al.*, 2015]. Therefore, when the crude extracts were tested for the DPPH free radical scavenging ability, the antioxidant activity of all crude extracts was expressed in terms of IC50 values (ppm) as shown in Table 2. IC50 values, which represent the minimum concentration at which 50% of DPPH were inhibited, are calculated from the plotting a graph between %inhibition and concentration of each crude extract. A higher DPPH radical scavenging activity is associated with a lower IC value.

Table 2. IC_{50} values of all *Stemona collinsae* Craib crude extracts[a]

Crude extracts	IC_{50}(ppm)
Leave	282.0±0.85
Stem	167.8±1.33
Root	118.7±1.12

[a] Values represent mean ± SD, n= 3 (p<0.05).

According to the data in Table 2, the IC_{50} values of root crude extracts were lower than those of leave and stem crude extracts, indicating that root crude extract had the strongest radical scavenging activity, while the lowest antioxidant activity was observed in the case of leave crude extract. These results well corresponded to the results of the studies in total phenolic and flavonoid contents in all crude extracts. This signified that the crude extracts of *Stemona collinsae* Craib root contained compounds such as polyphenolics that can donate electron/ hydrogen easily. The difference in scavenging activities of the extracts against the DPPH system could be explained by the presence of different compounds in the each part of *Stemona collinsae* Craib. It was previously reported that the crude extracts from root of *Stemona collinsae* had higher contents of major active components and biological activities than those from leave and stem [Xu Y.T. *et.al.*, 2006; Zhao W.M. *et.al.*, 1995]. This is the reason that the extracts from root has long been used in traditional medicinal plant for the treatment of respiratory diseases and to prevent human and cattle parasites [Xu Y.T. *et.al.*, 2006] and inhibited growth of many kinds of bacteria and fungi antifungal activity [Zhao W.M. *et.al.*, 1995].

Analysis of active compounds in Stemona collinsae Craib crude extracts via HPLC technique

The dried sample extraction and hydrolysis for analysis of active compounds were carried out as previously described by Mattila [Mattila P., 2007]. This extraction method differed from the extraction method for TFC, TFC and antioxidant activity testing. It can be explained that soluble phenolic acids (free and bound soluble forms) were extracted with methanolic acetic acid. The sum of bound soluble and insoluble formsplus free phenolic acids as aglycones were obtained after alkaline and acid hydrolyses which corresponding with standard active compounds.

The analysis of active compounds including phenolic acid and flavonoid compounds was carried out via HPLC technique for obtaining information on the active compound profiles. Standard phenolic acid compounds used in this work included gallic acid, *p*-hydroxybenzoic acid, ellagic acid, caffeic acid, *p*-coumaric acid, ferulic acid, and cinnamic acid, and the standard flavonoid compounds were catechin, epicatechin, luteolin, quercetin, and kaempferol. The choice of these compounds as standards is based on the availability of the stable and pure substances. In addition, these standard compounds were most abundant and showed the bioactivity properties as the chemopreventive compounds in plants. The chromatographic conditions for the quantification of these active compounds were optimized by conducting preliminary trials with the standard mix of the active compounds, with the aim of ensuring that all the compounds were well resolved. Three different wavelengths have been used for the quantification of phenolic acid compounds in this study: 280 nm for gallic acid cinnamic acids, catechin, *p*-hydroxybenzoic acid, ellagic acid and epicatechin; 320 nm for caffeic acid, ferulic acid and *p*-coumaric acids; 360 nm for luteolin, quercetin and kaempferol. The HPLC conditions used in this work can clearly differentiate all standard compounds in different retention times. The optimized time for the chromatographic run was 50 min (Table 3). To establish the calibration curves, five different concentrations of all standard active compounds of the phenolic acid groups and flavonoids were performed. The linearity of the standard curve was expressed in terms of the determination correlation coefficient (R^2) from the plots of the integrated peak area and concentration of the standard (in milligrams per liter). It was discovered that the correlation coefficient: R^2 of all standards ranged between 0.9960 and 0.9980. According to the R^2 values of the calibration curves for each standard compound, the linearity of all calibration curves was acceptable. The minimum concentration reliably detectable for the method ((LOD) and the minimum concentration reliably measurable (LOQ) were determined and shown in Table 3.

Table 3. Retention time (t_R), quantification absorbance (λ), correlation coefficient (R^2) of the calibration curves, LOD, and LOQ of all active compounds

Active compounds	t_R (min)	λ (nm)	LOD (mg/L)	LOQ (mg/L)	R^2
gallic acid	4.35	280	0.06	0.20	0.9993
p-hydroxybenzoic acid	11.23	280	0.09	0.30	0.9989
ellagic acid	21.18	280	0.25	0.80	0.9982
caffeic acid	12.90	320	0.03	0.10	0.9984
p-coumaric acid	19.45	320	0.03	0.10	0.9961
ferulic acid	21.57	320	0.06	0.20	0.9989
cinnamic acid	36.21	280	0.03	0.10	0.9994
catechin	9.32	280	0.25	0.80	0.9996
epicatechin	11.56	280	0.25	0.80	0.9969
luteolin	34.78	360	0.03	0.10	0.9977
quercetin	35.67	360	0.03	0.10	0.9979
kaempferol	41.89	360	0.03	0.10	0.9996

The contents of the active compounds in the unit of µg of active compound/100 g of dried sample in *Stemona collinsae* Craib crude extracts are shown in Figure 1.

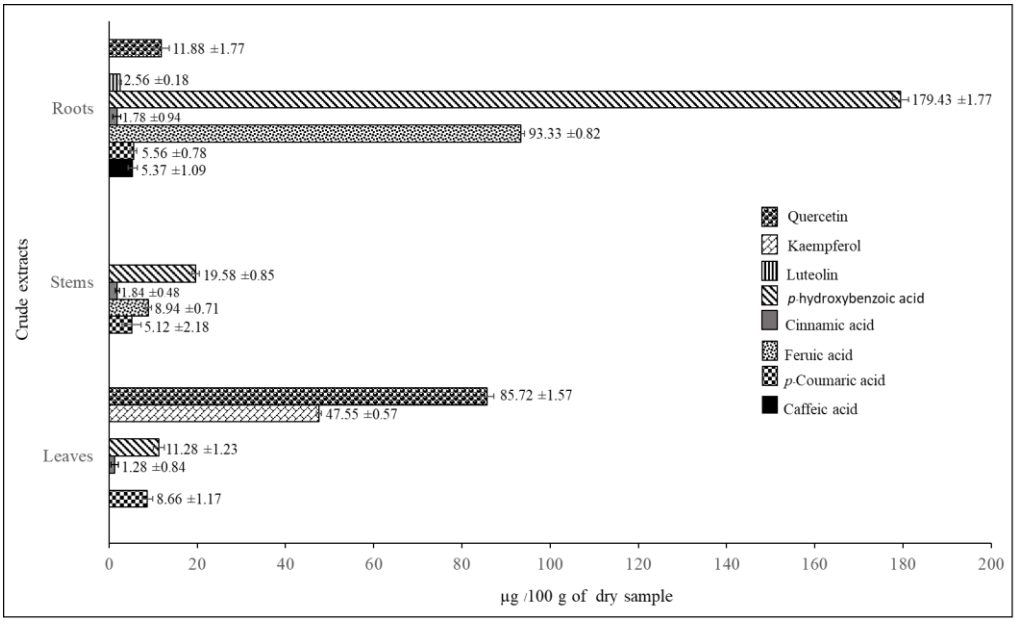

Figure 1. Quantitative analyses of glucosinolate compounds in the unit of mg /100 g of dried weight. The values represent mean ± SD, n= 3 (p<0.05).

Figure 1 shows the types and active compounds contents in the unit of µg of active compound/ 100 g of dried weight. The *p*-coumaric acid, cinnamic acid and *p*-hydroxybenzoic acid were found in all crude extracts. *p*-Hydroxybenzoic acid was the predominant phenolic compound found in all crude extracts, while the amount of cinnamic acid concentration was the lowest one in all crude extracts. The *p*-hydroxybenzoic acid as phenolic compound was found to be the main component having in the range from 11.28 to 179.43 µg/100 g of dried weight. In addition, the crude extract from root had the highest level of total and individual phenolic compounds, with *p*-hydroxybenzoic acid at 179.43 µg/100 g of dried weight. From these data, the difference in the phenolic compound content of root, stem and leave was rather significant. The amounts of phenolic compounds in crude extracts from root were drastically higher than those from stem and root. It can be explained that the crude extracts from root showed the greatest antioxidant activity and highest content of phenolic and flavonoid compounds corresponding to the result of total phenolic contents and antioxidant activity. In the case of flavonoid compounds, two flavonoid compounds including kaempferol and quercetin were mainly detected in crude extract from leave. Solely quercetin was found in crude extracts from root, whereas kaempferol and quercetin were not detected in stem crude extracts. It was worth to note that the different part of *Stemona collinsae* Craib had different types and amounts of active compounds. As a result, the numbers of active compound found in crude extracts from root were higher than those from stem and leaves.

Conclusions

This work focused on the investigation of total phenolic contents, flavonoid contents and antioxidant activity in *Stemona collinsae* Craib crude extracts from root, stem and leaves. The crude extract from root showed greatest antioxidant activity and

highest total phenolic and flavonoid contents, following with stem and leave crude extracts, respectively. The identification and quantification of the active compounds in crude extracts was studied via HPLC technique. The method was validated and showed acceptable quantitative performance in terms of correlation coefficient (R^2), LOD, LOQ. The differences in the individual active compound contents were observed among different crude extracts. Three phenolic compounds, including p-coumaric acid, cinnamic acid and *p*-hydroxybenzoic acid were present in all crude extracts. The *p*-hydroxybenzoic acid was the major phenolic compound in root and stem crude extracts, whereas quercetin as flavonoid compound was mainly detected in crude extracts from leave. In addition, crude extracts from root had higher amounts of phenolic acids than those from stem and leave. This study showed that *Stemona collinsae* Craib might be a good source of free-radical scavenging compound and the its extracts may help to prevent *in vivo* oxidative damage associated with diseases and illnesses.

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