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Research Article

Amino acids, bioactive compounds and biological activities of ten species from family Commelinaceae in Thailand

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Commelinaceae is used for ethnobotany. However, bioactive compounds information of this family is few data. The aim here was to determine the bioactive compounds and quantify the antioxidant activity from leaves 10 species. Twenty amino acids were identified using LC/MS/MS. The antioxidant contents were evaluated by the total phenolic and total flavonoid content assays. The individual phenolic acids, flavonoids and vitamin c were identified by HPLC. The antioxidant activities detected were DPPH scavenging and FRAP assay. The total amino acids found in most samples studied ranging from 239 to 1012 μg/g DW. Vitamin C contents were in the range of 23 to 195 mg/100 g DW. Total phenolic and total flavonoid contents ranged from 11.3 to 35.7 mg GAE/g DW and 56.7 to 368.7 mg RE/100 g DW, respectively. All the species studied possessed strong antioxidant properties (DPPH and FRAP). This result can be applied for further development of functional foods or cosmetics.

Keywords: amino acids; antioxidants; Commelinaceae; flavonoids; phenolic acids

Introduction

Commelinaceae is an important tropical and subtropical plant found worldwide. There are approximately 37 genera with over 600 species discovered (Edeoga and Ogbebor, 1999). The dominant characteristic of the family is succulent stems and sheathing leaves (Wilson, 1981). Many species of this family are grown ornamental plants and for ethnobotany (González-Avila *et al*., 2003; Mensah *et al*., 2006; Alonso-Castro *et al*., 2011; Myriam *et al*., 2011). Thailand has one of the richest Commetlinaceae floras in the world. North-eastern Thailand provides a unique environment for the nurture of specific genotypes, being unusually dry and elevated. The biologically active compounds of many of these genotypes have never been reported. However, reports on the amino acids and vitamin c have been slight with limited information of phytochemicals along with the biological activities of these plants.

In Thailand, the leaves of some Commelinaceae species are used in native dishes and some species are used in ancient folk medicine recipes. Numerous studies on their medicinal and nutritional properties have been conducted. The Commelinaceae species that are commonly consumed in Thailand include *Murdannia loriformis* (Hassk.) Rao & Kamm., *Tradescantia pallida* (Rose) D.R.Hunt, *Commelina bengalensis* L. and *C. clavate* C.B.Clarke. They are used as vegetables in many traditional Thai foods. The leaves of *C. diffusa* had the

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highest levels of alkaloids, ash, fat, and protein (Kamble, 2019). Currently, the development of functional foods from indigenous plants has been gaining great interest. For example, *Murdannia loriformis* (Hassk.) Rao & Kamm has been processed to a dried powder as an instant herbal tea beverage as it has been reported to possess medicinal properties for anti-inflammatory, analgesic and antipyretic activities (Kunnaja *et al*., 2014). Although there are many published reports related to the bioactive compounds of the Commelinaceae family, there has been little information on individual phenolic acids or phytochemicals along with the biological activities of Commelinaceae in Thailand. In addition, the composition and content of these compounds could be affected by the growth conditions or growth locations (Ghasemzadeh *et al*., 2010; Butsat *et al*., 2009). The aim of this work is present for the first reported, the individual amino acids, phenolic acids, flavonoids and antioxidant activity found in 10 of the widest spread and used medicinal plants in north-eastern Thailand.

Therefore, we aimed to generate information about the amino acids, bioactive compounds and biological activities in 10 varieties of Commelinaceae grown in north-eastern Thailand, which is the biggest plain region in Thailand. The total phenolic contents, total flavonoid contents, along with antioxidant activities were determined, and individual phenolic and flavonoid acids of the studied species were quantified. This present study expects to provide beneficial data for wider use of these plants. This research project should offer a useful foundation for future studies on the development foods product for functional food in the future.

Materials and Methods

All the chemicals and standards for HPLC analysis, standards of phenolic acids (*p*-hydroxybenzoic, gallic, caffeic, protocatechuic, sinapic, syringic, ferulic, *p*-coumaric, vanillic and chlorogenic acids), flavonoids (myricetin, rutin, apigenin, quercetin and kaempferol), amino acids (arginine, asparagine, alanine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, leucine, isoleucine, histidine, methionine, lysine, proline, phenylalanine, serine, tryptophan, threonine, valine and tyrosine) and ascorbic acid were purchased from Sigma–Aldrich Co. (St. Louis, MO., U.S.A.). All other high-purity solvents for HPLC analysis were supplied from Merck (Darmstadt, Germany).

Plant material and sample preparation

Whole plants of 10 edible species from Commelinaceae were collected from the north-eastern region of Thailand in 2020. Advice from local people was used as the basis for characterizing samples according to their uses, especially for being edible. They were identified by Dr. Surapon Saensouk, specialist plant taxonomist, where the specimens were positioned in the herbarium. The characteristics of the plants are given in Table 1. The leaves of selected species were thoroughly washed in tap water, freeze-dried and crushed into a powder using a dry grinder. The ground samples were kept at -20 °C until further analysis.

Amino acids by LC/MS/MS

Samples were analysed for amino acids using a method as previously reported by Chumroenphat *et al*. (2021). The LC/MS/MS was performed using a LCMS-8030 triple-quadrupole mass spectrometer (Shimadzu, Kyoto, Japan) operated in the ESI mode and a Shimadzu LC-20AC series HPLC system (Shimadzu, Kyoto, Japan). The 20 of individual amino acids was identified by their m/z values and by comparison with the retention time of external standards.

Sample extraction of phenolic contents and antioxidant activity

The extraction of phenolic contents and antioxidant activity were performed according to Chumroenphat *et al*. (2019).

Table 1. The characteristics of the selected plants

Sample name	Local name	Collector number	Part of use	Reference
C. axillaris (L.) D.Don. ex Sweet	Phak-plap-na	TC-2021-001	Leaves: vegetable; herbal medicine	Regis and Gabriel, 2017
C. bengalensis L	Phak-plap-bai-kwang	TC-2021-002	leaves: vegetable herbal medicine	Kokilavani *et al*., 2014
C. diffusa Burm.f.	Phak-plap-bai-cab	TC-2021-003	leaves: vegetable	Kamble, 2019
C. clavata C.B.Clarke	Phak-plap-bai-sak	TC-2021-004	leaves: vegetable	-
C. fragrans (Lindl.) Woodson	Vassana-lueang	TC-2021-005	leaves: vegetable	-
C. repens (Jacq.) L.	Yha-pai-nam-lek	TC-2021-006	leaves: vegetable	-
M. loriformis (Hassk.) Rao & Kamm.	Yha-pak-king	TC-2021-007	leaves: food; vegetable; herbal medicine	Cheeptham and Towers, 2002
T. fluminensis Vell	Yha-pai-nam	TC-2021-008	leaves: herbal medicine	Tan and Kwan, 2020
T. pallida (Rose) D.R.Hunt.	Hua-chai-sri-muang	TC-2021-009	leaves: herbal medicine	Tan and Kwan, 2020
T. spathacea Swartz.	Wan-kab-hoi-chiaw	TC-2021-010	leaves: herbal medicine	Tan and Kwan, 2020

Total phenolic contents (TPC)

The TPC test was done following the Folin-Ciocalteau method used in a previous study (Al-Duais *et al*., 2009) with some modified. Folin-Ciocalteu reagent was prepared 10% in distilled water and then 100 µl of that reagent was mixed with 20 µl of each extract were pipetted to the corresponding well of the 96-well plate. The mixture was incubated in incubator for 4 min and 75 µl of 10% sodium carbonate solution added. Then the mixture was left to stand at room temperature for 2 h. The solution absorbance was measured at 725 nm using a Varioskan Lux Multimode microplate reader (Thermo Fisher Scientific, USA). The results were expressed in mg gallic acid equivalents (mg GAE/g DW).

Total flavonoid contents (TFC)

Total flavonoid contents were determined using the colorimetric method described by Zhishen *et al*. (1999) with some modified. In brief, 25 µL of extract was mixed with 100 µL of purified water in the 96-well plate followed by the addition of 10 µL of a 5% $NaNO_2$ solution. After shanking for 5 min, 15 µL of a 10% $AlCl_3$ $6H_2O$ solution was added to the mixture with shaker for 6 min prior to the addition of 50 µL of 1 M NaOH and 50 µL of purified water. The mixture measured instantly at 510 nm by using a Varioskan Lux Multimode microplate reader (Thermo Fisher Scientific, USA). The results were expressed as mg rutin equivalents per 100-gram sample (mg RE/100 g DW).

Phenolic acids and flavonoids by HPLC

The individuals of phenolic acids and flavonoids were extracted and identified by HPLC according to Kaisoon *et al*. (2012). The phenolic acids and flavonoids in the extracts were identified by comparing their relative retention times with external standards.

Vitamin C by HPLC

The extraction of vitamin C was performed according to Siriamornpun and Kaewseejan (2017), A Shimadzu LC-20AC series HPLC system with diode array detector (Shimadzu, Tokyo, Japan) was used for analyses.

Antioxidant activities

DPPH free radical scavenging assay

The scavenging DPPH radials of the extracts were studied using a previously published method with some modifications (Rivero-Pérez *et al*., 2007). The extract or control (20 µL) of was added to 180 µL of 60 µM DPPH solution dissolved in methanol. The mixture was shaken vigorously and left to stand at an ambient temperature for 30 min in the dark. and then the absorbance was detected at 517 nm using a microplate reader (Varioskan Lux, Thermo Fisher Scientific, USA). Results were expressed as mg Trolox equivalents (TE) per one gram of dried sample (mg TE/g DW).

Ferric reducing/antioxidant power assay (FRAP)

The procedures used for this assay were according to the method measured based on an FRAP assay described from Li *et al*. (2017) with some modifications. An aliquot of 180 µL of FRAP reagent was briefly mixed with 5 µL of extract in the 96-well plate. FRAP reagent was freshly prepared by mixing 0.3 M acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl and 20 mM $FeCl_3 6H_2O$ in a 10:1:1 ratio at 37 °C. The mixture was shaken and incubated for 4 min at 37 °C. The absorbance was read at 593 nm using a microplate reader (Varioskan Lux, Thermo Fisher Scientific, USA). Results were expressed as mg $FeSO_4$ per one gram of dried sample (mg $FeSO_4$/g DW).

Statistical analysis

All data were analysed using a statistical program. These reported to be as the mean ± one standard deviation (SD) of three replicates and data were analysed using a one-way analysis of variance (ANOVA). The significance relative to the control.

Results and Discussion

The investigation of amino acids present in different kinds of plants has been a subject of interest in different studies. This work identifies both the qualification and quantitation using LC/MS/MS of 20 amino acids including nine essential amino acids in Commelinaceae for the first time, so providing useful information for further use of this plant. The results showed that the amino acids were found in all samples studied. On the other hand, cysteine and glycine were absent from all samples (Table 2). The highest total amino acids found were in *C. repens* (Jacq.) L. (1011.9 µg/g DW), *C. bengalensis* L. (867.8 µg/g DW) and *T. spathacea* Swartz. (762.0 µg/g DW), respectively. Glutamic acid and phenylalanine were found to have the highest content (137 µg/g DW). The total levels of amino acids found in Commelinaceae were considered to be lower than for other plants previously reported, such as in bean, potato, rice and mushroom (Ribeiro *et al*., 2008; Khalid *et al*., 2016; Huang *et al*., 2019; Ratseewo *et al*., 2020). However, this is an important nutrient for the human body.

The results from our present study have demonstrated that the amino acid content and composition varied greatly among varieties; however, free amino acid variability may also be related to different environmental conditions, using the discriminant and cluster analysis method. Therefore, further studies on this aspect are needed.

Table 2. Contents of amino acid in selected species from Commelinaceae analysed by LC/MS/MS

Amino acid content (μg/g DW)	Sample name									
	CA	CB	CC	CD	CF	CR	ML	TF	TP	TS
Alanine	17.43±1.25 i	19.18±0.52 i	6.70±0.26 j	21.80±1.77 i	3.40±0.31 h	17.05±2.33 k	12.18±1.24 k	8.38±1.35 k	36.39±2.99 f	53.16±3.33 e
Arginine	46.05±0.39 c	74.44±0.78 c	54.99±0.15 a	62.87±0.55 b	66.61±0.33 a	52.03±0.70 f	65.72±0.47 d	52.12±0.78 b	51.33±0.18 d	67.89±0.55 c
Asparagine	5.94±0.88 m	17.21±0.20 ij	5.03±1.08 jk	21.30±0.94 i	2.40±0.02 i	10.93±2.63 l	7.65±0.26 m	93.06±4.25 a	100.40±2.06 a	20.25±4.31 j
Aspartic acid	51.23±4.08 b	74.91±4.79 c	12.42±2.93 ef	58.24±3.70 c	4.75±0.84 h	59.28±1.01 e	77.94±5.88 c	34.48±1.74 e	43.08±2.08 e	45.23±1.53 f
Cysteine	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Glutamine	28.68±1.38 g	21.41±0.94 h	8.36±0.19 h	30.46±2.52 gh	7.15±0.35 f	27.80±1.81 i	25.49±3.23 h	7.07±0.71 k	41.83±1.83 e	31.15±1.96 i
Glutamic acid	41.34±1.99 d	137.64±10.08 a	53.87±0.86 h	34.41±2.11 g	10.35±0.92 e	96.22±5.38 d	110.18±8.71 a	29.41±1.18 f	61.42±4.54 c	86.87±3.58 b
Glycine	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Histidine	26.80±1.52 g	9.33±0.33 l	7.87±0.34 i	11.87±0.72 k	14.53±0.81 cd	32.05±2.53 h	6.83±0.26 n	14.06±0.22 j	23.55±1.87 h	4.31±0.72 m
Isoleucine	29.49±1.80f g	50.65±0.94 f	9.21±0.81 g	40.17±0.56 e	3.42±0.80 h	70.33±0.84 e	33.35±2.23 g	17.22±0.63 i	17.32±0.81 i	40.67±0.87 g
Leucine	30.06±1.65 f	52.31±2.70 f	13.29±0.83 e	40.27±0.29 e	7.44±0.60 f	68.47±0.99 e	34.65±2.08 g	19.65±0.32 h	21.74±1.82 h	36.41±1.22 h
Lysine	23.32±0.34 h	23.23±1.71 h	5.94±1.42 jk	32.15±2.28 g	7.48±0.56 f	29.54±0.98 h	20.78±1.44 j	8.65±0.58 k	42.97±2.12 e	32.94±1.46 i
Methionine	30.59±0.81 f	73.81±3.98 c	29.20±0.78 d	45.39±3.14 d	13.30±0.51 d	105.70±3.86 c	28.88±0.56 h	25.14±1.17 g	62.11±5.13 c	59.97±1.44 d
Phenylalanine	59.43±1.31 a	101.65±1.92 b	47.51±1.49 c	87.72±1.07 a	15.71±0.35 c	137.32±0.71 a	53.56±0.74 f	46.07±1.34 d	53.99±2.73 d	10.67±0.59 l
Proline	9.43±0.61 l	18.47±1.88 i	6.76±0.60 j	10.73±0.83 l	6.79±0.19 g	24.25±2.88 j	7.77±0.47 m	3.96±0.43 m	12.76±0.37 j	13.64±1.04 k
Serine	15.46±4.01 j	13.83±0.66 k	7.19±0.14 i	12.27±1.22 k	13.31±0.24 d	11.27±2.80 l	9.53±1.06 l	9.52±0.78 k	12.59±0.54 j	18.45±1.56 j
Threonine	12.82±1.40 k	17.13±0.16 ij	7.13±1.14 ij	16.46±1.87 j	3.41±0.03 h	12.66±1.01 l	9.55±0.74 l	5.42±0.25 l	11.47±0.77 j	14.36±1.14 k
Tryptophan	35.79±0.84 e	57.16±1.38 e	30.00±0.17 d	56.73±0.67 c	16.82±0.67 c	109.41±1.08 b	56.98±0.51 e	50.84±0.58 c	64.07±1.70 c	105.82±1.37 a
Tyrosine	34.90±1.17 e	60.21±2.73 d	22.72±0.64 e	29.37±1.45 h	35.81±0.75 b	103.38±5.00 c	88.36±5.26 b	16.25±0.85 i	68.61±1.71 b	82.95±1.65 b
Valine	25.55±1.22 g	45.30±3.91 g	14.81±1.24 e	37.90±2.61 f	6.34±0.37 g	43.12±2.10 g	24.40±1.35 hi	16.20±0.45 i	28.07±1.73 g	35.63±0.77 h
Total	553.78±4.57	867.87±2.20	342.99±0.84	650.12±1.57	239.01±0.48	1011.97±2.25	674.09±2.05	457.49±0.98	753.10±1.99	762.05±1.77

Values are expressed as mean ± SD of triplicate measurements (n = 3). Means with different letters are significantly different at $p < 0.05$ within the same column. CA: *C. axillaris* (L.) D.Don. ex Sweet; CB: *C. bengalensis* L.; CC: *C. clavata* C.B. Clarke; CD: *C. diffusa* Burm f; CF: *C. fragrans* (Lindl.) Woodson; CR: *C. repens* (Jacq.) L; ML: *M. loriformis* (Hassk.) Rao & Kamm.; TF: *T. fluminensis* Vell.; TP: *T. pallida* (Rose) D.R.Hunt.; TS: *T. spathacea* Swartz.

TPC and TFC are indicative of the levels of bioactive compounds that are beneficial to humans when found in high levels. The phenolic compounds have raised interest from the scientific community in recent times. In this study, the total phenolic contents were estimated in 10 selected species from Commelinaceae. The results are shown in Figure 1 (a). The levels of TPC in the evaluated Commelinaceae varied significantly, from 11.3 to 35.7 mg GAE/gDW. The highest values of TPC were found in *C. clavata* C.B. Clarke (35.7 mgGAE/g DW) and *T. spathacea* Swartz. (34.0 mgGAE/g DW), followed by *C. fragrans* (Lindl.) Woodson and *C. axillaris* (L.) D. Don. ex Sweet., while *M. loriformis* (Hassk.) Rao & Kamm. contained the lowest TPC compared to the other plants studied. In this study, the content of TPC was higher than those described in other species of Commelinaceae family (Tan *et al*., 2014).

Flavonoids are a diverse group of phenolic compounds distributed in higher plants, and they have been documented to have great antioxidant potential. As presented in Figure1(b), there were significant variations in the TFC in the selected species, which ranged from 56.7 to 368.7 mg RE/100 g DW. The highest TFC was found in *T. fluminensis* (368.7 mg RE/100 g DW), followed by *T. spathacea* Swartz. (134.7mg RE/100 g DW) and *C. clavata* (L.) D.Don. ex Sweet (55.89 mg RE/100 g DW). The lowest TFC levels appeared to have no statistical significance detected in *M. loriformis* (Hassk.) Rao & Kamm., *C. diffusa* Burm.f, *T. pallida* (Rose) D.R.Hunt and *C. repens* (Jacq.) L. The results section of this study suggested that flavonoids are not predominant in these selected species. Additionally, *T. spathacea* Swartz. and *C. clavata* C.B.Clarke were the richest source of phenolic compound and flavonoids.

Vitamin c is a good antioxidant, and many Commelinaceae species have been reported to contain vitamin c. Vitamin c contents in selected species from Commelinaceae are displayed in Figure 1(c). The amount of vitamin C ranged from 23.5 mg/100 g DW in *C. fragransto* (Lindl.) Woodson 195.4 mg/100 g DW in *T. pallida* (Rose) D.R.Hunt . Vitamin c is abundant in fruits (Kubola *et al*., 2011). Commelinaceae species can also be an alternative source of vitamin c, as indicated by this study. The findings obtained in this study conform to a previous study that presented a vitamin C content of 44.8 mg/100 g DW in *C. diffusa* Burm. f. (Kamble, 2019). Despite the fact that fruits are high in vitamin C. According to this study, Commelinaceae species can also be used as a source of vitamin C.

The Commelinaceae extracts were identified and quantified using HPLC. The distribution of phenolic acids and flavonoid compounds are presented in Table 3. The levels of total phenolic acid in the evaluated Commelinaceae varied significantly, from 189.9 to 2704.1 µg/g DW. The highest values of total phenolic acid were found in *C. clavata* C.B.Clarke (2704.1 µg/g DW), while *T. spathacea* Swartz. (189.9 µg/g DW) contained the lowest total phenolic acid compared to the other plants studied. Among all samples of Commelinaceae, *p*-hydroxybenzoic acid and sinapic acid were identified as the predominant phenolic acid found in *C. clavata* C.B.Clarke (2063.2 µg/g DW) and *C. fragrans* (Lindl.) Woodson (2039.3 µg/g DW). The minor phenolic acids, vanillic acid and chlorogenic acid, were detected in the Commelinaceae extracts. The five flavonoids (quercetin, kaempferol, rutin, apigenin and myricetin) were detected by HPLC. The quantifications of the five flavonoids based on calibration curves of authentic standards are presented in Table 4. Flavonoids were detected in all samples with significant differences among the samples ($p< 0.05$). Rutin was a major flavonoid in *C. clavata* C.B.Clarke (11460.44 µg/100g DW). Chorismate, the final product of the Shikimate pathway, is a precursor of the aromatic amino acid L-phenylalanine, from which benzoic and cinnamic acid derivatives have their biosynthetic origin. These phenolic acids are naturally found in plants and classified by constitutive carbon structures. They are derived and biosynthetic in origin from the aromatic amino acid L-phenylalanine, which is synthesized from chorismate, the final product in the shikimate pathway.

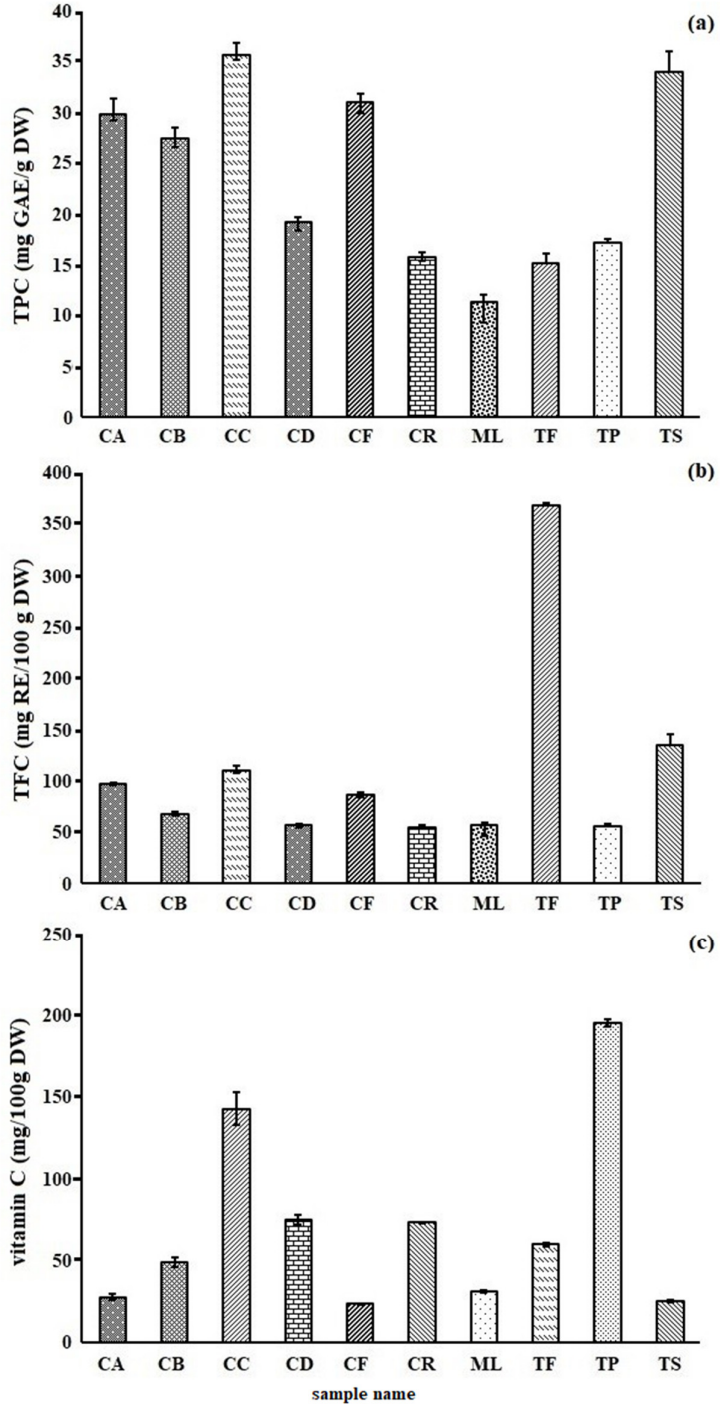

Figure 1. Contents of total phenolics, total flavonoids and vitamin c in Commelinaceae plants.; (a): total phenolic content; (b): total flavonoid content; (c): vitamin c content; CA: *C. axillaris* (L.) D.Don. ex Sweet; CB: *C. bengalensis* L.; CC: *C. clavata* C.B.Clarke; CD: *C. diffusa* Burm f; CF: *C. fragrans* (Lindl.) Woodson; CR: *C. repens* (Jacq.) L; ML: *M. loriformis* (Hassk.) Rao & Kamm.; TF: *T. fluminensis* Vell.; TP: *T. pallida* (Rose) D.R.Hunt.; TS: *T. spathacea* Swartz.

Table 3. Contents of phenolic acids in selected species from Commelinaceae

Sample name	Phenolic acid contents (μg/g, DW)										Total phenolic acid (μg/g, DW)
	GA	PCA	*p*-OH	ChA	VA	CA	SyA	*p*-CA	FA	SA	
CA	62.86 ± 1.62 e	ND	417.53 ± 26.78 c	ND	ND	62.96 ± 0.68 b	17.30 ± 0.15 b	12.81 ± 0.97 e	ND	910.86 ± 8.80 c	1484.32±3.90 c
CB	23.81 ± 0.64 h	11.37 ± 0.21 c	4.60 ± 1.18 g	ND	ND	146.65 ± 3.32 a	24.06 ± 1.93 a	183.48 ± 4.56 b	ND	1307.15 ± 16.84 b	1744.12±2.87 b
CC	34.82 ± 0.56 g	1.49 ± 0.29 f	2063.28 ± 30.04 a	ND	ND	3.15 ± 0.10 d	ND	9.56 ± 0.86 f	442.18 ± 5.98 b	149.67 ± 2.32 f	2704.15±1.44 a
CD	45.33 ± 0.42 f	1.95 ± 0.35e f	46.65 ± 0.53 g	13.15 ± 0.09 a	ND	2.64 ± 0.09 e	16.59 ± 0.03 b	2.86 ± 0.05 g	243.18 ± 1.72 c	160.65 ± 4.69 e	533.00±0.80 g
CF	35.75 ± 0.90 g	96.88 ± 0.27 a	94.45 ± 0.92 d	0.63 ± 0.33 d	ND	ND	8.66 ± 9.38 c	201.86 ± 2.90 a	148.96 ± 3.94 d	2039.35 ± 17.64 a	791.13±3.63 f
CR	166.09 ± 0.70 a	8.34 ± 1.28 d	61.82 ± 0.70 e	0.63 ± 0.36 d	ND	26.55 ± 0.21 c	23.57 ± 2.14 a	83.60 ± 2.49 d	664.31 ± 10.74 a	114.76 ± 1.46 h	1149.67±2.01 e
ML	75.01 ± 3.00 d	ND	50.02 ± 4.26 f	0.73 ± 0.13 d	ND	ND	8.64 ± 2.57 c	89.33 ± 1.00 c	94.89 ± 5.22 d	135.93 ± 6.49 g	454.55±2.27 h
TF	102.40 ± 0.57 c	8.82 ± 1.37 d	672.13 ± 1.67 b	2.45 ± 0.18 b	0.86 ± 0.28b	ND	ND	9.49 ± 0.93 f	421.27 ± 3.92 b	226.44 ± 1.12 d	1443.86±1.00 d
TP	152.55 ± 2.06 b	2.63 ± 0.82 e	39.52 ± 0.29 h	ND	ND	ND	ND	1.58 ± 0.03 g	12.07 ± 1.03 e	18.68 ± 1.87 j	227.03±0.61 j
TS	16.82 ± 1.87 i	25.74 ± 1.67 b	39.99 ± 0.41 h	1.00 ± 0.14 c	5.07 ± 0.15a	ND	ND	10.56 ± 0.40 f	ND	90.81 ± 0.46 i	189.99±3.47 i

Values are expressed as mean ± SD of triplicate measurements (n = 3). Means with different letters are significantly different at $p < 0.05$ within the same column.
CA: *C. axillaris* (L.) D.Don. ex Sweet; CB: *C. bengalensis* L.; CC: *C. clavata* C.B.Clarke; CD: *C. diffusa* Burm f; CF: *C. fragrans* (Lindl.) Woodson; CR: *C. repens* (Jacq.) L; ML: *M. loriformis* (Hassk.) Rao & Kamm.; TF: *T. fluminensis* Vell.; TP: *T. pallida* (Rose) D.R.Hunt.; TS: *T. spathacea* Swartz. ; GA: gallic acid; PCA: protocatechuic acid; *p*-OH; *p*-hydroxybenzoic acid; ChA: chlorogenic acid; VA: vanillic acid; CA: caffeic acid; SyA : syringic acid ; *p*-CA: p-coumaric acid; FA: ferulic acid; SA: sinapic acid. ND. = not detected.

Table 4. Contents of flavonoid compounds in selected species from Commelinaceae

Sample name	Flavonoid compounds (μg/100g g DW samples)					Total
	Rutin	Myricetin	Quercetin	Apigenin	Kaempferol	
C. axillaris (L.) D.Don. ex Sweet	990.25 ± 5.68 d	9933.43 ± 44.88 a	19.95 ± 1.18 g	1293.59 ± 37.78 f	277.35 ± 4.21 f	12514.57±18.75 b
C. bengalensis L.	399.09 ± 10.04 e	4977.82 ± 91.08 e	317.68 ± 14.91 b	980.79 ± 14.38 g	725.19 ± 25.57 a	7400.57±31.20 f
C. diffusa Burm.f.	2039.06 ± 26.47 a	1387.54 ± 23.61 h	30.87 ± 1.48 f	6280.97 ± 7.99 a	290.59 ± 9.07 e	10029.03±28.11 d
C. clavata C.B.Clarke	11460.44 ± 89.07 c	6217.15 ± 40.59 c	19.32 ± 0.08 g	1294.30 ± 82.24 f	343.11 ± 7.53 d	19334.32±47.91 a
C. fragrans (Lindl.) Woodson	582.97 ± 8.24 f	6533.98 ± 39.40 b	362.49 ± 5.01 a	2470.55 ± 34.53 b	149.67 ± 12.92 i	10099.66±40.02 c
C. repens (Jacq.) L.	1933.11 ± 30.35 b	3889.49 ± 54.09 fg	158.56 ± 4.30 d	1454.06 ± 7.96 e	212.90 ± 14.94 h	7648.12±35.93 e
M. loriformis (Hassk.) Rao & Kamm.	95.49 ± 1.54 i	313.98 ± 9.05 i	165.81 ± 3.56 c	291.57 ± 4.20 i	425.72 ± 20.05 b	1292.57±7.68 j
T. fluminensis Vell	54.86 ± 2.41 j	3930.59 ± 28.95 f	29.44 ± 2.27 f	1673.70 ± 35.25 d	724.88 ± 6.61 a	6413.47±15.10 h
T. pallida (Rose) D.R.Hunt.	131.13 ± 3.20 h	3873.22 ± 50.01 f g	17.12 ± 0.06 h	1928.89 ± 12.77 c	364.81 ± 34.50 c	6315.17±43.12 i
T. spathacea Swartz.	270.60 ± 3.57 g	5929.79 ± 36.58 d	33.92 ± 1.86 e	606.02 ± 21.76 h	259.05 ± 5.80 g	7099.38±13.92 g

Values are expressed as mean ± SD of triplicate measurements (n = 3). Means with different letters are significantly different at $p < 0.05$ within the same column.

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The antioxidant activities were expressed as DPPH radical scavenging activity shown by trolox equivalent concentration (TE) and FRAP value (Table 5).

DPPH is a stable free radical that has a purple colour. It has been used to evaluate the free radical scavenging activity. The deep purple colour of DPPH will become colourless or yellow after adding antioxidant material (Sánchez-Moreno *et al*., 1998). The colour changes occur due to transferring an electron or hydrogen atom to the DPPH by antioxidant materials. The absorbance of the remaining DPPH was determined at 517 nm using a microplate reader. In this study, the DPPH radical-scavenging activities were measured as mgTrolox equivalent of all samples (Table 5). The DPPH radical scavenging activity exhibited a wide range from 9.6 in *T. fluminensis* Vell. to 38.0mgTE/g DW in *C. fragrans*. These DPPH radical scavenging activity differences appear to have no statistical significance in *C. fragrans* (Lindl.) Woodson. *and C. clavata* C.B.Clarke, as they displayed the highest scavenging effect with 38.0 and 37.2 mg TE/g DW, respectively, followed by *C. bengalensis* L., *C. axillaris* (L.) D.Don. ex Sweet and *T. spathacea* Swartz. with the values of 21.2, 19.6 and 17.9 mg TE/g DW ($p< 0.05$).

FRAP, Ferric Reducing Antioxidant Power, is a direct method for determination of the antioxidant capacities (Benzie and Strain, 1996). Antioxidant is a reductant for the redox-linked colorimetric reaction. This method is based on the reduction of a ferric tripyridyltriazine (Fe^{3+}–TPTZ) complex to Fe^{2+}–TPTZ (blue-coloured) at a low pH. The change in absorption of Fe^{2+}–TPTZ was monitored at 593 nm using a microplate reader. The FRAP values of the Commelinaceae samples are presented in Table 5. It was found that *C. diffusa Burm.f.* had excellent reducing power (123.0 mg $FeSO_4$/g DW), after that *C. fragrans* (Lindl.) Woodson (104.7mg $FeSO_4$/g DW), *C. axillaris* (L.) D.Don. ex Sweet (92.3 mg $FeSO_4$/g DW), *C. bengalensis* L. (64.1 mg$FeSO_4$/g DW), *T. spathacea* Swartz (63.3mg $FeSO_4$/gDW), *T. pallida* (Rose) D.R.Hunt. (48.5 mg $FeSO_4$/gDW), *T. fluminensis* Vell. (42.6 mg $FeSO_4$/gDW), *C. diffusa* Burm.f (39.2 mg $FeSO_4$/gDW), *C. repens* (Jacq.) L. (28.2 mg $FeSO_4$/gDW) and then *M. loriformis* (Hassk.) Rao & Kamm. (17.6 mg $FeSO_4$/gDW). It has been observed that there was a wide variation in the antioxidant activities among Commelinaceae samples used in this study. Deviation in antioxidant activity values may be predictable due to variations in environmental and growth conditions.

Table 5. Antioxidant activities of Commelinaceae

Sample name	DPPH (mg TE/ g DW)	FRAP (mg $FeSO_4$/ g DW)
C. axillaris (L.) D.Don. ex Sweet	19.62±0.38 c	92.33±1.43 c
C. bengalensis L	21.23±1.01 b	64.14±1.22 d
C. diffusa Burm.f.	37.21±2.80 a	123.06±4.18 a
C. clavata C.B.Clarke	14.37±0.95 e	39.25±0.77 g
C. fragrans (Lindl.) Woodson	38.01±2.75 a	104.77±1.45 b
C. repens (Jacq.) L.	9.76±0.17 g	28.27±1.06 h
M. loriformis (Hassk.) Rao & Kamm.	10.60±0.45 f	17.60±0.50 i
T. fluminensis Vell	9.64±0.10 g	42.63±1.93 f
T. pallida (Rose) D.R.Hunt.	9.70±0.46 g	48.58±1.27 e
T. spathacea Swartz.	17.95±0.98 d	63.31±2.15 d

Values are expressed as mean ± SD of triplicate measurements (n = 3). Means with different letters (a, b, c, d) are significantly different at $p < 0.05$ within the same column. FRAP: Ferric reducing antioxidant activities; DPPH radical scavenging activities

Conclusions

This research has identified and compared key bioactive compounds and the biological activities of 10 selected species from Commelinaceae discovered in the north-eastern region of Thailand. The amino acid, bioactive compounds and antioxidants of some species of Commelinaceae have been revealed for the first time.

These genotypes were rich in phenolic compounds and flavonoids, with their concentrations varying widely. *C. clavata* C.B.Clarke and *C. fragrans* (Lindl.) Woodson were the richest source of phenolic acid and flavonoids, followed by *C. diffusa* Burmf. Our findings suggest that *C. clavata* C.B.Clarke was the most promising source of bioactive compounds. Regarding the biological activities, *C. clavata* C.B.Clarke appears to display the highest antioxidant activity, followed by *C. fragrans* (Lindl.) Woodson and *C. bengalensis* L. Gallic acid, *p*-hydroxybenzoic acid, p-coumaric acid, sinapic acid and all flavonoids were detected in all samples. The findings suggest that bioactive compounds (phenolic compounds and vitamin c) responsible for antioxidant activity. The results obtained from our study have provided useful information for the potential use of Commelinaceae as functional foods or cosmetics products. The results from our present study have demonstrated that the bioactive compounds and biological activities varied greatly among the varieties, however the bioactive compounds and biological activities were variable and may also be related to different environmental conditions using the discriminant and cluster analysis method. Therefore, further studies should include the influence of the environment and growth conditions as well as processing methods on these bioactive compounds and biological activities.

Authors' Contributions

TC and SS performed the conception and design of study. TC: analysis and/or interpretation of data designed. TC and SS wrote the manuscript and reviewed the final manuscript for journal submission. Both authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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