Pharmacogn J. 2018; 10(5): 979-982
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
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Original Article

Antioxidant Activities, Acute Toxicity and Chemical Profiling of Torch Ginger (*Etlingera elatior* Jack.) Inflorescent Extract

Bunleu Sungthong[1*], Buavaroon Srichaikul[2]

ABSTRACT

Aim/Background: The objectives of the study were to determine total phenolic contents, flavonoid contents, antioxidant activities and assess acute toxicity of torch ginger (*Etlingera elatior* Jack.) inflorescent hydroethanolic extract. **Methods:** The analysis of total phenolic contents, total flavonoid contents and antioxidant activities were analyzed spectrophotometrically using micro-titer plate reader. With regard to acute toxicity assessment, Wistar rats were fed with a single dose of torch-ginger either 1.0, 1.5 or 2.0 g extract/kg body weight in comparison with control group. **Results:** Total phenolic contents, flavonoid contents of the extract were 0.17±0.02 mM gallic acid equivalent/g extract and 0.30±0.01 mM quercetin equivalent/g extract, respectively. The antioxidant evaluation using DPPH radical scavenging assay, FRAP assay and ABTS radical scavenging assay were 0.14±0.08 mg/ml (EC_{50}), 0.13±0.01 mmol Fe^{2+} equivalent/g extract and 0.30±0.12 mM trolox equivalent/g extract, respectively. According to acute toxicity, no mortality or bizarre behavior had been observed throughout 14 days. Clinical chemistry including blood glucose, AST, ALT, BUN, creatinine, total cholesterol, triglyceride, HDL, LDL, total serum protein, albumin, globulin and total bilirubin were in normal ranges and comparable to the control (p<0.05). In conclusion, phenolic compounds and flavonoids of torch-ginger could be measured and indicated the quality of the extract as well as antioxidant activities. Regarding acute toxicity assessment, the extract was safe for experimental animals up to 2.0 g extract/kg body weight. **Conclusion:** Torch-ginger extract exhibited high amounts of phenolic contents, flavonoid contents, antioxidant activities and was safe in animal model.

Key words: Torch ginger, Antioxidant activities, Acute toxicity, Total phenolic contents, Total flavonoid contents.

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History

- Submission Date: 30-05-2018;
- Review completed: 28-06-2018;
- Accepted Date: 11-07-2018

DOI : 10.5530/pj.2018.5.166

Article Available online
http://www.phcogj.com/v10/i5

INTRODUCTION

Plant secondary metabolites, phenolic compounds and flavonoids, are commonly found in plants and possess various pharmacological activities. They are also used as functional foods to prevent cardiovascular disease, dyslipidemia, cancer and diabetes.[1] Several studies had been evaluated *in vitro* biological activities of plant extract such as antimicrobial,[2] antioxidant activities.[3-4] Long-term oxidative stress contributes to pathogenesis of chronic diseases such as diabetes, chronic kidney disease and some cancer.[3] There were also clinical efficacy of plant food supplement on diabetes patients.[5-6]

Torch ginger (*Etlingera elatior* Jack., Family Zingiberaceae) is normally grown in South-east Asia. It was generally used as herb or for cut flower production.[7] It was also contained enormous phenolic compounds and flavonoids as well as antioxidant properties.[8] Bioactive compounds from torch ginger can be extracted using various organic solvents; methanol, ethanol, acetone and water. Torch ginger extract was also expressed various pharmacological properties; antioxidant, antimicrobial, antifungal, tyrosinase inhibition, cytotoxic and hepatoprotective activities.[9] As generally used as food ingredient, toxicity should be evaluated either in rats or other organisms.[10] In a previous study, methanol extract of torch ginger was assessed in brine shrimps (*Artemia salina*) and showed no toxicity. In recent study, the aims of this research were to determined phenolic compounds, flavonoid contents, antioxidant activities and acute toxicity of torch ginger inflorescent extract.

MATERIALS AND METHODS

Plant materials

Torch ginger (*Etlingera elatior* Jack.) inflorescences were harvested in October 2013 in Samut Songkhram Province. The authentic sample was identified and collected in the herbarium of the Faculty of Pharmacy, Mahasarakham University, Thailand. The sample was washed with deionized water and cut in small pieces prior to air-dry condition at 50°C for 24 h. The sample was then ground and stored in air tight box before extraction.

Cite this article: Sungthong B, Srichaikul B. Antioxidant Activities, Acute Toxicity and Chemical Profiling of Torch Ginger (*Etlingera elatior* Jack.) Inflorescent Extract. Pharmacog J. 2018;10(5):979-82.

Extraction

The ground sample was extracted by means of ultrasonication. The ratio of solid to liquid ratio of sample to 50% ethanol was prepared at 1:50 w/v. Then, the prepared sample was placed into ultrasonic bath (Crest technology, Malaysia). The resultant was filtered and evaporated by rotary evaporator. Then, the sample was dried by lyophilization. The dry hydroexthanolic extract was kept under vacuum and protected from light.

Determination of total phenolic content (TPC)

Total phenolic content of the extract was analyzed by Folin Ciocâlteu (FC) assay with slight modification from previous studies.[11-12] The extract of 1 mg/ml in 80% ethanol (25 µL) was added to 125 µL of FC reagent in 96-well microtiter plate and subsequently added 100 µL of sodium carbonate (75 g/L). The mixture was then incubated for 2 h at ambient temperature. The absorbance was measured at 760 nm using microplate reader (Labortech, Germany). Total phenolic content was expressed in mM gallic acid equivalent per mg of extract (mM GAE/mg extract).

Determination of total flavonoid content (TFC)

Determination of total flavonoid content was carried out using aluminium chloride method as described previously.[13-14] Twenty five microliter of the extract (at a concentration of 1 mg/ml in 80% ethanol) was added to 96-well microtiter plate and then added 75 µL 95% ethanol. After that, 5 µL of 10% Aluminium chloride and 5 µL of 1 M potassium acetate were added into the solution. Finally, the solution was diluted with 140 µL of distilled water and incubated for 30 min before measurement. The absorbance of the resultant was measured at 415 nm using spectrophotometer (Labortech, Germany). The extract was calculated in mM quercetin equivalent per mg of extract (mM QE/mg extract).

DPPH radical scavenging assay

The effect of extract on reduction of free radical of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was determined as previously described with some modification.[8] The extract was prepared in methanol for 5 concentrations (15 – 500 µg/mL). The solution of different concentrations (750 µL) was added to equal volume of DPPH solution (60 µg/mL in methanol). The mixture was then thoroughly shaken and left in the dark place at room temperature for 20 min. Subsequently, the absorbance was measured at 517 nm against methanol. The percentage of radical scavenging was calculated according to the formula:

$$\text{Percentage} = \frac{A_{ctr} - A_{sample}}{A_{ctr}} \times 100$$

Where A_{ctr} is the absorbance of control (DPPH solution without extract) and A_{sample} is the absorbance of the DPPH solution with extract.

The percentage inhibition was plotted against concentration. Inhibitory concentration at 50% was calculated from the calibration line.

Ferric ion reducing antioxidant power assay (FRAP assay)

The antioxidant of extract using reducing power assay was modified from Wijekoon *et al.*[8] The FRAP reagent was consisted of 30 mL of 0.3M acetate buffer pH 3.6, 1 mL of TPTZ solution (10 mM 2,4,6-tris(2-pyridyl)-5-triazine in 40 mM HCl) and 1 mL of 20 mM ferric chloride solution. The extract at a concentration of 1 mg/ml (100 µL) was added to 3 mL of FRAP reagent. The mixture was then mixed and incubated for 10 min and measured the absorbance at 593 nm. The reducing power was calculated as millimoles of ferrous (II) per mg of extract (mM Fe^{2+}/mg extract).

2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) assay (ABTS assay)

The extract at a concentration of 1 mg/ml (in 80% Ethanol) was used to assess antioxidant capacity. The ABTS (2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid)) solution was mixed in equal volume of 7 mM ABTS and 2.45 mM potassium persulfate. The mixture was left in dark place for 16 h. Then, the resultant was diluted with methanol to obtain the absorbance of 1.00 at 734 nm. In the test method, 150 µL was added to 150 µL of ABTS solution and subsequently incubated at room temperature for 6 min. The absorbance of the sample was then measured at 734 nm. The antioxidant capacity was expressed as trolox equivalent in µg/mg extract (µg TEAC/ mg extract).

Acute toxicity assessment

Male and female Wistar Rats, six week-old, with weight between 200 – 250 g were obtained from North East Laboratory Animal Center (Khon Kaen, Thailand) and kept in at the Faculty of Sciences, Mahasarakham University. The rats were left in separate laboratory at 25±2°C with relative humidity of 40-60% and maintained 12 h light/dark cycle. The rats were fed with a standard diet from National Laboratory Animal Center (Nakhon Prathom, Thailand), and allowed to access to water *ad libitum*, and acclimated to laboratory conditions for 7 days.[15]

The experiment rats were divided into 4 groups, one control group and 3 treatment groups. The torch ginger extracts in 0.5% Tween 80 at concentrations of 1000, 1500 and 2000 g/kg body weight were fed orally using orogastric feeding device. After single dose administration, the animals were hourly observed for gross behavioral, neurological, autonomic, and toxic effects for 24 h and then twice daily for 14 days. The clinical signs of toxicity and mortality were investigated including the change in gait, posture, and response to handling and the presence of clonic or tonic movements, or bizarre behavior. At the day 14, blood sample for clinical chemistry tests including serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), blood creatinine, total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL), total serum protein (TP), albumin (ALB), globulin, total bilirubin, and serum glucose were collected directly from aorta under chloroform anesthesia. The weight of inner organs; liver, kidneys, heart and lungs, were assessed comparing with the control group.

Statistical analysis, means and standard deviations of each parameter were calculated and compared among each group, a control and 3 treatment groups. Normality, randomness and homogeneity of variance were evaluated prior to statistical testing (ANOVA, P value < 0.05). Scheffe's test was also performed to compare the difference among groups.

RESULTS AND DISCUSSION

Determination of phenolic compounds and flavonoids

Phenolic compounds and flavonoids were determined using spectrophotometric method. Basically, these compounds express high antioxidant according to their hydrogen atom transfer and/or electron donation.[16] The determination of phenolic compounds and flavonoids of the torch ginger extract was previously studied using water, methanol (100%, 90% and 50%) and acetone (100%, 90% and 50%) as extracting solvents. The highest amounts of both compounds were extracted using 50% of methanol and acetone.[8] The current study had used 50% ethanol as extracting solvent due to safety consideration. The polarity of 50% ethanol is similar to both 50% methanol and 50% acetone. The ethanol residue from extraction is less toxicity comparing to methanol or acetone. The amount of phenolic compounds was 0.17±0.02 mM gallic acid equivalent/mg extract (n=3). In case of flavonoid determination,

the compounds are commonly chelated with metal ion which initiated lipid peroxidation. Intensity of complexation between flavonoids and aluminium ion indicated the amount of flavonoids, and can be measured spectrophotometrically. Total flavonoids content from the method previously described was 0.30±0.01 mM quercetin equivalent/mg extract (n=3).

Antioxidant activities

Antioxidant activities were evaluated using DPPH radical scavenging assay, FRAP assay and TEAC assay. The mechanism of DPPH radical scavenging assay is based on hydrogen or electron donating of analytes to DPPH radicals.[16-17] The antioxidant activity using DPPH radicals is basically related to the intensity reduction of the purple radicals to yellow. The antioxidant activity was exhibited as percentage inhibition of radical comparing with the control. Determination of IC_{50} of the extract using 50% ethanol was prepared in two-fold dilution and calculated a regression line over 50% inhibition. The IC_{50} using DPPH radical scavenging assay of the extract was 0.14±0.08 mg/mL (n=3).

For FRAP assay, the reducing power of the extract was determined as the reduction of Fe^{3+}-TPTZ complex to Fe^{2+}-TPTZ in acidic condition.[17] The intensity of the reduce form can be measured at 593 nm in comparison with standard curve of Fe^{2+} in reaction well. FRAP value of the extract was 0.13±0.01 mM Fe^{2+} equivalent/mg extract (n=3). In ABTS assay, antioxidant compounds are reacted with ABTS in a present of potassium persulfate and measured at 734 nm using spectrophotometer. The intensity of the resultant correlated to the amount of antioxidant compounds in the extract. The antioxidant activity was calculated on a basis of intensity of a standard trolox in different concentrations. The trolox equivalent antioxidant capacity of the extract was 0.30±0.12 mM trolox equivalent/mg extract (n=3). Summary of total phenolic contents, flavonoid contents and antioxidant activities were shown in Table 1.

Table 1: Total phenolic contents, flavonoid contents and antioxidant activity of torch ginger extract (n= 3).

	TPC (mM GAE/mg extract)	TFC (mM QE/mg extract)	DPPH assay (IC_{50}, mg/mL)	FRAP assay (mM Fe^{2+}/mg extract)	ABTS assay (TEAC/ mg extract)
extract	0.17±0.02	0.30±0.01	0.14±0.08	0.13±0.01	0.30±0.12

Acute toxicity

Acute toxicity of torch ginger extract was evaluated in Wistar rats. The treatment groups were orally administered at concentration of 1000, 1500 and 2000 mg/kg body weight diluted with 0.5% Tween 80. The control group was fed with 0.5% Tween 80 without any additives. Survival and clinical observation after oral administration had been monitored hourly for 24 h. There was neither mortality nor abnormal clinical signs including scrubbing, unusual defecation or urination, vocalization on handling and bizarre behavior in the first day. After that, the mortality and clinical signs had been observed twice daily for 14 days. There was no mortality and abnormal clinical signs as well. At the end of day 14, blood samples were collected for clinical chemistry test. Clinical chemistry parameters; AST, ALT), ALP, BUN, blood creatinine, TC, TG, HDL, LDL, TP, ALB, globulin, total bilirubin and serum glucose of the treatment groups were not significant differences from the control group except alkaline phosphatase from 1000 mg extract/kg body weight treated group (p value ≥ 0.05) as shown in Table 2. Inner organs including liver, kidneys, heart and lungs were also weighed immediately after dissection to avoid dryness.[10] The weight of the organs from treatment groups were not significant differences comparing with the control group (p value ≥ 0.05)

Table 2: Effect of torch ginger extract on clinical chemistry parameters in Wistar rats.

parameters	control	1000 mg/kg body weight	ext1500 mg/kg body weight	ext2000 mg/kg body weight
Glucose(mg/dl)	146.60±8.15[a]	141.33±13.17[a]	140.00±11.68[a]	157.33±9.32[a]
BUN(mg/dl)	26.48±1.58[a]	28.06±1.98[a]	28.80±1.59[a]	27.75±1.36[a]
Creatinine(mg/dl)	0.84±0.02[a]	0.85±0.03[a]	0.91±0.03[a]	0.95±0.02[a]
Uric(mg/dl)	3.72±0.28[a]	3.81±0.36[a]	4.75±0.49[a]	3.36±0.23[a]
Cholesterol(mg/dl)	78.00±5.95[a]	73.66±5.37[a]	73.16±5.12[a]	73.16±5.38[a]
TG(mg/dl)	123.60±13.41[a]	120.83±8.59[a]	122.16±8.69[a]	117.33±15.95[a]
HDL(mg/dl)	16.60±1.12[a]	15.33±0.84[a]	15.50±1.23[a]	15.83±1.04[a]
LDL(mg/dl)	38.00±5.31[a]	37.16±2.99[a]	38.83±2.24[a]	37.00±3.17[a]
Total protein(mg/dl)	6.06±0.12[a]	5.83±0.17[a]	6.11±0.12[a]	5.76±0.05[a]
Albumin(g/dl)	3.86±0.06[a]	3.76±0.06[a]	3.90±0.08[a]	3.75±0.02[a]
Globulin(g/dl)	2.20±0.07[a]	2.06±0.11[a]	2.21±0.07[a]	2.01±0.04[a]
Total bilirubin(mg/dl)	0.30±0.03[a]	0.38±0.08[a]	0.38±0.08[a]	0.26±0.03[a]
AST(U/L)	78.60±2.60[a]	131.00±25.66[a]	106.16±14.62[a]	77.50±3.14[a]
ALT(U/L)	29.20±0.96[a]	32.66±2.80[a]	41.00±7.67[a]	28.00±1.61[a]
ALP(U/L)	100.60±4.10[a]	142.00±13.80[b]	113.16±8.05[ab]	110.50±5.84[ab]

Statistical testing was evaluated using ANOVA. Scheffe's test was also performed to compare the difference among groups.

[a, b] different alphabet indicated statistical significance (P value < 0.05).

Table 3: Effect of torch ginger extracts on the weight (gram) of inner organs.

organ	control	1000mg/kg body weight	ext1500mg/kg body weight	ext2000mg/kg body weight
Liver	4.84±0.31[a]	4.99±0.38[a]	5.13±0.38[a]	5.13±0.60[a]
Kidneys	0.76±0.04[a]	0.85±0.04[a]	0.07±0.03[a]	0.89±0.06[a]
Heart	0.44±0.02[a]	0.41±0.01[a]	0.42±0.02[a]	0.42±0.02[a]
Lungs	0.58±0.04[a]	0.61±0.02[a]	0.59±0.02[a]	0.71±0.09[a]

Statistical testing was evaluated using ANOVA. Scheffe's test was also performed to compare the difference among groups.

[a, b] different alphabet indicated statistical significance (P value < 0.05).

as shown in Table 3. According to the dose of the extract, the acute toxicity test was performed up to 2000 mg extract/kg body weight which was relatively high. The results revealed that there was no evidences of mortality, abnormal clinical signs or bizarre behavior found during the experiment. Therefore, we decided to stop increasing the dose of extract for acute toxicity test.

CONCLUSION

Torch ginger inflorescent extract exhibited in high phenolic and flavonoid contents. Their antioxidant activities was also expressed in high capacity in the test models. According to acute toxicity test using Wistar rat, oral administration of the extract was safe up to 2000 mg/kg body weight. The extract is beneficial for further development of food supplementation product for antioxidant purposes.

ACKNOWLEDGEMENT

The authors would like to thanks the Thailand Research Fund, grant No. RDG5650116 for financial support, and Mahasarakham University Development Fund for facilities.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

IC_{50}: The concentration required to reduce by 50% response; **AST**: Serum aspartate aminotransferase; **ALT**: Serum alanine aminotransferase; **ALP**: Alkaline phosphatase; **BUN**: Blood urea nitrogen; **TC**: Total cholesterol; **TG**: Triglycerides; **HDL**: High density lipoprotein cholesterol; **LDL**: Low density lipoprotein cholesterol; **TP**: Total serum protein; **ALB**: albumin.

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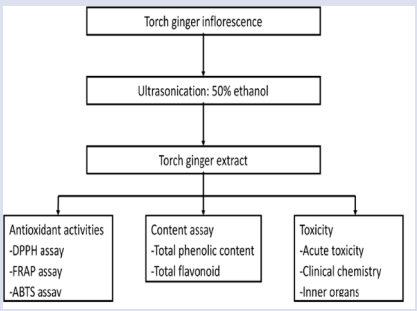
GRAPHICAL ABSTRACT



SUMMARY

- The present research provides information about total phenolic contents, flavonoid contents, antioxidant activities and acute toxicity of torch-ginger (*Etlingera elatior* Jack.) inflorescent hydroethanolic extract.

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