Chemical Components and Antioxidant Activities of Thai Local Vegetables

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Abstract

Proximate compositions, phenolic compounds and antioxidant activities of five Thai local vegetables were investigated. All studied samples showed the similar proximate profiles, in which moisture and carbohydrate were the major components. However, *Tiliacora triandra* Diels. also contained high content of fiber and protein. *Careya sphaerica* Roxb. had the highest total phenolic content (TPC) among all samples tested ($p < 0.05$). The main phenolic acids found in the vegetables were hydrocinnamic acids, including ferulic acid (1 to 16 mg/g), *p*-coumaric acid (4 to 7 mg/g), sinapic acid (0.9 to 5 mg/g) and syringic acid (1 to 6 mg/g). *Careya sphaerica* Roxb. extract exhibited the highest ferric reducing antioxidant power (FRAP) (3355.84 μmol $FeSO_4$/100g sample) ($p < 0.05$). DPPH radical scavenging activities of *Careya sphaerica* Roxb.(85.6 %inhibition) and *Cratoxylum formosum* (Jack) Dyer (82.6 %inhibition) extracts were comparable ($p > 0.05$). This research provided useful information for screening Thai local vegetables as potential sources of bioactive components for consumers and public health workers.

Keywords: local vegetables, phenolic acids, phenolic compounds, antioxidants

1. Introduction

The uses of local vegetables as food flavoring and seasoning agents have been found in many Southeast Asian countries such as Thailand [1]. Food flavor is usually the result of chemical components such as moisture, protein, carbohydrate, fat, ash, and fiber contents presented in vegetables, possessing diverse chemical and physicochemical properties. Vegetables containing vitamins, carotenoids, flavonoids and other phenolic compounds have been known as good sources of natural antioxidants [2-3]. The antioxidant activities could be obtained from leave, roots, rhizome, flowers, fruits, seeds and bark [4]. Local vegetables have also been recognized as antimicrobial, anti-inflammatory, anti-mutagenic and anti-carcinogenic potentials [5-9]. Phenolic compounds have strong antioxidant activities associated with their ability to scavenge free radicals, break radical chain reactions and chelate metals [10]. Reduced risk of cardiovascular disease and cancers correlated with increased consumption of food containing phenolic compounds has been reported [11]. Vegetables found in Thailand such as Yanang leaves (*Tiliacora triandra* Diels.), Pak Thew (*Cratoxylum formosum* (Jack.) Dyer.) and Pak Paew (*Polygonum odoratum*) etc. always contribute in Thai dishes including hot and sour fish soup, meat salad (in Thai called Laab), rice curry salad and other dishes from Northeast Thailand featuring bamboo shoots (in Thai called Naw Mai). The use of those local vegetables mostly found in dietary cultures where local vegetables are used regularly [12].

Although, these local vegetables were used in various dishes such as Thai curry and spicy soups with local style cooking in Northeastern Thailand. However the knowledge about chemical composition, phenolic acids, phenolic compounds and antioxidant properties of local vegetables (*Polygonum odoratum* or Pak Paew, *Tiliacora triandra* Diels. or Yanag leaves, *Cratoxylum formosum* (Jack.) Dyer or Pak Tew, *Careya sphaerica* Roxb or Kradon and *Syzygium gratum* (Wight) S.N. *Mitra var. gratum* or Pak Mek) consumed in Northeastern Thailand is scared. Therefore, the aim of this research was to investigate the chemical components and antioxidant properties of local vegetables from Northeastern Thailand. The results gained can be useful information for consumers, food industries and public health workers.

2. Materials and methods

2.1 Chemicals

The compounds 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tripiridyl-s-triazine (TPTZ), Folin–Ciocalteu's reagent, standards of gallic, ferulic, phydroxybenzoic, protocatechuic, *p*-coumaric, caffeic, syringic, sinapic, chlorogenic and vanillic acids were purchased from Sigma–Aldrich Fine Chemicals (St. Louis, MO). The phosphoric acid, methanol and acetonitrile used in the HPLC analysis were purchased from Merck (Darmstadt, Germany). All other solvents purchased from Fisher Scientific were of the highest available purity.

2.2 Plant materials (local vegetables) and sample preparation

Vegetables including *Polygonum odoratum* or Pak Paew, *Tiliacora triandra* Diels or Yanag leaves, *Cratoxylum formosum* (Jack.) Dyer. or Pak Tew, *Careya sphaerica* Roxb. or Kra don and *Syzygium gratum*(Wight)S.N. *Mitra var. gratum* or Pak Mek were randomly selected from three representative markets in the Ubon Ratchathani province, in the

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northeastern region of Thailand. At each market, 3 kg samples were sampled from three representative outlets. Single composite samples for each representative market, were prepared by combining about 500g of homogenized single sample of the same sample variety from three representative outlets and then homogenizing again to obtain a uniform single composite sample. All samples were freeze-dried using freeze-drier (Model Duratop^{TM}lP/Dura Dry^{TM}lP, FTS® System, Inc., Stone Ridge, NY, USA) and stored in a freezer (-20°C) until further analysis. Analyses were conducted in triplicate ($n = 3$).

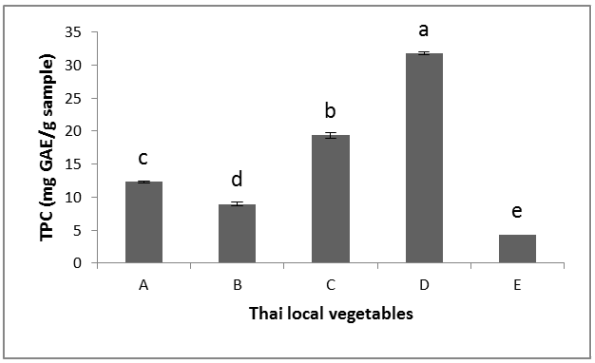
2.3 Determination of proximate composition

The proximate compositions of the samples, including moisture, ash, fat, fiber, and protein contents were determined according to the methods of AOAC 1999 [13]. Moisture content was determined by drying to a constant weight at 105°C. The crude lipid content was determined by extracting the sample with petroleum ether with a Soxhlet apparatus. The protein content was determined by the micro-Kjeldahl method. Carbohydrate contents were obtained by difference in ash, fat, fiber, and protein.

TABLE 1 PROXIMATE COMPOSITION OF THAI LOCAL VEGETABLES

Vegetables	Content (%)*					
	Moisture	Carbohydrate	Crude ash	Crude fat	Crude fiber	Crude protein
Polygonum odoratum	81.90±0.48^{a**}	12.22±0.11^{c}	1.50±0.03^{a}	0.40±0.01^{c}	1.88±0.05^{c}	2.10±0.06^{c}
Tiliacora triandra Diels.	65.73±0.27^{c}	11.09±0.06^{d}	1.54±0.02^{a}	0.83±0.03^{a}	9.80±0.84^{a}	11.01±0.53^{a}
Cratoxylum formosum (Jack.)Dyer.	83.10±0.39^{a}	11.49±0.08^{d}	0.91±0.01^{b}	0.64±0.06^{b}	1.98±0.07^{c}	1.88±0.03^{d}
Careya sphaerica Roxb.	75.67±0.31^{b}	18.44±0.14^{a}	1.51±0.04^{a}	0.42±0.02^{c}	1.86±0.04^{c}	2.10±0.08^{c}
Syzygium gratum (Wight) S.N.*Mitra var. gratum.*	76.35±0.34^{b}	14.42±0.12^{b}	1.47±0.02^{a}	0.38±0.01^{c}	4.24±0.39^{b}	3.14±0.17^{b}

Means ± SD (n=3).
*Different superscripts in the same column indicate the significant differences ($p < 0.05$).



Polygonum odoratum (A),
Tiliacora triandra Diels.(B),
Cratoxylum formosum (Jack.) Dyer. (C),
Careya sphaerica Roxb. (D),
Syzygium gratum (Wight) S.N.*Mitra var. gratum.*(E),

Bars represent the standard deviation (n=3).

**Different letters on the bars indicate significant differences *($p < 0.05$).*

Figure 1 Total phenolic content (TPC) of Thai local vegetables.

2.4 Determination of total phenolic content (TPC)

Total phenolic content of the prepared samples was determined following the method of Abu Bakar *et al.* [14] with some modifications. The prepared samples (1 g) were extracted for 2 h with 10 mL of 80% ethanol at room temperature on an orbital shaker (Heidolph NIMAX 1010, Schwabach, Germany) set at 180 rpm. The mixture was centrifuged at 1400 × g for 20 min and the supernatant was decanted into a 30 mL vial. The pellet was re-extracted under identical conditions. Supernatant was combined and used for total phenolics contents. Total phenolics content was determined using Folin–Ciocalteu reagent [14]. Briefly, 300 µL of extract was mixed with 2.25 mL of Folin–Ciocalteu reagent diluted (10-fold) with distilled water and allowed to stand at room temperature for 5 min prior to addition of 2.25 mL of 6% sodium carbonate solution into the mixture. After 90 min at room temperature, the absorbance was measured at 725 nm using spectrophotometer (Model RF-1500, Shimadzu Co., Kyoto, Japan). Total phenolic content of the extracts was calculated and expressed as mg gallicacid equivalents in 1 g of dried sample (mg GAE/g DW) based on the gallic acid standard curve.

2.5 Identification and quantification of phenolic compounds

2.5.1 Extraction of vegetable phenolics

The phenolic compounds in the vegetable samples were extracted using a modification of the procedure described by Bengoechea *et al.* [15]. Each sample (5 g) was mixed with 50 mL of methanol/HCl (100:1, v/v) which contained 2% tertbutylhydroquinone, in inert atmosphere (N_2) during 12 h at 35°C in the dark. The extract was then centrifuged at 4000 rpm/min using a refrigerated centrifuge (Beckman Coulter, Avanti J-E Centrifuge, Palo Alto, CA, USA), and the supernatant was evaporated to dryness under reduced

pressure (35–40°C). The residue was redissolved in 25 mL of water/ethanol (80:20, v/v) and extracted four times with 25 mL of ethyl acetate. The organic fractions were combined, dried for 30–40 min with anhydrous sodium sulfate, filtered through the Whatman-40 filter, and evaporated to dryness under vacuum (35–40°C). The residue was redissolved in 5 mL of methanol/ water (50:50, v/v) and filtered through a 0.45 μm filter before injection (20 μL) into the HPLC aperture. Samples were analyzed in triplicate.

2.5.2 HPLC–DAD system for analysis of phenolic compounds

RP-HPLC system for analysis of phenolic compounds was performed using Shimadzu LC-20AC pumps, SPD-M20A with diode array detector and chromatographic separations were performed on a LUNA C-18 column (4.6×250 mm i.d., 5 μm). The composition of solvents and the gradient elution conditions used were described previously by Schieber *et al.* [16] with slight modification. The mobile phase consisted of purified water with acetic acid (pH 2.74) (solvent A) and acetonitrile (solvent B) at a flow rate of 0.8 mL/min. Gradient elution was performed as follows: from 0 to 5 min, linear gradient from 5 to 9% solvent B; from 5 to 15 min, 9% solvent B; from 15 to 22 min, linear gradient from 9 to 11% solvent B; from 22 to 38 min, linear gradient from 11 to 18% solvent B; from 38 to 43 min, from 18 to 23% solvent B; from 43 to 44 min, from 23 to 90% solvent B; from 44 to 45 min, linear gradient from 90 to 80% solvent B; from 45 to 55 min, isocratic at 80% solvent B; from 55 to 60 min, linear gradient from 80 to 5% solvent B and a re-equilibration period of 5 min with 5% solvent B used between individual runs. Operating conditions were as follows: column temperature, 38°C, injection volume, 20 μL, and UV-diode array detection at 280 nm (hydroxyben zoic acids), 320 nm (hydroxycinnamic acids) and 370 nm (flavonols) at a flow-rate of 0.8 mL/min. Spectra were recorded from 200 to 600 nm. Phenolic compounds in the samples were identified by comparing their relative retention times and UV spectra with those of authentic compounds and were detected using an external standard method. Standards namely: gallic, ferulic, p-hydroxybenzoic, protocatechuic, *p*-coumaric, caffeic, syringic, sinapic, chlorogenic and vanillic acids were purchased from Sigma–Aldrich Fine Chemicals (St. Louis, MO).

2.6 Antioxidant activities determination

2.6.1 DPPH free radical scavenging activity

The antioxidant activity of the extracts, on the basis of the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, was determined as per the method of Braca *et al.* [17]. Ethanolic extract (0.1 mL) was added to 3 mL of a 0.001 M DPPH in methanol. The absorbance at 517 nm was determined after 30 min, and the percent inhibition of activity was calculated as $[(A_o\text{-}A_e)/A_o] \times 100$ (A_o = absorbance without extract; A_e = absorbance with extract).

2.6.2 Ferric reducing antioxidant power (FRAP)

The total reducing capacity was determined using the FRAP assay as described by Benzie and Strain [18]. The FRAP reagent initially prepared consists of 300 mM acetate buffer, pH 3.6, 10 mM iron reagent (TPTZ) solution in 40 mM HCl, and 20 mM $FeCl_3 6H_2O$ solution. The fresh working solution was warmed at 37 °C before using. The extracts (100 μL) were allowed to react with 1.9 mL of the FRAP solution. After incubation for 4 min, the absorbance was read at 593 nm using a spectrophotometer. The results were calculated by standard curves prepared with known concentrations of $FeSO_4$, and were expressed as μmol $FeSO_4$/g.

2.7 Statistical analysis

All experiments were run in triplicate determinations. Data were subjected to analysis of variance (ANOVA) and mean comparison was carried out using Duncan's multiple range test (DMRT) [19]. Statistical analyses were performed using the Statistical Package for Social Science (SPSS 11.0 for windows, SPSS Inc., Chicago, IL, USA).

3. Results and Discusion

3.1 Proximate composition of Thai local vegetables

Proximate composition of five Thai local vegetables is shown in Table 1. In general, moisture is the main component found in all tested samples, ranging from 65% to 83% (wet weight basis). Other compositions of all samples including fat and ash contents were similar. However, the content of carbohydrate in *Careyas phaerica* Roxb. was highest compared with that of other samples. The higher fiber (9%) and protein (11%) contents were also found in *Tiliacora triandra* Diels. when compared with that of other tested samples. The different chemical composition might affect on different flavor of various vegetables. Apart from fiber and protein, high contents of vitamin A, B, C and E and minerals (calcium, sodium and potassium) in some vegetables (spinach, asparagus, tomatoes, sweet potatoes, mushroom, green beans, and corn beets) have been reported [20].

3.2 Total phenolic content (TPC)of Thai local vegetables

Ethanolic extracts obtained from five vegetables were evaluated for the presence of phenolic compounds. The samples were evaluated using the Folin-Ciocalteu assay, which was suggested as a fast and reliable method to quantify phenolics in foods [21]. TPC was determined in comparison with standard gallic acid and the results expressed in terms of mg gallic acid equivalent (GAE)/g DW. The levels of TPC in the evaluated vegetables varied from 3 mg GAE/g DW in *Syzygium gratum* (Wight) S.N.*Mitra var. gratum* to 32 mg GAE/g DW in *Careya sphaerica* Roxb. (Fig. 1). The highest TPC value was found in *Careya sphaerica* Roxb. (32 mg GAE/g

DW), followed by *Cratoxylum formosum* (Jack.) (20 mg GAE/g DW) compared to other vegetables. However, high phenolic content was found in villous amomum, *Fructus amomi* (83.47 mg GAE/g) [22]. The different phenolic content in various plant species might be different from cultivars. Phenolic compounds are the main bioactive compounds in fruits and vegetables [23]. Recently, phenolic compounds have been considerable attention as potential antioxidant activities and free-radical scavenging abilities, which potentially have beneficial implications in human health[24].

TABLE 2 PHENOLIC ACIDS OF THAI LOCAL VEGETABLES

Vegetables	Phenolic acids* (mg/g DW)										
	Hydrobenzoic acids								Hydrocinnamic acids		Total
	GA	PCCA	p-HO	ChA	VA	CFA	SyA	p-CA	FA	SNA	
Polygonum odoratum	3.41 ±0.12^{e**}	4.27 ±0.24^{b}	4.28 ±0.36^{b}	1.85 ±0.03^{d}	3.79 ±0.28^{c}	ND	4.01 ±0.14^{b}	6.72 ±0.15^{a}	1.26 ±0.03^{e}	4.28 ±0.21^{b}	33.87
Tiliacora triandra Diels	4.11 ±0.26^{d}	4.31 ±0.25^{d}	6.78 ±0.56^{b}	ND	7.82 ±0.42^{a}	ND	6.52 ±0.21^{b}	7.31 ±0.34^{a}	1.23 ±0.04^{e}	5.47 ±0.73^{c}	43.55
Cratoxylum formosum (Jack.) Dyer.	1.19 ±0.04^{e}	5.08 ±0.32^{d}	9.98 ±0.86^{b}	7.48 ±0.46	ND	ND	ND	ND	16.44 ±1.06^{a}	5.00 ±0.02^{d}	46.17
Careya sphaerica Roxb.	1.59 ±0.05^{e}	1.66 ±0.06^{e}	12.30 ±0.51^{b}	ND	1.85 ±0.04^{e}	ND	1.45 ±0.04^{e}	4.31 ±0.18^{c}	16.70 ±1.02^{a}	3.89 ±0.12^{d}	49.75
Syzygium gratum(Wight) S.N. Mitra var. gratum.	1.21 ±0.03^{d}	0.87 ±0.01^{e}	1.35 ±0.06^{d}	0.86 ±0.02^{e}	5.05 ±1.06^{c}	11.73 ±1.04^{a}	1.07 ±0.02^{e}	ND	9.24 ±0.32^{b}	0.91 ±0.01^{e}	32.29

GA= Gallic acid; PCCA=Protocatechuic acid; p-HO=*p*-hydroxybenzoic acid; ChA= Chorogenic acid; VA= Vanillic acid; CFA= Caffic acid; SyA=Syringic acid; p-CA= *p*-Coumaric acid; FA=Ferulic acid; SNA=Sinapic acid.
ND= not detectable
*Means ± SD (n=3).
**Different superscripts in the same row indicate the significant differences ($p < 0.05$).

3.3 Identification of phenolic acids of Thai local vegetables

Phenolic acids are hydroxylated derivatives of hydrobenzoic acid and hydrocinnamic acid, which often occur in plants as esters, glycosides and bound complexes [25]. In the analyzed samples, it was possible to identify 10 phenolic acids including gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, chorogenic acid, vanillic acid, caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid and sinapic acid. The distribution of phenolic acids in all vegetables is depicted in Table 2. The highest concentrations of total phenolic acids were found in *Careya sphaerica* Roxb.(49 mg/g DW), followed by *Cratoxylum formosum* (Jack.) Dyer. (46 mg/g DW), compared with other samples. The main phenolic acids (hydrocinnamic acids) in these samples were ferulic acid, *p*-Coumaric acid, syringic acid and sinapic acid. Ferulic acid was the major hydrocinnamic acid derivative, ranging from 1 to 16 mg/g DW (Table 2). High levels of ferulic acid are found in herbs, vegetables, fruits, cereals, and coffee [26]. Ferulic acid is an abundant dietary antioxidant which may offer beneficial effects against cancer, cardiovascular disease and diabetes [26]. Generally, hydroxylbenzoic acid, gallic acid, vanillic acid and *p*-hydroxybenzoic acid occurred in low quantities (Table 2), excepted that for chorogenic acid, *p*-hydroxybenzoic acid and protocalechuic acid contents were found in high content in *Cratoxylum formosum* (Jack.) (Table 2). Natural antioxidants are important ingredients that facilitate the control of the oxidative deterioration of foods [27]. Vegetables extracts containing high amounts of total and individual phenolics, were found to exhibit antioxidant activities [28]. The antioxidant potential of phenolic compounds is dependent on the number and arrangement of the hydroxyl groups as well as the presence of electron donating substituent in the ring structure [29]. In general, phenolic compounds are considered a positive quality of fruits and vegetables [30]. However, phenolic compounds are not considered vital nutrients for humans, and their potential benefit to human health is still under discussion. Their nutritional benefits are often attributed to their substantial antioxidant activity [30].

3.4 Antioxidant activities of Thai local vegetables

3.4.1 DPPH radical scavenging activity

The DPPH radical is widely used to evaluate the radical scavenging activity of antioxidant compounds. The ability to act as donor of hydrogen atoms in the transformation of the DPPH radical to its reduced form was investigated for different vegetables extracts. The antioxidant activities of Thai local vegetables are shown in Table 3. The results showed that the DPPH radical scavenging activity of vegetables ranged from 77% to 85% Table 3. It was noted that *Careya sphaerica* Roxb. and *Cratoxylum formosum* (Jack.) Dyer extracts have high inhibition activities. This result suggested that both extracts were a very potent radical scavenger. The hydrogen

atom or electron-donation ability of the corresponding extracts and some pure compounds was measured from the bleaching of a purple-colored methanol solution of DPPH [31]. Higher radical scavenging activity found in *Careya sphaerica* Roxb. and *Cratoxylum formosum* (Jack.) Dyer. extracts was coincidental with higher amount of phenolic compounds in those extracts than that of other samples tested (Fig. 1). The varied radical scavenging activity of the vegetables extracts might be depended on the amount of total phenolic content in each sample (Fig. 1). A well correlation between antioxidant activity and phenolics content in plants extracts was reported [32]. Several flavonoids and polyphenols have been isolated from plant extracts with potent DPPH radical scavenging activities [33]. Marwah *et al.* [34] reported that DPPH radical scavenging activity of plants depends upon species.

3.4.2 Ferric reducing antioxidant power (FRAP)

Ferric reducing antioxidant power (FRAP) method is based on the reduction of a ferroin analogue, the Fe^{3+} complex of tripyridyltriazine $Fe(TPTZ)^{3+}$ to the intensely blue-color from Fe^{2+} complex $Fe(TPTZ)^{2+}$ by antioxidants in acidic medium [19]. The formation of blue color from Fe^{2+}–TPTZ complex (Fe^{2+} tripyridyltriazine) increases the absorbance at 593 nm. The FRAP values of the extracts of Thai local vegetables are shown in Table 3. The FRAP value of different local vegetables extracts indicated that the *Careya sphaerica* Roxb. extract had the greatest reducing power, followed by *Cratoxylum formosum* (Jack) Dyer, compared with that of other samples tested(Table 3). Higher FRAP values in both *Careya sphaerica* Roxb. and *Cratoxylum formosum* (Jack.) Dyer extracts might be due to their higher ferulic acids and total phenolic acids contents than other samples (Table 2). Phenolic acids was positively associated with the FRAP values [32]. Polyphenolic compounds found in edible plants have been reported to have multiple biological effects, including antioxidant activity [35]. Like other plants, local vegetables contained phenolic compounds that have a strong antioxidant activity (hydrogen atom donation). Plant extracts, generally used for their flavoring characteristics, often have strong H-donating activity thus making them extremely effective antioxidants. This antioxidant activity is most often due to phenolic acids, phenolic diterpenes, flavonoids and volatile oils (eugenol, carvacrol, thymol, and menthol). Some plant pigments including anthocyanin and anthocyanidin can chelate metals and donate H to oxygen radicals thus slowing oxidation via 2 mechanisms [36].

4. Conclusion

Selected Thai local vegetables in this study showed different chemical compositions and antioxidant activities. Ferulic acid was dominant in *Careya sphaerica* Roxb. and *Cratoxylum formosum* (Jack.) Dyer. *Careya sphaerica* Roxb. extract exhibited highest antioxidant activities. The information gained from this work could be useful for general consumers, food industries and researchers to utilize local vegetables as sources of bioactive compounds.

Acknowledgement

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Table 3 Antioxidant activities of Thai local vegetables

Vegetables	Antioxidant activities*	
	DPPH (% inhibition)	FRAP (µmol $FeSO_4$/100g sample)
Polygonum odoratum	77.39±0.28^{b**}	1745.43±5.42^{c}
Tiliacora triandra Diels.	77.89±0.32^{b}	975.32±3.28^{d}
Cratoxylum formosum (Jack.) Dyer.	82.60±0.84^{a}	2186.75±6.53^{b}
Careya sphaerica Roxb.	85.58±0.52^{a}	3355.84±7.36^{a}
Syzygium gratum (Wight) S.N.*Mitra var. gratum.*	78.35±0.63^{b}	429.26±1.73^{e}

*Means ± SD (n=3).
**Different superscripts in the same column indicate the significant differences ($p < 0.05$).

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