Chemical Constitutions and Antioxidant Activities of Tomato Leaf Extracts

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ABSTRACT The present study aimed to determine the contents of five flavonols and two glycoalkaloids as well as the antioxidant activities of leaf ethanol extracts of 50 tomato accessions. The antioxidant activity was assessed using different tests: 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), 2,2-diphenyl-1-picryl hydrazyl (DPPH), nitric oxide (NO), and total polyphenol content (TPC). Flavonols and glycoalkaloids contents were determined using a high performance liquid chromatography-diode array detector system. Among five flavonols and two glycoalkaloids, naringenin and tomatine were detected in tomato leaves at high concentrations. Of the 50 tomato accessions, IT 229711, IT2365203, and IT 207224 were found to have the highest contents of quercetin, kaempferol, and tomatine, respectively. Leaf extract of IT189949 exhibited the highest relative antioxidant capacity index (RACI). Among the five flavonols, myricetin showed positive correlations with DPPH, ABTS, and NO, while isorhamnetin had positive correlation with DPPH. These results will expand the chemical constitution database and provide information on tomato leaves. They are valuable for the development of functional foods or feed-additives.

Keywords Antioxidant activity, Flavonol, Glycoalkaloids, Leaf extracts, Tomato

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill) is a widely studied species. However, only a few research studies have focused on tomato leaves (Taveira *et al.* 2012). Although this plant material is considered a by-product of tomato production, it contains several bioactive metabolites, including steroidal alkaloids (Friedman 2002) and phenolics such as hydroxycinnamic acids and flavonoids (Slimestad and Verheul 2009; Ferreres *et al.* 2011). These compounds are known to be involved in host-plant defenses. In addition, they have several pharmacological and nutritional functions in humans (Friedman 2002).

Flavonoids are structurally diverse nonvolatile plant specialized metabolites, including chalcones, flavanones, flavones, flavonols, and anthocyanins (Buer *et al.* 2010).

Flavonols, a subclass of flavonoids, include kaempferol, quercetin, and myricetin. They are relatively widespread and abundant in plants (Herrmann 1976). Flavonols are effective radical scavengers and potential chain-breaking antioxidants in lipid oxidation reactions (Hopia and Heinonen 1999).

Steroidal alkaloids are secondary metabolites of Solanaceae. In tomato, these compounds have characteristic C27 cholestane skeleton with an oxa-azaspirodecane system (spirosolane) in 22S or 25S configuration (Taveira *et al.* 2014). Tomatine is the major alkaloid. It is composed of aglycone tomatidine and a tetrasaccharide residue containing d-galactose, two molecules of d-glucose, and d-xylose. Steroidal alkaloids from tomato have several biological activities, including antiviral, anti-fungal, antibacterial, anti-inflammatory, cholesterol lowering, and

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immunopotentiating properties (Friedman 2002; Khalid *et al.* 2004; Milner *et al.* 2011; Friedman 2013; Singh *et al.* 2013).

Antioxidant compounds have received attention from natural-product consumers and researchers due to their pharmacological properties. Antioxidants can lower oxidative stress caused by reactive oxygen species (Nordberg and Arnér 2001). There is an increasing interest in natural antioxidant products for use as medicines and food additives (Mossi *et al.* 2004; Willcox *et al.* 2004). Phytochemicals such as polyphenols and carotenoids are important because of their contributions to human health with multiple biological effects such as antioxidant, anti-mutagenic, anticarcinogenic, and cytoprotective activities (Ajila and Prasada Rao 2008). The objective of this study was to isolate and identify flavonols and glycoalkaloids from tomato leaves of 50 accessions and determine their antioxidant properties through 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), total polyphenol content (TPC), and nitric oxide (NO) assays.

MATERIALS AND METHODS

Plant materials

Fifty tomato accessions were obtained from the National Agro-biodiversity Center of the Rural Development Administration, Republic of Korea (<http://genebank.rda.go.kr>). These accessions were grown in an experimental field in 2014. Leaf samples were dried at 40°C for 4 days. Crude extracts were produced from 7 g of oven-dried tomato leaves using an ASE-200 (Dionex, Sunnyvale, CA, USA) extractor. Extractions were performed in 40-ml 75% ethanol under nitrogen gas at a pressure of 1,500 psi and temperature of 70°C. Extracted samples were dried using a Genevac HT-4X vacuum concentrator (Genevac, Suffolk, UK).

Analysis of flavonol aglycones

Fifty milligrams of extract were mixed with 10 ml of 1N HCl at 80°C for 2 hours followed by addition of 10 ml absolute EtOH. The mixture was shaking at 150 rpm for 2

hours. After centrifugation at 13,000 rpm for 10 minutes, each specimen was filtered through a 0.45 µm syringe filter and analyzed with Agilent 1260 Infinity high performance liquid chromatography (HPLC) system (Agilent Technologies, Santa Clara, CA, USA). The analysis was performed using a prodigy ODS column (250×4.6 mm, 5 µm particle, Phenomenex). HPLC conditions were as follows: solvent A, 0.5% H₃PO₄/H₂O; solvent B, Methanol; gradient, 30% (B) for 0 to 40 minutes, 20% (B) for 40 to 42 minutes, hold at 20% (B) for 42 to 44 minutes, and 50% (B) for 44 to 50 minutes; column temperature, 30°C; and flow rate, 1 ml/min. The filter detector was set at 370 nm.

Analysis of steroid glycoalkaloids

Fifty milligrams of extract were mixed with 15 ml of 0.5 N HCl at 80°C for 2 hours. After centrifugation at 5,000 rpm for 15 minutes, clean supernatants were transferred to new tubes. Each specimen was mixed with 15 ml of ammonia solution and incubated at 80°C for 2 hours. After centrifugation at 5,000 rpm for 15 minutes, clean supernatants were transferred to new tubes and incubated at 4°C for 12 hours. After centrifugation at 5,000 rpm for 15 minutes, clean supernatants were transferred to new tubes and evaporated to remove ammonia solution. The residue was reconstituted in 2 ml tetrahydrofuran/acetonitrile/20 mM KH₂PO₄ (50:25:25, v/v/v). After centrifugation at 5,000 rpm for 15 minutes, each specimen was filtered through a 0.45 µm syringe filter and analyzed with the Agilent 1260 Infinity HPLC system. The analysis was performed using a prodigy ODS column (250×4.6 mm, 5 µm particle, Phenomenex). HPLC conditions were as follows: solvent A, Acetonitrile, solvent B, 50 mM Ammonium phosphate (pH 3.0 O; gradient, 75% (B) for 0 to 12 minutes, 65% (B) for 12 to 15 minutes, 55% (B) for 15 to 17 minutes, 35% (B) for 17 to 25 minutes, and 80% (B) for 25 to 30 minutes; column temperature, 30°C; and flow rate, 1 ml/min. The filter detector was set at 208 nm.

DPPH assay

DPPH radical-scavenging activities of the extracts were assessed using the method of Lee and Lee (2004) with slight modifications. Briefly, DPPH solution (150 µl; 150 µM in anhydrous EtOH) was added to 100 µl of sample

solution. The mixture was shaken vigorously and left to stand at 25°C in the dark for 30 minutes. Absorbance at 517 nm was measured using a spectrophotometer (Epoch; Bio-Tek, Winooski, VT, USA). Results were expressed as IC₅₀ and compared to ascorbic acid standard.

ABTS assay

ABTS radical-scavenging activity was estimated using the method of Re *et al.* (1999) with modifications. Briefly, the ABTS radical cation was generated by adding 7 mM ABTS to 2.45 mM potassium persulfate followed by overnight incubation in the dark at room temperature. The ABTS radical cation solution was diluted with MeOH to obtain an absorbance of 0.7 ± 0.02 at 735 nm. The diluted ABTS radical cation solution (190 μ l) was added to 10 μ l of sample solution. After 6 minutes of incubation at room temperature, the absorbance at 735 nm was measured using a spectrophotometer (Epoch; Bio-Tek). Trolox was used as standard. Results were expressed in μ g of Trolox equivalent per mg of dried sample.

Total polyphenol content assay

TPC was measured using modified Folin-Ciocalteu method (Waterhouse 2001). Folin-Ciocalteu reagent (100 μ l) was added to 100 μ l of sample solution and reacted at room temperature for 3 minutes. After adding 100 μ l 2% sodium carbonate, the mixture was incubated at room temperature for 30 minutes. Absorbance was measured at 750 nm on a spectrophotometer (Epoch; Bio-Tek) with distilled water as blank. Total phenolic content was reported as milligrams of gallic acid equivalents (GAE) per gram of dry weight sample (mg GAE g^{-1} dry seed).

Nitric oxide-scavenging activity

The NO-scavenging activity of each plant extract was determined using the method of Tsai *et al.* (2007). In order to determine the effect of tomato leaf extract on NO production, 1×10^5 RAW 264.7 cells were seeded into 96-well culture plates. Following 24 hours of incubation, adherent cells were washed three times with phosphate buffer saline. These cells were then incubated in prepared Dulbecco's modified Eagle's medium containing extracts from various concentration of tomato leaves with or

without lipopolysaccharide (LPS) (100 ng/ml). Following 24 hours of incubation, the medium was collected for nitrite assay. Cell viability was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide method (Mosmann 1983). Medium nitrite concentration was measured as an indicator of NO production using Griess reaction (Kim *et al.* 1995). The NO-suppressing effect of herbal tea extracts was expressed as the IC₅₀ which denotes the concentration of herbal tea extracts causing 50% inhibition of NO production by LPS-activated RAW 264.7 cells.

Statistical analysis

All experiments were designed to include three replicates. Least Significant Difference was used to determine the differences among the 50 tomato accessions using IBM SPSS Statistics ver. 20 (IBM Co., Armonk, NY, USA). Clustering analysis and correlation analysis were also performed. Hierarchical clustering was performed using the R statistical software environment (<http://www.r-project.org>). Distances were calculated using complete linkage clustering and Pearson's correlation analysis. A $P < 0.05$ was considered to be statistically significant.

RESULTS

Flavonol and glycoalkaloid contents in the 50 tomato accessions

The main descriptive statistics of five flavonols and two glycoalkaloids are summarized in Table 1. Quercetin content had the largest variance (114.9%), with values ranging from 9.0 to 413.2 mg/100 g. Quercetin was detected in 24 tomato accessions, with IT229711 showing the highest content (429.7 mg/100 g, Supplementary Table 1). Kaempferol was detected in all tomato accessions, with content values ranging from 3.2 to 81.2 mg/100 g. IT236523 showed the highest kaempferol content (81.2 mg/100 g) while IT201650 had the lowest kaempferol content (3.2 mg/100 g). Myricetin had the smallest variance (27.7%) among five flavonols whose content values ranging from 1.3 to 4.8 mg/100 g. Of the 50 tomato

accessions, myricetin were detected in all accessions except two (IT265355 and IT265357). Naringenin had the highest level among five flavonols in tomato leaves, although it was detected in 15 tomato accessions. Isorhamnetin was only detected in seven accessions. Its content ranged from 1.0 to 10.3 mg/100 g.

Tomatine and tomatidine were detected in all tomato

leaves. Their levels ranged from 209.4 to 925.6 mg/100 g and 25.0 to 110.4 mg/100 g, respectively. Among the 50 tomato accessions, IT207224 had the highest tomatine content (925.6 mg/100 g). A total of 13 tomato accessions had high tomatidine contents. They did not show significant ($P > 0.05$) difference.

Table 1. Descriptive statistics of five flavonols, two glycoalkaloids, and four antioxidant activities in leaf extracts of 50 tomato accessions.

Group	Variable	No. acc.	Minimum	Maximum	Average	SD ²⁾	CV (%)	Skewness	Kurtosis
Flavonol (mg g ⁻¹)	Myricetin	48	1.3	4.8	2.9	0.8	27.6	-0.2	-0.3
	Quercetin	24	9.0	413.2	119.9	137.7	114.8	1.2	-0.1
	Naringenin	15	23.2	1,306.4	429.7	410.3	95.5	0.9	-0.5
	Kaempferol	50	3.2	81.2	15.7	11.8	75.2	3.9	19.9
	Isorhamnetin	7	1.0	10.3	6.7	2.9	43.3	-1.4	3.4
Glycoalkaloid (mg g ⁻¹)	Tomatine	50	209.4	925.6	483.1	164.8	34.1	0.6	0.1
	Tomatidine	50	25.0	110.4	61.3	20.3	33.1	0.7	0.1
Antioxidant activity	DPPH (%)	50	10.3	39.5	22.7	6.5	28.6	0.8	0.4
	ABTS (%)	50	21.3	59.7	40.9	8.0	19.6	0.0	0.0
	TPC (mg GAE g ⁻¹)	50	16.8	59.9	30.7	7.9	25.9	1.4	2.9
	NO (IC ⁵⁰)	50	24.8	71.5	36.9	7.2	19.5	0.3	1.1

²⁾SD: standard deviation, CV: coefficient of variation, DPPH: 2,2-diphenyl-1-picryl hydrazyl, ABTS: 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid), TPC: total polyphenol content, GAE: gallic acid equivalents, NO: nitric oxide.

Fig. 1. Relative antioxidant capacity index of leaf extracts of 50 tomato accessions.

Antioxidant activities of 50 tomato accessions

In antioxidant activities, NO had the smallest variance (19.5%), with values ranging from 24.8 to 71.5 μ l (IC₅₀). DPPH and ABTS values ranged from 10.3 to 39.5 and 21.3% to 59.7%, respectively. Among the 50 tomato accessions, IT 191046 had the highest level of DPPH, while IT033117 and IT189949 had the highest level of ABTS. TPC ranged from 16.8 to 59.9 (mg GAE/100 g), with IT207214 having the highest level (59.9 $\pm$ 0.0 mg GAE/100 g). To compare data obtained by different chemical methods used to evaluate antioxidant activities, relative antioxidant capacity index (RACI) was calculated (Fig. 1). Results of NO expressed as IC₅₀ were converted using 1/IC₅₀ before RACI calculation. Our results revealed that IT189949 had the highest RACI (1.58), followed by IT191046 (1.46). Positive values of RACI were obtained for 21 accessions. The tomato accession that showed the lowest RACI was IT259255 (−1.32).

Principal component analysis analysis

Results of principal component analysis (PCA) analysis are summarized in Table 2. The first four main principal components (PCs) were extracted from complicated

components. Total cumulative variance of these four factors amounted to be 74.3%. These components had Eigen values > 1. The PCA grouped these tomato leaves into four main components, with PC1, PC2, PC3, and PC4 accounting for about 32.1%, 21.7%, 11.2%, and 9.3% of the variation, respectively (Table 2). The first PC was related to flavonols. It had contributing traits such as quercetin, naringenin, and isorhamnetin content. The second PC was related to antioxidant activity. It had contributing traits such as DPPH and ABTS. The third PC was related to tomatine and TPCs. The fourth PC was related to kaempferol and tomatine contents. The distribution of tomato accessions in PCA analysis is shown in Fig. 2. After placing an ellipse around the data representing 95% confidence interval using Hotelling's T² statistic, all tomato accessions were observed except four accessions (IT229711, IT235444, IT265355, and IT265357). The four tomato accessions showed higher flavonol contents than other accessions.

Correlation among the parameters of 50 tomato accessions

The correlations among five flavonols, two glycoalkaloids, and four antioxidant activities are summarized in Table 3.

Table 2. Principal component analysis of five flavonols, two glycoalkaloids, and four antioxidant activities explained by the first four main components.

Principal component	1	2	3	4
Eigen value	3.53	2.38	1.23	1.03
% of variance	32.1	21.7	11.2	9.3
Cumulative %	32.1	53.7	64.9	74.3
Component matrix				
Myricetin	−0.298	0.333	−0.071	0.081
Quercetin	0.484	0.157	0.020	−0.132
Naringenin	0.475	0.204	0.074	−0.132
Kaempferol	0.194	0.322	0.156	0.592
Isorhamnetin	0.441	0.272	0.109	0.131
Tomatine	−0.202	−0.088	0.424	0.575
Tomatidine	−0.017	−0.328	−0.264	0.284
DPPH ²⁾	−0.218	0.420	−0.357	0.014
ABTS	−0.172	0.494	−0.298	0.132
TPC	−0.193	0.142	0.643	−0.177
NO	−0.251	0.298	0.272	−0.363

²⁾DPPH: 2,2-diphenyl-1-picryl hydrazyl, ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), TPC: total polyphenol content, NO: nitric oxide.

Fig. 2. Two-dimensional scatter diagram of principal component analysis (PCA) for leaf extracts of the 50 tomato accessions based on five flavonols, two glycoalkaloids, and four antioxidant activities.

Table 3. Correlation coefficients between traits in leaf extracts of the 50 tomato accessions.

	Myricetin	Quercetin	Naringenin	Kaempferol	isorhamnetin	Tomatine	Tomatidine	DPPH	ABTS	TPC	NO
Quercetin	−0.646**										
Naringenin	−0.548	0.895**									
Kaempferol	0.079	0.099	0.416								
isorhamnetin	−0.518	0.725	0.975**	0.128							
Tomatine	0.100	−0.469*	−0.160	0.012	−0.490						
Tomatidine	−0.244	0.079	0.078	−0.181	−0.145	0.075					
DPPH ²⁾	0.393**	−0.156	−0.050	0.104	0.073	−0.060	−0.131				
ABTS	0.528**	−0.027	0.257	0.182	0.775*	0.012	−0.179	0.702**			
TPC	0.181	−0.182	−0.170	0.039	−0.194	0.189	−0.163	0.089	0.072		
NO	0.375**	−0.195	0.033	−0.129	0.107	0.136	−0.173	0.377**	0.349*	0.430**	
RACI	0.522**	−0.178	0.042	0.074	0.384	0.095	−0.233	0.765**	0.749**	0.560**	0.760**

***Significant at $P < 0.05$ and $P < 0.01$, respectively.

²)DPPH: 2,2-diphenyl-1-picryl hydrazyl, ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), TPC: total polyphenol content, NO: nitric oxide.

Kaempferol did not show any correlation with the 12 parameters. Naringenin showed positive correlations with quercetin ($r=0.859, P<0.01$) and isorhamnetin ($r=0.975, P<0.01$). Quercetin had negative correlations with myricetin ($r=-0.646, P<0.01$) and tomatine ($r=-0.469, P<0.05$). Among the 12 characters, myricetin showed positive correlations with the following three antioxidant activities: DPPH ($r=0.393, P<0.01$), NO ($r=0.375, P<0.01$), and RACI ($r=0.522, P<0.01$). ABTS had

positive correlation with isorhamnetin ($r=0.775, P<0.05$).

Clustering analysis of 50 tomato accessions based on flavonols and glycoalkaloids contents

All tomato accessions were clustered into four groups based on five flavonols and two glycoalkaloids except IT236523 (Fig. 3, Table 4). IT236523 showed the highest kameferol content (81.2 ± 1.3 mg/100 g). Group I consisted of five accessions with the highest quercetin

Fig. 3. Hierarchical clustering analysis of five flavonols and two glycoalkaloids in leaf extracts of 50 tomato accessions.

content (342.2 ± 90.8 mg/100 g) and naringenin content (960 ± 203 mg/100 g) but the lowest myricetin content (1.89 ± 0.90 mg/100 g). Group II consisted of 13 accessions with the highest tomatine content (702 ± 102 mg/100 g) without detectable naringenin. Group III consisted of 14 accessions with high myricetin content (3.37 ± 0.64 mg/100 g). Group IV consisted of 17 accessions with the highest tomatidine content (79.3 ± 19.4 mg/100 g). Among the five flavonols, only isorhamnetin was detected in Group I. The four groups did not show significant difference in antioxidant activities.

DISCUSSION

Information concerning phenolic substances found in plant byproducts is limited, especially the constituents of plant fractions. Most studies have evaluated phenolic constituents of fruit byproducts (Elbadrawy and Sello 2011; Taveira *et al.* 2012). Few studies have focused on whole tomato plant, although the properties of tomato fruits have been extensively studied (Silva-Beltrán *et al.* 2015). A recent investigation has revealed that tomato plant leaves have several active metabolites, including phenolic compounds and steroidal alkaloids (Taveira *et al.* 2012).

Table 4. Average values±SD of each group clustered based on five flavonols, two glycoalkaloids, and four antioxidant activities.

Group	No. acc	Flavonols					Glycoalkaloids		Antioxidant activities				
		Myricetin	Quercetin	Naringenin	Kaempferol	Isorhamnetin	Tomatine	Tomatidine	DPPH ²⁾	ABTS	TPC	NO	RACI
I	5	1.89±0.90a	342.2±90.8b	960±203b	25.4±11.6c	7.9±1.5	334±131a	51.9±18.4a	22.6±9.7a	40.2±8.4a	27.3±7.9a	37.1±1.7a	-0.26±0.33a
II	13	3.06±0.86b	32.8±37.4a	-	14.2±4.9ab	-	702±102c	58.6±13.0a	23.6±8.1a	43.8±9.0a	33.8±8.1a	34.1±4.7a	0.20±0.93a
III	14	3.37±0.64b	68.2±56.9a	161±93a	15.9±5.8b	-	393±91ab	44.8±8.1a	21.3±4.7a	39.0±6.9a	29.4±5.0a	37.7±10.7a	0.04±0.78a
IV	17	1.88±0.66ab	90.5±129.7a	174±174a	10.1±3.5a	-	426±84b	79.3±19.4b	23.0±5.9a	39.7±7.7a	30.1±9.7a	38.0±6.0a	-0.13±0.55a

The same letter in each column indicates no significant difference by Duncan's multiple range test, $P < 0.05$.

Values are presented as mean±standard deviation.

²⁾DPPH: 2,2-diphenyl-1-picrylhydrazyl, ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), TPC: total polyphenol content, NO: nitric oxide, RACI: relative antioxidant capacity index.

Sánchez-Rodríguez *et al.* (2012) have reported that quercetin and kaempferol are major components in tomato leaves. In this study, we investigated five flavonols and two glycoalkaloids in 50 tomato leaves. We confirmed that these tomato leaves didn't contain only quercetin and kaempferol, but also abundant naringenin and tomatine (Table 1).

In this study, flavonols contents of 50 tomato accessions were evaluated (Supplementary Table 1). Among the 50 tomato accessions, IT229711, IT265355, and IT236523 showed the highest contents of quercetin, naringenin, and kaempferol, respectively. Flavonoids, in particular those belonging to the class of flavonols (such as kaempferol and quercetin), are potentially health-protecting components in human diet due to their high antioxidant capacity and their ability of inducing human protective enzyme systems (Dugas *et al.* 2000; Duthie and Crozier 2000; Ng *et al.* 2000; Shih *et al.* 2000). These findings suggest that flavonoids might offer protection against major diseases such as coronary heart diseases and cancer (Hertog and Hollman 1996; Steinmetz and Potter 1996; Trevisanato and Kim 2000). In addition, several epidemiological studies have suggested a direct relationship between cardioprotection and consumption of flavonols from dietary sources (Hertog *et al.* 1993; Keli *et al.* 1996). Our results showed the possibility of using tomato leaves as new sources of antioxidant materials.

Tomato leaves contain glycoalkaloids similar to solanine. Toxic glycoalkaloids are not usually detectable in the fruit (Friedman and Levin 1995). Tomatidine, the basic aglycone of glycoalkaloids (α -tomatine and dehydrotomatine) in the tomato plant, can aid the defense of tomato plant against bacteria, fungi, viruses, and insects (Barceloux 2009). Friedman *et al.* (2000) have reported that tomatine has a strong affinity to cholesterol in vitro. They suggested that the low oral toxicity of tomatine might be due to its ability of forming an insoluble complex with cholesterol in vivo which can then be eliminated through feces. Kozukue *et al.* (2004) have suggested that biosynthesis/degradation of dehydrotomatine and α -tomatine may be under distinct genetic control in the leaves of tomato plants. In this study, the contents of tomatine and tomatidine in the 50 tomato accessions were measured (Table 1). Tomatine and toma-

tidine contents ranged from 209.4 to 925.6 mg/100 g and 25.0 to 110.4 mg/100 g, respectively. Various contents of glycoalkaloids in these 50 tomato accessions might have been caused by various genetic background.

In this study, the antioxidant capacities of 50 tomato leaves were measured using four evaluation methods (DPPH, ABTS, TPC, and NO). Many evaluation methods for antioxidant capacity have been developed. Their merits and disadvantages have been fully discussed in several reviews (Halliwell *et al.* 1995; Frankel and Meyer 2000; Prior *et al.* 2005; Roginsky and Lissi 2005; MacDonald-Wicks *et al.* 2006). However, each method of measuring antioxidant capacity has its own limitations because multiple reaction mechanisms and different phase locations can affect the measurements of antioxidant capacity (Sun and Tanumihardjo 2007). Therefore, Sun and Tanumihardjo (2007) have proposed RACI. The key advantage of RACI is that it is a numerical scale that can integrate multiple chemical methods, thus allowing comparison of antioxidant capacity of a large number of samples. Our results showed that the 50 tomato leaves had different rankings in antioxidant capacity (Supplementary Table 1). To compare data obtained by different chemical methods used to evaluate antioxidant activity, we used RACI of the 50 tomato leaves (Fig. 1). It appeared that the results of RACI could be used to select tomato accessions with high antioxidant activity in leaves so that new breeding materials can be developed.

Our results revealed the presence of five flavonols and two glycoalkaloids in tomato leaves. In addition, antioxidant activities of tomato leaves were determined using four evaluation methods (DPPH, ABTS, TPC, and NO). In the agro-industry, various beneficial properties of plant byproducts have been documented (Silva-Beltrán *et al.* 2015). This study opens up new possibilities of developing tomato leaves that contain phytochemicals by selection or commercial breeding. This strategy could be used to improve other underutilized crops.

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