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Phytochemical profile and antioxidant activity of torch ginger (*Etlingera elatior*) inflorescence extract after *in vitro* simulated digestion

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ABSTRACT

Background : Torch ginger (*Etlingera elatior*) is an edible flower whose inflorescence is utlized for culinary purposes to enchance the taste of traditional dishes containing polyphenols and antioxidant compounds. However, investigation on the phytochemical profile and antioxidant activity of torch ginger inflorescence extract following simulated gastrointesinal digestion is still limited.

Objective: This study aims to determine the phytochemical profile and evaluate the antioxidant activity of the inflorescence extract after *in vitro* simulated digestion.

Methods: Torch ginger inflorescence (TGI) was extracted by ultrasound-assisted extraction with different solvents (water, 50%, and 80% aqueous ethanol). Total phenolics content (TPC), total flavonoid content (TFC), and antioxidant activity were determined by ORAC, DPPH, FRAP, and metal ion (Fe^{2+}) chelating activity. The solvent extraction that gave the highest

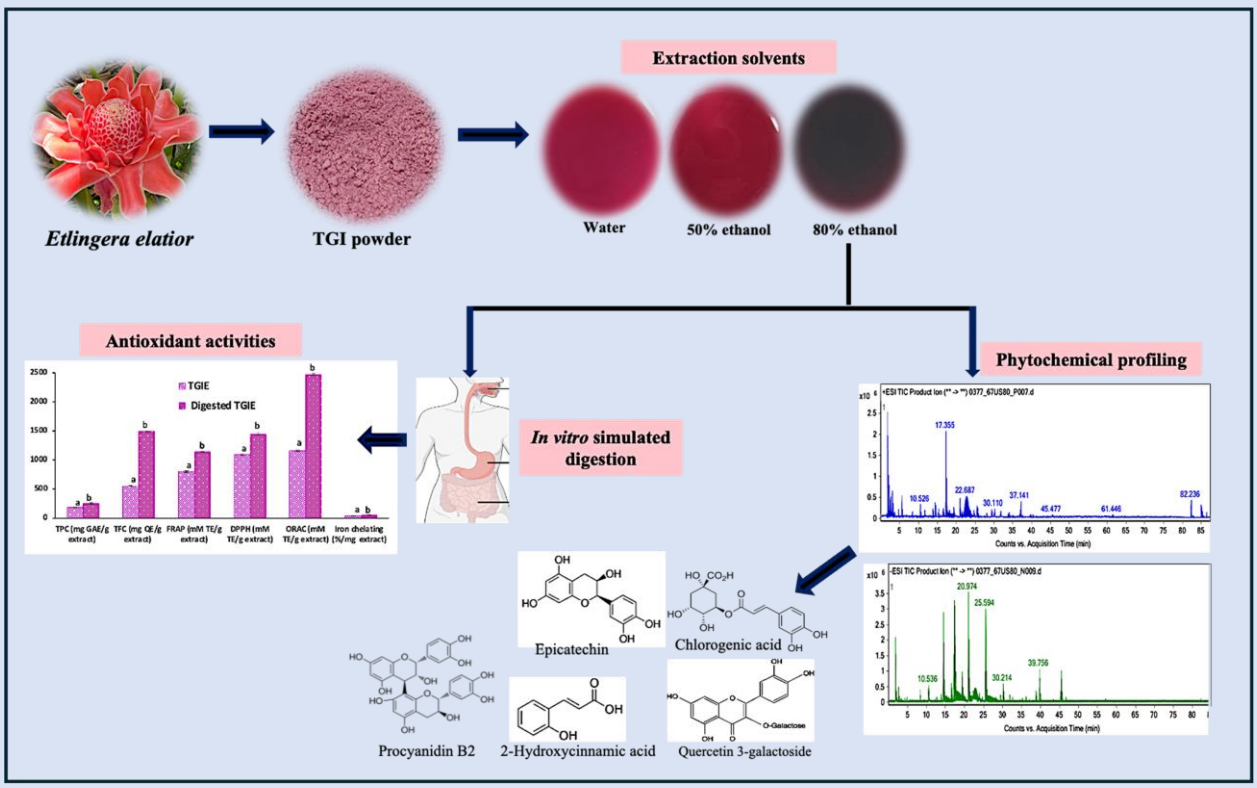
value of TPC, TFC, and antioxidant activities was used for an *in vitro* digestion and identification of phytochemicals profile by LC-ESI-QTOF-MS/MS. Furthermore, the TPC, TFC, and antioxidant capacities of digested TGI extract were compared to those of undigested extract.

Results: TGI contained 90.24% moisture. The 80% aqueous ethanol extract exhibited the highest antioxidant capacity, with an ORAC value of 1,156.61 ± 11.55 mM TE/g extract, DPPH radical scavenging capacity of 1,087.68 ± 14.37 mM TE/g extract, FRAP value of 799.30 ± 1.45 mM TE/g extract, and Fe2+ chelating capacity of 42.32 ± 3.48% /mg extract. The 39 phytochemicals were identified for 9 flavonoids and 5 phenolic acids. The putative bioactive compounds for antioxidant, anticancer, anti-inflammatory, and anticholesterol were detected in TGIE, such as catechin, 2-hydroxycinnamic acid, astragalin, chlorogenic acid, coumarin, and procyanidin B2. After passing through an *in vitro* simulated mouth, gastric, and intestinal digestion, the TGI extract exhibited higher values of TPC, TFC, and antioxidative capacities than the undigested extract.

Conclusion: This study reviewed the phytochemical components presented in the 80% aqueous ethanol TGIE. The expressed antioxidant capacity was increased when the TGIE passed through the *in vitro* simulated digestion, which could potentially represent a promising source of endogenous antioxidants in food and nutraceutical applications.

Keywords: Antioxidant, edible flower, gastrointestinal digestion, phytochemicals, Torch ginger inflorescence

Graphic Abstract



INTRODUCTION

Torch ginger (*Etlingera elatior*) is a popular edible plant in Southeast Asia. It is generally known as "kecombrang" in Indonesia, "daa laa" in Thailand, and "bunga kantan" in Malaysia. The inflorescences exhibit distinctive flavors and aromas that enhance traditional dishes such as "Khao Yum", a specialty of Southern Thai cuisine, or "sambal kecombrang" in Indonesia [1-2]. A previous study on the health effects of torch ginger reported that torch ginger inflorescence (TGI) exhibited total phenolic and flavonoid contents of 1.05 mg/100 g extract, 2.29 ± 0.00 mg GAE/g extract and 42.50 ± 2.64 mg RE/g extracts, respectively. Furthermore, it possessed the lowest IC50 with 2.86 ± 0.02 mg/mL with DPPH inhibition 68.70 ± 1.26%, among eight culinary flowers of the ginger family (Zingiberaceae) [3]. Interestingly, torch ginger exhibits antimicrobial, antifungal, anticancer, antibacterial, antidiabetic, and prebiotic-like as modulated gut microbiota [4-5]. Ethanolic extract of torch ginger inflorescence was assessed for acute toxicity in Wistar rat and was indicated to be safe for use with 2.0 g extract/kg body weight [6].

It is believed that consuming functional foods is essential for preventing various diseases. Functional food contains bioactive compounds, mainly found in plants, such as flavonoids and phenolic compounds, which have ability to promote health benefit in human body [64]. Flavonoids and phenolic acids are among the extensively studied antioxidant compounds, collaborating to mitigate age-related, intestinal, and chronic diseases. Antioxidants in foods prevent oxidation caused by reactive oxygen species (ROS). However, their effectiveness following the digestive system is a pivotal factor. Our digestive system has the potential to influence the form and rate of released antioxidant substances, thereby influencing their bioavailability throughout the digestive tract [7-9]. The study of the release of antioxidant compounds after food digestion can be conducted through *in vitro* digestion. To date, no studies have investigated digested TGIE for their antioxidant activities.

Given the above, the first aim of this study was to determine the optimum extraction type of solvent. The second objecctive was to determine the phytochemical found in a selected optimum solvent by LC-ESI-QTOF-MS/MS. Lastly, phenolic content, flavonoids content, and antioxidant activity should be evaluated after *in vitro* simulated digestion.

MATERIAL AND METHODS

Materials and chemicals: TGI were obtained from local farmers in the Songkhla Province, which is Southern Thailand. The light pink inflorescences of torch ginger were harvested on the bloom stage with uniform maturity (see Fig.1). Gallic acid monohydrate ≥ 98%, quercetin hydrate ≥ 95%, and (±)-6-Hydroxy-2,5,7,8-tetramethyl-chromane-2-carboxylic acid 97%, were purchased from Sigma-Aldrich Corp. (Milan, Italy). α-Amylase from human saliva, trypsin from porcine pancreas (~1500 U/mg), pepsin from porcine gastric mucosa, and α-chymotrypsin from bovine pancreas ≥40 units/mg protein were purchased from purchased from Sigma-Aldrich (St. Louis, USA).

Figure 1: Torch ginger (*Etlingera elatior*) inflorescences harvested on the bloom stage (A), the inner (B) and outer (C) petals were used in experiment.

Preparation of TGIE : Cleaned and air-dried TGI petals were subjected to freeze drying at -50 °C for 48 h. The freeze-dried TGI petals were grounded into fine powder using a crusher (High-Speed Crusher, Daming, DMF 10-A, China). The grinned TGI was sieved by FRIST Analytical Sieve (355 µm, Idar-Oberstein, Germany) to remove fiber and homogenize the particle size powder called TGI powder. Then, it was kept at 4°C until further use. The ultrasound-assisted extraction (UAE) of phytochemicals from TGI powder was slightly modified [10]. TGI powder was extracted with various solvents: water, 50%, and 80% aqueous ethanol (v/v). The solvent ratio was 1:40 (w/v). Extraction was carried out with a power of 230 W for 30 min using UAE (Hielscher, 1000hdT). The mixtures were centrifuged (Hettich Zentrifugen Mikro 22R, Tuttlingen, Germany) at 4000 x g for 15 min at 25ºC. The supernatant was collected, and solvents were removed by a rotary evaporator (Buchi, Vacuum Pump V-700). The obtained crude extract from TGI powder, called TGI extract (TGIE), was kept at 4° until further use.

Proximate analysis: Proximate analysis of TGI was performed according to [63] for total fat, protein, moisture, and ash content. Total carbohydrate was calculated using the following equation: Carbohydrate content (%) = 100 - (%total fat + %total protein + %moisture content + %ash). While total energy was calculated as: Total energy (Kcal/100g) = (protein x 4 kcal/g) + (fat x 9 kcal/g) + (carbohydrate x 4 kcal/g).

Determination of total phenolic content: Total phenolic content (TPC) was determined following previous studies [11]. Briefly, 20 µL of TGIE was mixed with 40 µL of the Folin-Ciocalteu reagent (10%) and incubated at room temperature for 6 min. Forty µL of Na_2CO_3 solution (7.5%) and 100 µL of distilled water were added to the mixture and incubated at room temperature in the dark for 30 min. The absorbance was measured at 765 nm using a multimode microplate reader (Varioskan LUX, Thermo Fisher Scientific, Waltham, MA, USA, SkanIt RE 6.1.1 software).

Determination of total flavonoid content : Total flavonoids content (TFC) was determined by following previous studies [11,12]. Firstly, 20 µL of a 5% $NaNO_2$ was added to 150 µL TGIE and incubated for 6 min at room temperature. Then 20 µL of 10% $AlCl_3$ solution was added to the mixture, and incubated again for 6 min. Finally, 100

µL of 1 M NaOH was added. After incubation in the dark for 20 min at room temperature, the mixture was centrifuged at 16,000 x g for 5 min at 25 °C and placed into 96-well plates. The absorbance was measured at 510 nm using a multimode microplate reader.

Oxygen radical absorbance capacity (ORAC): Determination of ORAC was modified following previous studies [12, 13]. First, 25 µL of sample was mixed with150 µL of a fresh fluorescein solution 8.16 x 10^{-5} mM in a black 96-well plate. The mixture was incubated in a multimode microplate reader without shaking at 37 °C for 30 min. Then, the fluorescence was measured at 485 nm (excitation) and 520 nm (emission). Immediately, 25 µL of 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) 153 mM was added. Without incubation, the fluorescence was measured again at 2 min time intervals for 90 min.

DPPH free radical scavenging activity: The determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical followed previous studies [11]. DPPH solution was prepared in methanol at a concentration of 200 µM and mixed vigorously in the dark at room temperature for 5 h. The DPPH solution 180 µL was mixed with 20 µL of samples in 96-well plates and incubated in a dark room for 30 min at room temperature. The absorbance was measured at 517 nm using a multimode microplate reader.

Ferric reducing antioxidant power assay (FRAP assay): The determination of FRAP was adapted from a previous study [11]. Acetate buffer (300 mM, pH 3.6), 10 mM 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$ were prepared for stock solutions. The FRAP reagent was prepared by mixing the stock solutions in 10:1:1 ratio. Then, 20 µL of TGIE were mixed with 180 µL FRAP reagent in 96-well ***plates.*** The mixture was incubated for 30 min in the dark at room temperature. The absorbance was measured at 593 nm using a multimode microplate reader.

Ferrous iron chelating: The Ferrous (Fe^{2+}) chelating activity was modified from a previous study [5]. Ethylenediaminetetraacetic acid (EDTA) was used as a standard and prepared in distilled water. Briefly, 20 µL of TGIE was mixed with 100 µL distilled water, 40 µL of 0.3 mM $FeSO_4.7H_2O$ solution, and 40 µL of 0.8 mM ferrozine. Then, the mixture was incubated in the dark for 10 min at room temperature. Absorbance was measured at 562 nm using a multimode microplate reader.

Liquid chromatography-mass spectrometry (LC-ESI-QTOF-MS/MS) profiling: A liquid chromatography analysis was conducted on TGIE 80% aqueous ethanol by following a previous study [14]. The separation process was processed by an Agilent ZORBAX Eclipse Plus C18 Rapid Resolution HD 2.1 x 150 mm, with a particle size of 1.8 µm. Dual AJS ion source was used for ionization. Mobile phase A was made up of water: formic acid (99.8:0.2, v/v), whereas mobile phase B contained acetonitrile, formic acid, and water in the ratio of 50:0.2:49.8 (v/v/v). Every sample extract or standard had an injection volume of 2 µL and a flow rate of 0.2 mL/min. A separate 2000 V was established for the nozzle and nitrogen gas temperature was set at 325 °C. The flow rate of sheath gas was 13L/min. Thirty-five psi was the nebulization pressure for nitrogen gas. With the collision energy (10, 20, and 40 V), a fixed mode MS/MS analysis covering a full mass scan range of m/z 50 to 1300 was carried out. MassHunter workstation software (Qualitative Analysis, version B.08.00, Agilent) was used for data collection and identification of polyphenols. Compounds detected by LC-ESI-QTOF-MS/MS with over

80 library identification scores were taken into consideration for further characterization and m/z validation.

***In vitro* digestion procedure:** In vitro simulated digestion was modified following a previous study [15]. All digestion processes were performed in MEMMERT water bath (Schwabach, Germany) at 37 °C and mixed with an overhead stirrer (IKA RW 20 Digital). The TGIE was dissolved with distilled water 1:30 (w/v), and then mixed with simulated saliva fluid (SSF) containing 0.117 g/L NaCl, 0.149 g/L KCl, and 2.1 g/L $NaHCO_3$. At the pH of 7.0, α-amylase (1 mg) was added and incubated for 1 min. Simulated gastric fluid (SGF) consisted of 8.775 g/L NaCl, and 1.5 g/L gastric mucin 1:1 (v/v) was added to the oral outcome. The pH of the mixture was lowered to 1.8 – 2, and pepsin (1 g/L) was added and stirred for 1 h. The gastric results were combined with simulated intestinal fluid (SIF) which was 8.776 g/L NaCl and 6.804 g/L KH_2PO_4 1:1 (v/v). The pH was adjusted to 8 and 11 unit/mL trypsin, and 24 unit/mL α-chymotrypsin were added to the solution and incubated for 2 hr. To stop the reaction, the temperature was increased to 90 °C for 10 min, and then cooled on ice for 30 min. The samples were centrifuged (Hitachi High-speed Refrigerated CR 22-G III) at 25 °C for 30 min at 12,000 rpm. The supernatant was freeze dried for 48 h at -50 °C. The freeze-dried powder called digested-TGIE was kept at 4 °C for further experiment.

Statistical analysis: All results were expressed as mean ± SD of three replicates of analysis. The data was analyzed using one-way ANOVA following Tukey HSD post-hoc test. Statistical analysis was performed using IBM SPSS 22.0 software (IBM Corp, Armonk, NY, USA). Significant differences were considered at $p < 0.05$.

RESULTS

Proximate composition, total phenolic and flavonoid: The result on proximal composition of fresh torch ginger inflorescence including inner and outer petals was presented in Table 1.

Table 1: Proximate compositions of fresh torch ginger inflorescence

Composition	Results (g/100g FW)
Total Protein	1.25
Total Fat	0.71
Moisture content	90.24
Ash content	1.17
Carbohydrate	6.64
Total energy*	37.90

*(Kcal/100g)

The effect of solvent on extraction yield, total phenolic content (TPC), and total flavonoid content (TFC) are shown in Table 2. The results showed that water extraction gave the highest yield. However, extraction with 80% aqueous ethanol gave the highest TPC and TFC.

Table 2: Extraction yield, total phenolic and flavonoid contents of torch ginger inflorescence extracts with different solvent

Solvents	%yield TGIE (w/w)	TPC (mg GAE/100g FW)	TFC (mg QE/100g FW)
Water	68 ± 2.02[c]	275.41 ± 1.03[a]	919.34 ± 30.02[a]
50% Ethanol	36 ± 1.65[b]	587.54 ± 7.51[b]	1,272.61 ± 9.20[b]
80% Ethanol	33 ± 2.72[a]	610.30 ± 0.50[c]	1,385.34 ± 25.49[c]

Data is showed as means ± SD of three experimental replicates. Different letters indicate that the means in each column show significant difference ($p < 0.05$); 50% and 80% Ethanol are aqueous ethanol extraction.

Antioxidant activities: The results showed ORAC, DPPH, FRAP, and ferrous iron chelating among different solvents, which the extraction with 80% aqueous ethanol gave the highest value in a dose-dependent manner (Fig 2).

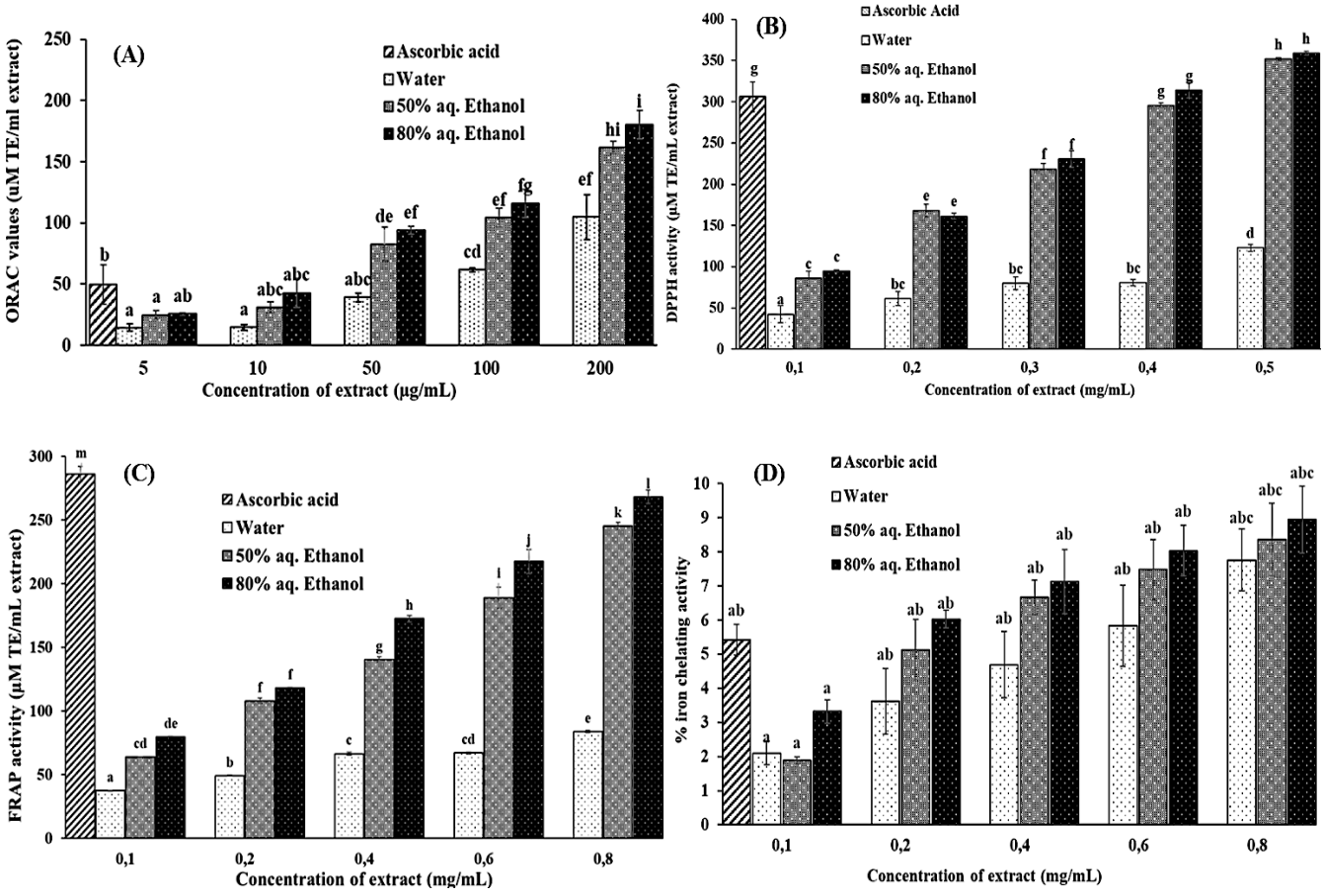


Figure 2: Antioxidant activities of TGIE with different solvents and concentration increased in a dose-dependent manner measured by the ORAC (A), DPPH (B), FRAP (C), and Iron chelating activity (D). Data is showed as means ± SD of three experimental replicates; Different letters indicate that the means show significant difference ($p<0.05$).

Qualitative characterization of phytochemicals: The result of TPC, TFC, and antioxidant capacities of TGIE from different solvents extraction suggested that 80% aqueous ethanol was selected for next steps. Qualitative

analysis and identification of polyphenols was conducted by LC-ESI-QTOF-MS/MS in negative and positive ionization modes. Chromatographic analysis was performed for identification and characterization of polyphenols and showed in Figure 3A and 3B. Phytochemicals were tentatively characterized and identified using Agilent LC-MS mass hunter qualitative software and the Personal Compound Database and Library. Score results ≥ 80 and mass error ± 5 ppm compounds were chosen for further m/z verification and MS/MS identification. In this study, 39 phytochemicals from TGIE 80% aqueous ethanol extraction were identified through LC-MS/MS (Table 3) including 9 flavonoids, 5 phenolic acids, 11 amino acids, 5 carboxylic acids, 3 nucleotides, and 3 nutrients.

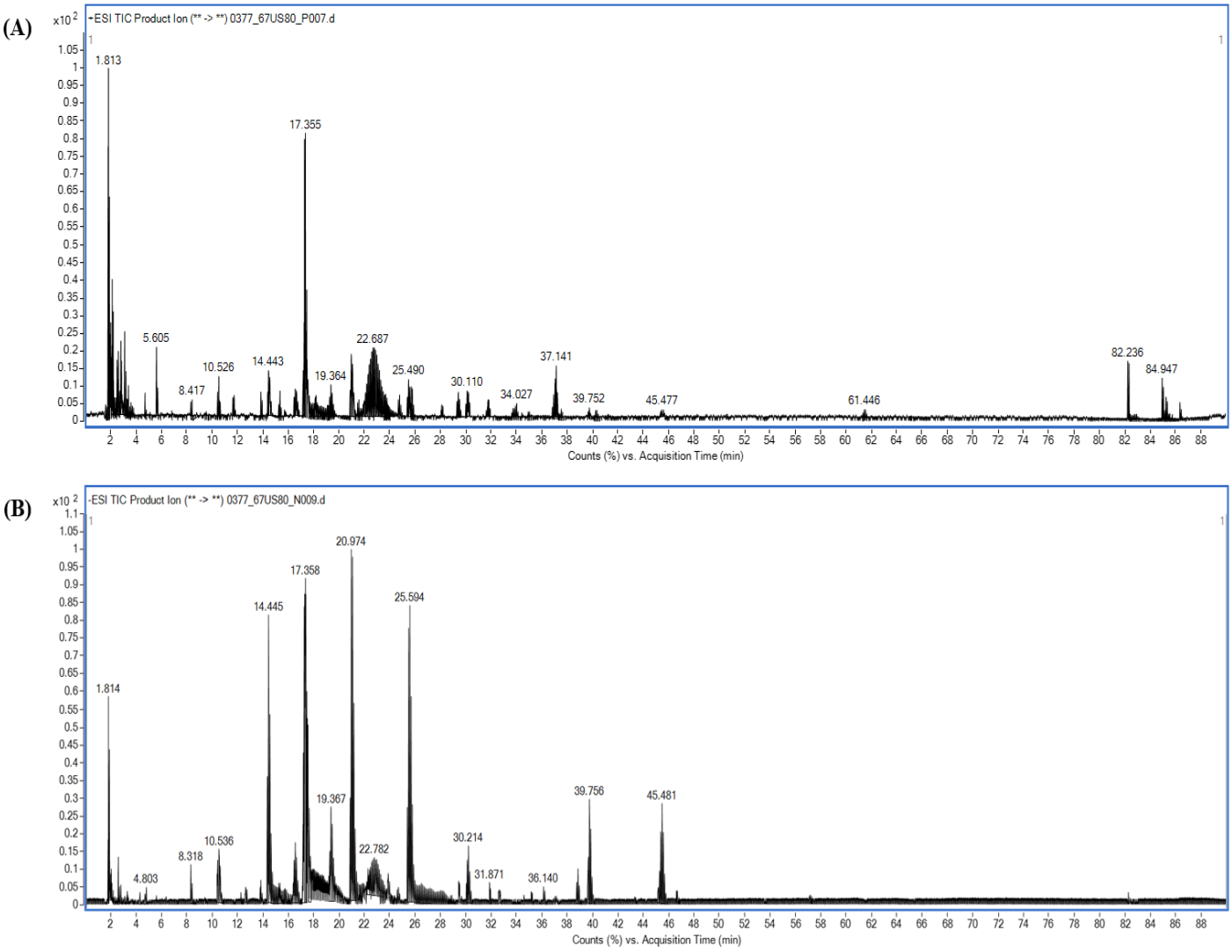


Figure 3: Chromatogram of product ion in positive mode (A) and negative mode (B) by LC-ESI-QTOF-MS/MS.

Tentative phytochemicals and their biological properties*:* The tentative phytochemical compounds with their health effects are listed in Table 4.

Table 3: Characterization of tentative phytochemical compounds in torch ginger 80% aqueous ethanol extract by LC-ESI-QTOF-MS/MS

No.	Tentative Compounds	Molecular Formula	RT (min)	Ionization Mode	MW (g/mol)	m/z	MS/MS Product Ions	Score (DB)	Diff (DB) (ppm)
Acetamide									
1	2-Phenylacetamide	C_8H_9NO	3.127	$[M+H]^+$	135.0681	136.0753	65, 91	95.04	2.27
Alkaloid									
2	Trigonelline	$C_7H_8NO_2$	2.273	$[M+H]^+$	138.0553	138.0547	63, 138	90.43	1.55
Amino Acid									
3	L-Histidine	$C_6H_9N_3O_2$	1.722	$[M-H]^-$	155.0701	154.0626	93	96.69	-4.03
4	Glutathione, oxidized	$C_{20}H_{32}N_6O_{12}S_2$	2.526	$[M-H]^-$	612.1526	611.1453	74, 128, 143, 213, 272, 306, 404, 482, 611	93.01	-1.04
5	Pyroglutamic Acid	$C_5H_7NO_3$	3.053	$[M-H]^-$*	129.043	128.0358	128	100	-3.51
6	Guanine	$C_5H_5N_5O$	3.303	$[M+H]^+$	151.0491	152.0563	135	89.37	1.89
7	Aminocaproic Acid	$C_6H_{13}NO_2$	3.405	$[M-H]^-$	131.095	130.0877	130	70.12	-2.9
8	L-Leucine	$C_6H_{13}NO_2$	3.491	$[M+H]^+$	131.0944	132.1017	86	90.53	1.7
9	Pro-Leu	$C_{11}H_{20}N_2O_3$	3.629	$[M+H]^+$	228.1476	229.1549	70, 142	96.61	-0.95
10	L-Phenylalanie	$C_9H_{11}NO_2$	5.663	$[M+H]^+$	165.0791	166.0864	77, 120	97.81	-0.83
11	D-Trypotophan	$C_{11}H_{12}N_2O_2$	11.716	$[M-H]^-$	204.0907	203.0834	116, 203	98.63	-3.78
12	L-Tryptophan	$C_{11}H_{12}N_2O_2$	11.764	$[M+H]^+$	204.0896	205.0968	74, 118, 146, 188	98.51	1.56
13	Pro Arg Thr	$C_{15}H_{28}N_6O_5$	53.620	$[M+H]^+$	372.2126	373.2198	145, 205	88.02	-1.38
Carboxylic acid									
14	Anthranilic Acid	$C_7H_7NO_2$	1.984	$[M+H]^+$	137.0479	138.0552	65, 138	82.65	-1.86
15	Quinic Acid	$C_7H_{12}O_6$	1.961	$[M-H]^-$	192.0641	191.0568	85, 191	90.74	-3.72
16	Malic Acid	$C_4H_6O_5$	2.501	$[M-H]^-$	134.0222	133.0149	71	98.73	-4.93
17	Citric Acid	$C_6H_8O_7$	2.601	$[M-H]^-$	192.0247	191.0202	87	94.32	-2.09
18	Succinic Acid	$C_4H_6O_4$	3.204	$[M-H]^-$	118.0265	117.0192	73	99.74	1
Flavonoids and derivates									
19	Pyrocatechol	$C_6H_6O_2$	8.615	$[M-H]^-$	110.037	109.0297	108	88.55	-1.75
20	Procyanidin B2	$C_{30}H_{26}O_{12}$	14.702	$[M+H]^+$*	578.1425	579.1499	55, 127, 289, 291, 409, 427	88.48	-0.07
21	(±)-Catechin	$C_{15}H_{14}O_6$	17.83	$[M-H]^-$	290.0809	289.0736	109, 123, 125, 205, 245	91.2	-6.3
22	Kaempferol-7-o-glucoside	$C_{21}H_{20}O_{11}$	22.011	$[M-H]^-$*	448.1018	447.0945	57, 125, 147, 284, 285,	96.12	-2.67

No.	Tentative Compounds	Molecular Formula	RT (min)	Ionization Mode	MW (g/mol)	m/z	MS/MS Product Ions	Score (DB)	Diff (DB) (ppm)
23	Astragalin	$C_{21}H_{20}O_{11}$	22.385	$[M+H]^+$	448.1013	449.1087	287	89.02	-1.63
24	Fisetin	$C_{15}H_{10}O_6$	23.113	$[M+H]^+$	286.0477	287.0551	135, 137, 213	85.9	-0.96
25	Epicatechin	$C_{15}H_{14}O_6$	25.775	$[M+H]^{+*}$	290.0796	291.0869	55, 123, 139, 207	97.08	-1.79
26	Quercetine 3-galactoside	$C_{21}H_{20}O_{12}$	39.513	$[M-H]^-$	464.096	463.0888	151, 300, 301	94.03	-1.14
27	Isoquercitrin	$C_{21}H_{20}O_{12}$	39.860	$[M+H]^+$	464.0955	465.1027	85, 145, 153, 303	90.35	0.01
Monosaccharide phosphate									
28	Mannose 1-phosphate	$C_6H_{13}O_9P$	1.747	$[M-H]^-$	260.0307	259.0235	78, 96, 169, 170, 259	99.8	-3.81
Nucleotide									
29	Adenosine	$C_{10}H_{13}N_5O_4$	2.914	$[M+H]^+$	267.0971	268.1043	57, 136	98.83	-1.19
30	Inosine	$C_{10}H_{12}N_4O_5$	3.304	$[M-H]^-$	268.0817	267.0744	92, 135, 267	99.18	-3.44
31	5'-Deoxy-5'-(methylthio)adenosine	$C_{11}H_{15}N_5O_3S$	10.358	$[M+H]^+$	297.0898	298.097	61, 136	99.26	-0.74
Nutrient									
32	Choline	$C_5H_{14}NO$	1.897	$[M+NH_4]^+$	104.1076	104.1071	58, 60, 104	93.07	-0.74
33	Sucrose	$C_{12}H_{22}O_{11}$	2.235	$[M+Na]^+$	342.1169	365.1062	203	99.39	-2.1
34	Niacinamide	$C_6H_6N_2O$	2.298	$[M+H]^+$	122.0478	123.055	80	96.54	1.96
Phenolic Acid and derivates									
35	2-Hydrioxycinnamic acid	$C_9H_8O_3$	3.152	$[M+H]^+$	164.0472	165.0545	77, 95, 123	85.59	0.68
36	4-Hydroxycoumarin	$C_9H_6O_3$	10.647	$[M+H]^+$	162.0319	163.0392	89	83.15	-1.45
37	Chlorogenic Acid	$C_{16}H_{18}O_9$	17.275	$[M+Na]^{+*}$	354.0959	377.0854	163, 215	97.66	-2.26
38	Coumarin	$C_9H_6O_2$	28.913	$[M+H]^+$	146.0368	147.0441	65	83.59	-0.23
39	Phloridzin	$C_{21}H_{24}O_{10}$	42.627	$[M-H]^-$	436.1377	435.1304	81, 167, 247, 315	88.27	-1.64

* = Compounds were identified in negative and positive ionization modes, only data from a single mode was presented. RT = Retention Time; MW = Molecular Weight.

Table 4: Putative phytochemical compounds presented in 80% aqueous ethanol TGIE and their biological activities.

Phytochemical	Biology Activity	Mechanism	Plant Source	References
(±)-Catechin	-antioxidant	- Inhibited interaction of FOXO3 with IKKα promotor to suppress IKKα. - Inhibited IKKα-p53, ameliorated cell apoptosis and alleviated oxidative damage. - Reduced oxidative stress by enhanced antioxidant defense and attenuated the AMK-induced nephrotoxicity	Tea, green tea, grape	[16-19]
	-anti-cancer	- Bound with HPV16E6 involved in cervical development.		
2-Hydroxycinnamic acid	-antioxidant	- Reduced ROS level in Caco-2 cells. - Induced the antioxidant enzymes (CAT, SOD1, SOD2, Mgst10) by activating the Nrf/keap1. - Eliminated H_2O_2-induced oxidative stress in HDF and L929 cells.	Coffee, *Stachys tundjeliensis*, *Bougainvillea* × *buttiana*	[20-23]
4-Hydroxycoumarin	-antioxidant	- Inhibited AChE activity. - Inhibited NO activity by using LPS-induced RAW 264.7 cells.	Olive leaves, *Gerbera delavayi*	[24-26]
	-anticancer	- Inhibited COX-2 activity. - Inhibited EGFR and P13K/mTOR in cancer cell.		
Astragalin	-antioxidant	- Inhibited oxidative stress and promoted Nrf2 in LO2 cells. - Increased Nrf-2 and reduced Keap-1. - Restored all the PS-MPs instigated hepatic damages.	*T*, *Chinense Graviola pericarp*	[27-31]
	-hepatoprotective	Reduced lipid peroxidation and enhanced antioxidant genes by modulating the lnc XIST/miR-155-p/Nrf2 axis.		
	-anti-cancer	Regulated cell movement, migration, angiogenesis, and PI3K-AKT signal pathway.		
	-anti-inflammatory	Reduced the lung inflammatory in mice LPS-induced by regulating $NRLP_3$, caspase-1, IL-1β and COX-2.		
Chlorogenic Acid	-antioxidant	- Ameliorated the cerebral cortex apoptosis by bax, bcl2, and bax/bcl2, modulated miR-27a/smurf1/TNF-α axis. - Stimulated the proliferation of Prevotella and Faecal bacterium, increased total SCFA and beneficial bacteria.	-	[32-35]
	-anti-epileptic	- Inhibited neurotoxicity and seizures by suppressing glutamate and preserving of AMPK/sirtuin 1/PGC-1α-mediated mitochondrial biogenesis and PINK1/Parkin-induced mitophagy.		
	-anti-allergy	- Reduced allergenicity of tropomyosin by covalent or non-covalent binding, which modify its secondary structure and covers the linear epitopes of tropomyosin.		
	-anti-obesity	- Inhibited phosphorylation of adipogenesis-related kinases, changed in transcriptomic, lipidomic and inhibit the differentiation of 3T3-L1 preadipocytes.		
Coumarin	-anti-cancer	- Inhibited GC cells activity such as proliferation, migration and invasion. Targeting p21, E cadherin, and CDK1 by downregulating lncRNAs, SNHG6 and CASC11, also upregulating ncRuPAR and mir-340–5p.	Black poplar and *Eurasian aspen*	[36-38]

	-antioxidant	- Reduced H_2O_2-induced oxidative stress in CHO-K1 cells. - Suppressed COX/5-LOX, oxidative stress, and human erythrocyte biocompatibility.		
Epicatechin	-antioxidant -anti-cancer	- Reduced oxidative damage in AML 12 cells H_2O_2-induced. - Eliminated the proliferation and oxidative damage by blocking phosphorylation of P13K pathway. - Inhibited cell viability of A549 cells by modulating P13K-Akt-mTOR signaling pathway.	Tea	[39-40]
Fisetin	-anti-cholesterol	- Modulated cholesterol homeostasis enhancing TICE flux (activating PPARδ) and inhibited cholesterol absorption. - Promoted cholesterol excretion by increasing ABCB1 and ABCG5/G8. - Inhibited NPC1L1 expression.	Strawberries	[41]
Isoquercitrin	-antioxidant	- Inhibited osteoclast genesis and bone loss by activating Nrf2 blocked RANKL-induced ROS.	-	[42]
Kampeferol-7-o-glucoside	-antioxidant	- Attenuated LPS-induced NF-κB activation by decreasing p65 nuclear translocation, inhibiting κBα (IκBα) and IKKα/β phosphorylation	*Cudrania tricuspidata*	[43]
	-anti-inflammatory	- Decreased LPS-induced activator AP-1 by inhibiting c-Fos expression. - Abrogated LPS-induced phosphorylation of signal transducer and activator of transcription (STAT) 1 (Ser 727, Tyr 701) and STAT3 (Tyr705), inhibiting the phosphorylation of JAK1 and JAK2. - Inhibited NF-κB, AP-1, and JAK-STAT pathways in LPS-treated RAW 264.7		
Phlorizin	-antioxidant	- Upregulated neurotrophic factors, reduced oxidative stress and increased cholinergic signaling. - Reversed abnormal levels of GSH, BDNF, MDA and AChE in the brain of diabetic animals.	-	[44]
Procyanidin B2	-antioxidant	- Prevented CdCl2-induced oxidative stress by increasing antioxidant enzymes and reducing proinflammatory cytokines. - Reduced cadmium deposition, increase in MT1, MT2, and MT3, protected CdCl2-damaged by promoting beneficial microbiota. - Inhibited sFlt-1 secretion, ameliorates endothelial dysfunction and impaired angiogenesis via Nrf2/PPARγ axis.	-	[45-46]
Pyrocatechol	-antioxidant	- Inhibited NO production and CCL2 secretion by suppressing iNOS and CCL2 in RAW264.7 cells.	Coffee	[47]
	-anti-inflammation	- Inhibited the LPS-induced activation of NF-κB by preventing its nuclear localization and induced the expression of Nrf2.		
Hyperoside	-antioxidant	- Protected inflammation and fibrosis by decreasing HMGB1 protein and reduced expression of Toll-like receptor 4, PARP-1, and NF-κB p65 mRNA. - Inhibited the cytoplasmic translocation of HMGB1 and nuclear localization of NF-κB p65 in the hepatic tissues of mice. - Blocked on hepatic fibrosis by PARP-1-HMGB1 pathway regulation.	-	[49]

Effect of *in vitro* simulated digestion: After simulated digestion process, TPC, TFC, and antioxidant properties were determined to observe the effect of *in vitro* simulated digestion on bioactive compounds and their biological activities. The comparison before and after *in vitro* simulated digestion is shown in Table 5. The result exhibited that phytochemicals and antioxidant capacities of *in vitro* simulated TGIE were significantly higher than before digestion.

Table 5: TPC, TFC, and antioxidant capacity of TGIE and digested-TGIE passed through the in vitro simulated digestion.

Antioxidant Activities	TGIE	digested-TGIE
TPC (mg GAE/g extract)	184.68 ± 0.6[a]	254.13 ± 12.62[b]
TFC (mg QE/g extract)	551.84 ± 4.72[a]	1,498.80 ± 3.01[b]
ORAC (mM TE/g extract)	1,156.61 ± 11.55[a]	2,472.04 ± 13.74[b]
DPPH (mM TE/g extract)	1,087.68 ± 14.37[a]	1,136.97 ± 27.88[b]
FRAP (mM TE/g extract)	799.30 ± 1.45[a]	1,446.12 ± 10.17[b]
Iron chelating (%/mg extract)	42.32 ± 3.48[a]	53.61 ± 3.73[b]

Data is showed as means ± SD of three experimental replicates. Different letters indicate that the means in each row show significant difference ($p < 0.05$).

DISCUSSION

The proximate compositions of fresh TGI in this study from Southern Thailand were represented in g FW with 90.24% of moisture. Previous studies [3,50] reported that TGI moisture was 89.9% (from Malaysia) and 95.12 g/100 g (from Northern Thailand). Higher moisture content can increase the likelihood of plants becoming perishable. The ash content in flowers indicates the levels of inorganic materials, such as minerals [3]. This indigenous flower also contains low fat (<1%). Thus, the consumption of TGI might be adequately recommended for people suffering from obesity. The extraction yield of TGIE with various solvents showed a significant difference. Solvent polarity, pH, temperature, extraction time, and sample substances may affect the yield extraction. The yield of ethanol, acetone, and methanol extraction was increased by adding more water to the solvent. Extraction yield of 50%>75% aqueous ethanol by 32.94 ± 1.86 and 26.08 ± 1.35, respectively [50]. This phenomenon may be caused by the presence of organic solvents and water, which facilitate the extraction of compounds soluble in both water and organic solvent. Different solvents also affected the bioactive compounds in the extract. Phenolic acids in plants play a vital role as free radical scavengers. In general, the mechanism of polyphenol compounds involves donating hydrogen and iron ion chelating abilities [2]. The results showed that 80% aqueous ethanol extract had the highest TPC, followed by 50% of aqueous ethanol and water extract, where the TPC decreased with increasing water content. Our results are on par with previous studies in torch ginger inflorescence [2, 5], also [51] reported in *L. aromatica* extract, wherein water extract was the lowest value of phenolic compounds when compared with acetone, methanol, and aqueous ethanol. As well as TFC, the best solvent for extracting flavonoids was 80% aqueous ethanol. A study reported that all of the polyphenol in plants, flavonoids are the most prevalent

and extensively dispersed category with the most potent antioxidant properties [2]. Antioxidant activities of TGIE were determined by different reaction mechanisms including ORAC, DPPH free radicals scavenging, providing single electrons transfer by FRAP, and iron chelating activity. The antioxidant activities of the extract show a positive correlation with flavonoid and phenolic acid content in a dose-dependent manner, as indicated in the the previous study [12]. As the 80% aqueous ethanol TGIE had the highest values compared to other solvents, consequently, it was chosen for LC-ESI-QTOF-MS/MS to conduct qualitative analysis and identification of polyphenols in both negative and positive ionization modes and further *in vitro* simulated digestion.

This study found that 39 compounds were 9 flavonoids and 5 phenolic acids identified in torch ginger 80% aqueous ethanol extract. Epicatechin was found on ESI^+ and ESI^- ionization mode, with a high score (lib). Flavonoids has been known for their potential as anti-inflammatory and antioxidant. A recent study [52] found that the effect of epicatechin in GK T2DM rats successfully preserved glucose homeostasis and decreased tissue damage by modulating the gut-liver axis and liver insulin signaling pathway. It inhibited the proliferation of LPS-producing bacteria, reduced serum LPS, and increased the insulin signaling pathway and serum insulin. Chlorogenic acid was also identified by ionization ESI^+ and ESI^-. It has been shown to reduce the allergenicity of various foods, from plant sources to animal products, by suppressing TLR 4 and TLR7/8 signaling pathways [53-59]. The phytochemical profiles discovered in torch ginger 80% aqueous ethanol extract support extending the intensity determination of specific biological substances to acquire more value-added applications, such as nutraceuticals and medical products.

Oral, gastric, small intestinal, and eventually large intestinal fermentation are typically simulated to imitate digestion. Instead of focusing on other variables, these quick techniques replicate physiological circumstances *in vivo*, including the concentrations of digestive enzymes, pH, digesting time, and salt concentrations. [60]. Indeed, the bioactive ingredients released during digestion in the gastrointestinal lumen, and subsequently accessible for intestinal absorption, may be denoted as the bioaccessibility fraction within a food matrix. A study showed a rise in antioxidant activities as well as TPC and TFC after simulated digestion. The digestion process may cause this result by most likely facilitating the release of bioactive substances depending on the pH, temperature, and activity of digestive enzymes. A study investigated the antioxidant activities of CM (cardoon meal) and CC (cardoon cake) significantly increased after digestion [61]. Another study performed on seaweed showed that after intestinal digestion the TPC and TFC significantly increased [8], and in coffee pulp flour and extract [62]. Our results suggest that the *in vitro* simulated digestion process may have contributed to the liberation of polyphenol substances from undigested insoluble fractions, thereby enhancing overall antioxidant activity. The TPC, TFC, and antioxidant activities were correlated increasing after passed through the *in vitro* simulated of mouth, gastric, and intestinal digestion.

CONCLUSION

The present study reviewed that the quantity and type of antioxidant compounds extracted vary depending on the type of solvent. The *in vitro* simulated digestion significantly increased TPC, TFC, and consequently increased a different mechanism of antioxidative capacity compared with before digestion. Taken together, torch ginger inflorescence could be considered a potential for functional food, with its ingredients potentially providing health benefits, particularly in preventing NCDs.

Abbreviations: GK: Goto-Kakizaki, T2DM: type 2 diabetic; NCDs: non communicable diseases.

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