

Comparison of Antioxidant Activities in Tomato Leaves and Stems

Kyung Jun Lee¹, Gi-An Lee¹, Jong-Ro Lee¹, Jong-Wook Chung², Yang-Hee Cho¹,
Hee-Kyoung Kang³ and Kyung-Ho Ma^{1*}

¹National Agrobiodiversity Center, NAS, RDA, Jeonju 54874, Korea

²Department of Industrial Plant Science & Technology, Chungbuk National University, Cheongju 28644, Korea

³Department of Horticulture, College of Industrial Science, Kongju National University, Yesan 32439, Korea

Abstract - This study was conducted to investigate the antioxidant activity in the leaves and stems of 50 tomato accessions, in order to examine the possibility of using tomato by-products as a functional material. The extracts of the leaves (LE) and stems (SE) were analyzed for DPPH, ABTS, and total polyphenol content (TPC). Antioxidant activities and TPC differed significantly between the LE and SE of the 50 tomato accessions. TPC in LE and SE showed wide variation, ranging from 24.4 to 60.6 and 12.5 to 18.8 mg GAE/g, respectively. The DPPH and ABTS antioxidant activities of LE ranged from 10.0 to 38.2% (scavenging effect) and 20.8 to 59.0 mg ASC/g, respectively, while the DPPH and ABTS measurements of SE were 1.4 to 8.8% and 2.2 to 22.5 mg ASC/g, respectively. As assessed by the relative antioxidant capacity index (RACI), IT033117 and IT203466 had the highest antioxidant activity in LE and SE, respectively. These results will expand the knowledge of antioxidant activity and provide information on tomato accessions valuable for the development of functional foods and food additives.

Key words - Antioxidant activity, Leaves extracts, Stem extracts, Tomato

Introduction

Tomato (*Solanum lycopersicum* L.) is widely consumed, either raw or after processing, and can provide a significant proportion of the total antioxidants in one's diet (Martinez-Valverde *et al.*, 2002). Tomato contains carotenoids, such as lycopene, β -carotene, and vitamin E, which are known as effective antioxidants (Engelhard *et al.*, 2006). Increasing the production of tomato fruits causes growth to the same extent in leaves and stems; parts of the plants are used as compost, but large amounts are wasted (Kobayashi *et al.*, 2012). Optimal use is a big challenge, because wise use of resources and recycling is becoming ever more necessary (Kobayashi *et al.*, 2012). Functional constituents in tomato plants can be used as food preservatives and biological control agents. In addition, it has been reported that the aroma of plants differs according to the picking season and site, and that antibacterial activity varies greatly (Angioni *et al.*, 2006; Chorianopoulos *et al.*, 2006).

A wealth of scientific evidence has shown that, under

conditions of oxidative stress, reactive oxygen species (ROS), such as superoxide, hydroxyl, and peroxyl radicals, are generated, and the balance between antioxidation (reduction) and oxidation is believed to be critical for maintaining a healthy biological system (Davies, 2000). ROS play an important role in the etiology and pathophysiology of human aging and diseases such as cancer, coronary heart disease, Alzheimer's disease and other neurodegenerative disorders, atherosclerosis, cataracts, and inflammation (Gey, 1990; Ames, 1983; Davies, 2000; Finkel and Holbrook, 2000; Khodr and Khalil, 2001; Kim *et al.*, 2016). In a healthy state, there is a dynamic balance between the production of free radicals in the body and the generation of antioxidants that scavenge or quench them to protect the body against their deleterious effects. The concentration of antioxidants present under normal physiological conditions may be insufficient to neutralize free radicals generated under pathological conditions. Therefore, it is important to enrich one's diet with antioxidants to protect against harmful diseases. Hence, there has been increased interest in the food industry and in preventive medicine in the development of "natural antioxidants"

*Corresponding author. E-mail : khma@korea.kr
Tel. +82-63-238-4870

from plant materials (Amaeze *et al.*, 2011; Yang *et al.*, 2015).

Antioxidant compounds have received attention from consumers of natural products and researchers owing to their pharmacological properties. Antioxidants lower the oxidative stress caused by reactive oxygen species (ROS) (Nordberg and Arner, 2001). There is 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 and their multiple biological effects, such as antioxidant, antimutagenic, anticarcinogenic, and cytoprotective activities (Ajila and Rao, 2008).

The objective of this study was to explain the possibility of using tomato by-products such as leaves and stems as a functional material. In this study, the antioxidant activity present in the leaves and stems extracts of 50 tomato accessions were measured by DPPH radical scavenging activity, ABTS radical scavenging activity, and total polyphenol content.

Materials and Methods

Plant materials

Fifty tomato accessions (*Solanum lycopersicum* L.) were obtained from the National Agro-biodiversity Center of the Rural Development Administration (RDA), Republic of Korea (<http://genebank.rda.go.kr>). The accessions were grown in a field in 2014. The leaves and stems of the 50 tomato accessions were dried at 40°C for 4 days. Seven grams of dried tomato leaves and stems were extracted using an ASE-200 (Dionex, CA, USA) extractor. Extractions were performed in 40 ml 75% ethanol under nitrogen gas at a pressure of 1,500 psi at 70°C. Extracted samples were dried using a HT-4X vacuum concentrator (Genevac, Suffolk, UK).

DPPH assay

DPPH radical-scavenging activity was assessed using a previously published method with some modifications (Lee and Lee, 2004). 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 min. Absorbance at 517 nm was measured using a spectrophotometer (Epoch; Bio-Tek, Winooski, VT, USA).

ABTS assay

ABTS radical-scavenging activity was estimated using a previously published method with some modifications (Re *et al.*, 1999). 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 methanol (MeOH) to obtain an absorbance of 0.7 ± 0.02 at 735 nm. The diluted ABTS radical cation solution (190 µl) was added to 10 µl of sample solution. After 6 min, absorbance at 735 nm was determined using a spectrophotometer (Epoch; Bio-Tek, Winooski, VT, USA). Ascorbic acid was used as a standard, and the results are expressed in ascorbic acid equivalent.

Total polyphenol content assay

Total polyphenol content was measured using the modified Folin-Ciocalteu method (Waterhouse 2001). Folin-Ciocalteu reagent (100 µl) was added to 100 µl of sample solution and reacted at room temperature for 3 min. After adding 100 µl of 2% sodium carbonate, the mixture was incubated at room temperature for 30 min. Absorbance was measured at 750 nm using a spectrophotometer (Epoch; Bio-Tek, Winooski, VT, USA). Total phenolic content is reported as mg of gallic acid equivalent (GAE) per gram of dried weight sample (mg GAE/g).

Statistical analysis

Least Significant Difference (LSD) was used to detect significant differences among the 50 tomato accessions using SPSS Statistics 20 (SPSS Inc., Chicago, IL, USA). Cluster analysis was performed using the R statistical software (<http://www.r-project.org>). PAST3 software was used for principal component analyses (PCA) (Hammer *et al.*, 2001).

Results

Antioxidant activities of leaves (LE) and stem extracts (SE) from 50 tomato accessions

Total polyphenol content (TPC) and antioxidant activities of LE and SE in the 50 tomato accessions are summarized in Table 1 and Table 2. The TPC of LE and SE ranged from 35.9 ± 6.5 and 14.1 ± 6.3 mg GAE/g, respectively. Among the 50

Table 1. Total polyphenol content and antioxidant activities of leaves and stem extracts in 50 tomato accessions

		Min	Max	Average	SD	CV (%)	Kurtosis	Skewness
Leaves	TPC ^z	24.4	60.4	35.9	6.5	18.0	3.27	1.43
	DPPH ^y	10.0	38.2	21.9	6.3	28.6	0.40	0.76
	ABTS ^x	20.8	59.0	40.3	8.0	19.8	0.05	-0.02
Stems	TPC	12.5	18.8	14.1	1.2	8.8	3.74	1.63
	DPPH	1.4	8.8	4.4	2.0	45.5	-0.41	0.80
	ABTS	2.2	22.5	13.2	4.5	34.2	-0.44	0.07

^zTPC, total polyphenol content (mg GAE/g); ^yDPPH (scavenging effect,%); ^xABTS (mg ASC/g).

Table 2. ABTS and DPPH antioxidant activities and polyphenol contents of stem and leaves extracts of 50 tomato accessions

IT No.	Group	TPC ^z		DPPH ^y		ABTS ^x		RACI	
		(mg GAE/g)		(scavenging effect, %)		(mg ASC/g)		Leaves	Stems
		Leaves	Stems	Leaves	Stems	Leaves	Stems	Leaves	Stems
IT033117	I	37.5 ± 0.7	15.6 ± 1.3	36.0 ± 1.3	6.8 ± 1.6	56.7 ± 2.0	8.4 ± 2.0	1.5	0.5
IT033130	I	41.0 ± 0.6	17.4 ± 0.2	32.8 ± 0.3	8.4 ± 1.6	50.2 ± 2.3	18.1 ± 1.7	1.3	1.9
IT033131	III	37.7 ± 0.3	14.5 ± 0.5	17.2 ± 0.5	5.6 ± 1.1	28.4 ± 1.5	18.8 ± 1.7	-0.7	0.7
IT116984	II	41.7 ± 1.0	14.5 ± 0.8	24.9 ± 0.2	1.6 ± 1.2	36.9 ± 1.2	13.9 ± 2.9	0.3	-0.3
IT156677	II	39.0 ± 0.6	14.0 ± 0.5	19.9 ± 0.4	3.1 ± 0.9	40.6 ± 0.9	11.0 ± 1.4	0.1	-0.4
IT173906	II	49.8 ± 0.3	13.6 ± 1.7	25.4 ± 1.2	3.1 ± 1.3	47.6 ± 1.2	12.8 ± 1.0	1.2	-0.4
IT173907	II	48.3 ± 0.8	12.9 ± 0.2	17.8 ± 0.5	3.9 ± 0.7	49.8 ± 1.2	14.6 ± 2.4	0.8	-0.3
IT173908	II	47.8 ± 1.2	13.7 ± 0.0	23.2 ± 0.4	3.3 ± 1.0	51.7 ± 1.0	17.0 ± 1.0	1.2	0.0
IT181222	II	34.5 ± 0.2	15.5 ± 0.2	22.3 ± 0.9	3.9 ± 0.9	45.4 ± 1.6	12.4 ± 0.4	0.2	0.2
IT189945	II	43.2 ± 0.9	13.1 ± 0.9	24.6 ± 0.3	2.3 ± 0.9	34.6 ± 1.4	10.2 ± 2.7	0.3	-0.8
IT189949	I	33.7 ± 0.4	16.4 ± 0.2	36.6 ± 0.3	8.1 ± 0.7	59.0 ± 1.6	17.9 ± 1.8	1.4	1.6
IT191046	I	35.5 ± 0.6	15.5 ± 0.2	38.2 ± 0.4	8.3 ± 0.7	52.5 ± 1.0	15.5 ± 1.9	1.3	1.2
IT191047	I	35.1 ± 1.7	15.4 ± 0.3	33.6 ± 0.3	2.3 ± 0.9	47.9 ± 1.2	6.4 ± 1.0	0.9	-0.5
IT199433	III	35.4 ± 0.5	14.6 ± 0.1	24.1 ± 0.8	8.1 ± 0.7	36.2 ± 2.4	7.7 ± 3.3	-0.1	0.3
IT199437	III	33.8 ± 0.1	14.0 ± 0.2	25.1 ± 0.4	8.3 ± 0.7	42.2 ± 1.4	18.6 ± 1.8	0.1	1.0
IT199442	III	31.8 ± 0.2	13.7 ± 0.4	15.9 ± 0.6	3.0 ± 1.6	34.8 ± 0.3	21.4 ± 0.8	-0.8	0.3
IT199445	II	36.9 ± 0.5	13.6 ± 0.1	23.7 ± 0.5	2.2 ± 0.9	42.0 ± 0.2	21.0 ± 0.8	0.2	0.1
IT199447	III	29.3 ± 0.5	15.7 ± 0.3	19.5 ± 0.7	5.5 ± 1.4	34.1 ± 1.3	17.0 ± 1.4	-0.7	0.9
IT199449	III	36.0 ± 1.6	14.5 ± 0.3	21.1 ± 1.2	4.9 ± 0.3	29.1 ± 1.0	13.0 ± 1.9	-0.5	0.2
IT199451	II	33.1 ± 0.3	13.3 ± 0.1	23.2 ± 0.9	4.8 ± 0.6	39.6 ± 1.7	6.2 ± 1.7	-0.1	-0.6
IT199462	III	33.9 ± 0.1	14.2 ± 1.0	20.8 ± 1.2	6.3 ± 0.5	39.3 ± 1.5	10.2 ± 0.8	-0.2	0.1
IT199463	III	37.7 ± 0.7	14.9 ± 0.2	15.6 ± 1.0	6.8 ± 1.0	35.4 ± 1.8	16.8 ± 0.4	-0.5	0.9
IT200626	II	30.9 ± 0.2	14.4 ± 0.1	25.1 ± 1.3	6.2 ± 0.8	46.3 ± 0.8	11.0 ± 0.4	0.2	0.2
IT201650	III	32.8 ± 0.1	14.0 ± 0.3	19.6 ± 0.1	5.2 ± 0.1	40.8 ± 2.6	11.5 ± 1.7	-0.3	0.0
IT203253	II	32.0 ± 1.2	13.4 ± 0.1	28.0 ± 1.2	3.9 ± 0.5	42.0 ± 2.1	19.7 ± 0.4	0.2	0.2
IT203259	II	33.1 ± 0.4	14.6 ± 0.1	28.1 ± 1.0	3.3 ± 0.4	49.8 ± 2.8	15.2 ± 2.4	0.6	0.1
IT203262	II	24.4 ± 0.2	13.8 ± 0.1	26.7 ± 1.1	5.7 ± 0.8	48.4 ± 0.8	11.9 ± 2.0	0.0	0.0
IT203466	I	36.3 ± 0.4	18.8 ± 0.2	24.9 ± 1.1	7.8 ± 1.0	44.4 ± 2.4	15.7 ± 0.8	0.3	2.0
IT203468	III	44.2 ± 0.6	13.3 ± 0.2	19.5 ± 1.4	3.7 ± 0.4	38.2 ± 1.8	22.5 ± 1.8	0.2	0.4
IT207213	II	33.2 ± 0.5	14.7 ± 0.2	18.0 ± 0.8	2.9 ± 0.7	41.7 ± 1.7	10.4 ± 2.1	-0.3	-0.3
IT207214		60.4 ± 0.0	12.9 ± 0.1	22.0 ± 0.5	2.3 ± 1.7	32.1 ± 1.1	2.2 ± 0.8	0.9	-1.5
IT207224	III	34.1 ± 0.3	13.1 ± 0.4	15.1 ± 1.6	1.4 ± 1.3	37.8 ± 1.2	15.9 ± 3.4	-0.6	-0.6
IT207227	II	43.1 ± 0.6	13.8 ± 0.0	16.2 ± 0.6	2.8 ± 2.0	40.5 ± 1.8	11.7 ± 1.0	0.1	-0.4
IT207229	II	32.3 ± 1.2	14.4 ± 0.3	15.9 ± 0.4	3.1 ± 2.1	45.9 ± 2.1	9.5 ± 3.1	-0.3	-0.4
IT207234	II	33.7 ± 1.2	13.1 ± 0.1	13.3 ± 0.5	2.3 ± 0.1	36.7 ± 1.3	7.9 ± 2.4	-0.7	-1.0
IT207236	III	27.9 ± 0.1	13.5 ± 0.3	18.8 ± 0.7	2.8 ± 1.7	37.9 ± 1.6	17.9 ± 1.1	-0.7	-0.1

Table 2. ABTS and DPPH antioxidant activities and polyphenol contents of stem and leaves extracts of 50 tomato accessions (Continued)

IT No.	Group	TPC ^z		DPPH ^y		ABTS ^x		RACI	
		(mg GAE/g)		(scavenging effect, %)		(mg ASC/g)		Leaves	Stems
		Leaves	Stems	Leaves	Stems	Leaves	Stems		
IT207239	II	28.8 ± 0.4	13.0 ± 0.3	18.0 ± 1.3	3.7 ± 1.2	43.4 ± 3.0	8.4 ± 2.0	-0.4	-0.8
IT207288	III	32.5 ± 0.3	12.8 ± 0.1	16.4 ± 0.8	2.6 ± 0.5	27.2 ± 1.6	20.3 ± 1.0	-1.0	-0.1
IT211836	II	28.9 ± 1.7	13.1 ± 0.1	22.4 ± 1.5	3.7 ± 1.4	34.5 ± 2.2	7.5 ± 1.7	-0.6	-0.8
IT229536	II	36.4 ± 0.5	13.0 ± 0.1	21.7 ± 1.2	3.1 ± 1.0	38.2 ± 2.5	8.6 ± 0.7	-0.1	-0.8
IT229651	III	33.6 ± 0.3	13.5 ± 0.1	14.8 ± 1.3	4.5 ± 1.3	24.9 ± 2.9	11.9 ± 0.7	-1.1	-0.2
IT229694	II	42.5 ± 0.2	12.8 ± 0.0	22.0 ± 1.5	3.7 ± 1.1	34.1 ± 1.0	10.8 ± 2.1	0.1	-0.7
IT229711	II	27.5 ± 0.8	12.8 ± 0.1	26.9 ± 1.1	3.1 ± 0.2	43.8 ± 1.4	7.5 ± 1.4	0.0	-1.0
IT235444	III	32.1 ± 0.3	13.8 ± 0.2	20.4 ± 0.5	4.6 ± 0.7	35.3 ± 2.8	12.6 ± 0.7	-0.5	-0.1
IT236523	II	38.7 ± 0.5	13.6 ± 0.2	28.7 ± 1.0	3.2 ± 0.9	47.4 ± 2.0	15.5 ± 1.9	0.8	-0.2
IT247989	II	38.2 ± 0.4	14.3 ± 0.1	16.3 ± 0.4	2.9 ± 1.0	35.1 ± 1.4	12.4 ± 1.0	-0.4	-0.3
IT259085	III	33.8 ± 0.8	12.5 ± 0.1	16.8 ± 0.8	4.1 ± 1.4	29.2 ± 1.9	11.9 ± 1.1	-0.8	-0.6
IT259255	III	30.3 ± 0.8	12.5 ± 0.1	10.0 ± 0.5	4.0 ± 1.0	20.8 ± 0.5	11.3 ± 3.4	-1.7	-0.6
IT265355	II	32.6 ± 0.1	13.5 ± 0.1	15.6 ± 0.5	5.0 ± 2.0	44.0 ± 2.5	11.7 ± 2.8	-0.4	-0.2
IT265357	III	29.8 ± 0.3	14.6 ± 0.0	14.2 ± 0.4	8.8 ± 1.6	39.6 ± 0.6	16.1 ± 0.4	-0.8	1.1
LSD		1.14	0.69	1.43	1.8	2.82	2.89		

tomato accessions, IT207214 (60.4 ± 0.0 mg GAE/g) and IT203466 (18.8 ± 1.3 mg GAE/g) had the highest TPC in LE and SE, respectively (Table 2). TPC in LE was 2.54-fold higher than it was in SE. DPPH antioxidant activities of LE and SE in the 50 tomato accessions were found to range from 10.0 ± 0.5 to $38.2 \pm 0.4\%$ and from 1.4 ± 1.3 to $8.8 \pm 1.6\%$ (scavenging effect), respectively. Of the 50 accessions, IT191046 in LE and IT265357, IT033130, IT191046, IT199437, IT189949, IT199433, and IT203466 in SE had the highest DPPH antioxidant activity (Table 2). ABTS antioxidant activities varied from 20.8 ± 0.5 to 59.0 ± 1.6 and 2.2 ± 0.8 to 22.5 ± 1.8 mg ASC/g in LE and SE, respectively (Table 1). Among the 50 tomato accessions, IT033117 and IT189949 in LE and IT203253, IT207288, IT199445, IT199442, and IT203468 in SE had the highest ABTS antioxidant activities (Table 2). The integration of antioxidant capacity results derived from different chemical methods allowed us to calculate the Relative Antioxidant Capacity Index (RACI). The results are shown in Table 2. We found that IT033117 had the highest RACI (1.5), followed by IT189949 (1.4), IT247989 (1.3), IT191046 (1.3), IT033130 (1.2), and IT173906 (1.2), with IT259255 having the lowest RACI (-1.7) in LE. In SE, IT203466 had the highest RACI (2.0), followed by IT033130 (1.9), IT189949 (1.6), and IT191046 (1.2), with IT207214 having the lowest RACI (-1.5).

Table 3. Eigenvalue and percent of total variation and component matrix for the principal component axes

Principal Components	1	2
Eigen value	2.46	1.27
% of Variance	40.9	21.2
Cumulative %	40.9	62.1
Component Matrix		
TPC_L ^z	-0.035	0.636
DPPH_L ^y	0.511	0.340
ABTS_L	0.494	0.325
TPC_S	0.524	-0.150
DPPH_S	0.446	-0.378
ABTS_S	0.140	-0.458

^zTPC, total polyphenol content; ^yL, Leaves extracts; S, Stem extracts.

PCA analysis

PCA was performed for antioxidant activities, as shown in Table 3. A cumulative variance of 62.1% was accounted for by the first two axes, with an Eigenvalue >1.0. The first PC was more related to DPPH and ABTS of LE and DPPH and TPC of SE. The second principal component exhibited positive effects for TPC of LE and negative effects for ABTS of SE. The distribution of the 50 tomato accessions in the PCA analysis is shown in Fig. 1. Placing an ellipse around the data that represented the 95% confidence interval (CI) using Hotelling's T2 statistic showed that all tomato accessions

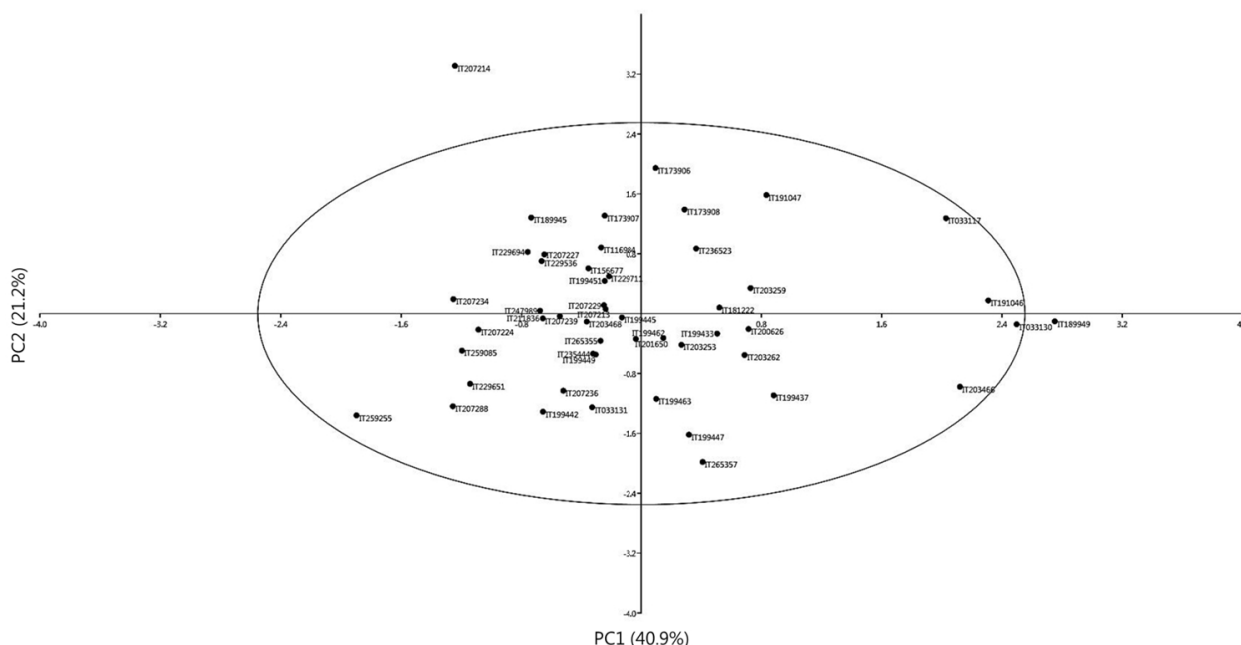


Fig. 1. 2D scatter diagram of principal component analysis (PCA) of 50 tomato accessions based on antioxidant activity.

Table 4. Average values ± SD of each group clustered based on TPC and antioxidant activities of leaves or stem extracts in the 50 tomato accessions

Group	n	TPC ^z _L ^y	TPC_S	DPPH ^x _L	DPPH_S	ABTS ^w _L	ABTS_S
I	6	36.5 ± 2.5ns ^v	16.5 ± 1.4b	33.7 ± 4.7c	7.0 ± 2.4c	51.8 ± 5.5c	13.7 ± 5ns
II	25	36.4 ± 6.7	13.7 ± 0.7a	21.9 ± 4.5b	3.5 ± 1.1b	42.4 ± 5.3b	12.0 ± 3.7
III	18	33.7 ± 3.7	13.9 ± 0.9a	18.1 ± 3.7a	5.0 ± 2.1a	34.0 ± 6.0a	15.3 ± 4.2

except two (IT189949 and IT207214) were in the 95% CI range. IT207214 showed the highest TPC in LE (60.4 ± 0.0 mg GAE/g), while IT189949 had the highest level of ABTS antioxidant activity (59.0 ± 1.6 mg ASC/g).

Clustering analysis

The fifty tomato accessions were classified into three groups according to their TPC and antioxidant activities (Table 4 and Fig. 2). Among 50 tomato accessions, IT207214 was an outlier that could not be classified into any of the three groups. In TPC and antioxidant activities of LE and SE, there was no significant difference of TPC in LE and ABTS in SE among three groups (Table 4). Group I contained six accessions, IT189949, IT191046, IT033130, IT203466, IT033117, and IT191047, and had the highest TPC and antioxidant activities. Group II contained 25 accessions and showed middling levels

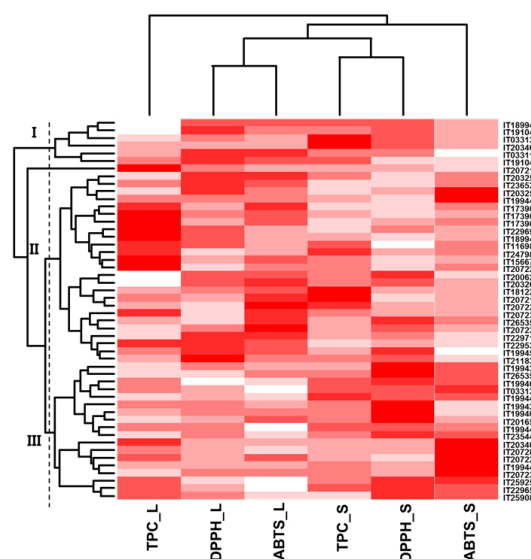


Fig. 2. Hierarchical clustering analysis of antioxidant activity in 50 tomato accessions. L, leaves extracts; S, stem extracts.

of antioxidant activities. Group III comprised 18 tomato accessions with low antioxidant activities.

Discussion

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

Total polyphenol content (TPC) was determined by the Folin-Ciocalteu method in this study. Tomato plants contain a large variety of phenolic derivatives. These compounds are essential for plant growth and reproduction. In addition, phenolic compounds are natural antioxidants that may be found in all parts of a plant and function as antibiotics and natural pesticides (Gupta and Sharma, 2006). Silva-Beltran *et al.* (2015) reported that leaves extracts of two tomato varieties exhibited higher phenolic contents, with values of 83.4 and 125.5 mg GAE/g, than did extracts from the stems and roots. In our study, leaves extracts showed 2.54-fold higher phenolic content than did stem extracts (Tables 1 and 2). A greater concentration of phenolic compounds is most likely found in leaves because of the stress caused by UV irradiation and other factors, which makes it necessary for the vacuoles of plant dermal tissues to defend against these factors (Chandra and Ramalingam, 2011). Rivero *et al.* (2003) reported that leaves are exposed to more direct light and UV radiation than are other parts of a plant and, therefore, exhibit a greater concentration of phenolic compounds.

Isolation of antioxidant compounds from plants is possible through extraction with different solvents and depends on the nature of the solvents used for extraction (Tatiya *et al.*, 2011). In this study, the antioxidant activities of leaves and stem extracts were assessed by measuring three compounds (DPPH, ABTS, and TPC). Many methods for evaluating

antioxidant activity have been developed. Their merits and disadvantages have been 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. Therefore, Sun and Tanumihardjo (2007) proposed a relative antioxidant capacity index (RACI). The key advantage of the RACI is that it is a numerical scale that can integrate multiple chemical methods, thus allowing comparison of antioxidant capacity over a large number of samples. Our results showed that leaves taken from the 50 different tomato accessions had different antioxidant capacities (Table 2). To compare data obtained by different chemical methods used to evaluate antioxidant activity, we used the RACI to compare the 50 tomato leaves (Fig. 1). It appears that the results of RACI analysis can be used to select tomato accessions with high antioxidant activity in leaves so that new breeding methods can be developed.

In the result of RACI, IT033117 tomato by-products showed the highest antioxidant activity among 50 tomato accessions (Table 2). Also, six tomato accessions in group I contain IT033117 showed higher antioxidant activity and TPC when compared to other groups among tomato by-products (Table 4). Son *et al.* (2011) reported that 771 tomato samples collected from five countries showed the sweetness of juice range from 2.0 to 11.5%, averaging 5.6% and fruit weight from 1 to 442 g, averaging 15g, in which the tomato samples collected from Korea showed the sweetness of juice range from 6.5 to 7.5% (in Brix). Similarly in the present study, six tomato accessions in group I showed various sweetness of juice and fruit weight with range from 3.7% (IT203466) to 6.3% (IT033130) (in Brix) and 6.7 g (IT033130) to 138.9 g (IT191047) (data not shown). IT033117 showed 5.5% sweetness and 11.8 g fruit weight, which is seemed to be a good source for developing a new variety. Among the six tomato accessions in group I, IT033130 and IT203466 seemed to be difficult to use for commercial breeding, because of their lesser sweetness (IT203466, 3.7%) and smaller fruit weight (IT033130, 6.7g). Although they are showed limited to utilize as a tomato fruit,

our results revealed that the possibility of their use as antioxidants.

We conclude that the antioxidant activities of leaves and stem extracts differ among the 50 tomato accessions (Table 2). The antioxidant activities of byproducts in many plants have been investigated using various evaluation methods, such as DPPH, ABTS, FRAP, and ORAC (Taveira et al. 2012; Kim et al., 2014; Zhen et al., 2016). Those studies reported that antioxidant data are meaningful at the sectional level and suggested that variations in antioxidant activities can be useful on an industrial scale. The results of our study indicate that tomato byproducts have significant antioxidant activities. Also, leaves extracts contain more powerful antioxidants than do stem extracts. Although a broader investigation of tomato byproducts is needed to confirm this characterization, our study is of interest as it identifies a new source of natural antioxidant products.

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