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Determination of Lipophilic and Hydrophilic Antioxidant Activities in the Crude Extracts of Ten Varieties of Tomatoes

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ABSTRACT

In this study, both lipophilic and hydrophilic antioxidant activities in ten varieties of tomato (*Lycopersicon esculentum* Mill.) crude extracts were determined using three common assays, namely 2,2-diphenyl-1-picryl-hydrazyl (DPPH), 2,2'-azinobis(3-ethyle-benzothiaziline-6-sulfonate) (ABTS) and ferric reducing antioxidant power (FRAP). All the tomato samples exhibited the same activity as that of Trolox. The highest total antioxidant activity (both lipophilic and hydrophilic antioxidant) was found in Black Cherry Kham Kaen while that of Mo Kho 40 sample was the lowest. The average values in terms of TEAC were 910.2, 989.4 and 1174 as determined by ABTS, DPPH and FRAP, respectively. These results demonstrated the potential role of high antioxidant property found in all tomato samples
Keywords : *ABTS, Antioxidant, DPPH, FRAP, tomato*

1. Introduction

Nowadays there is an increasing interest to extract and isolate natural antioxidant compounds especially phenolic compounds that are pharmacologically potent and play an important role as a health-protecting factor. They neutralize the free radicals, which are unstable molecules that are linked with the development of a number of degenerative diseases. On the other hand, the interest in antioxidants is growing because of their antimicrobial activity. Despite advanced food production and preservation techniques, the spoilage and poisoning of foods by microorganisms is still the problem. The consumer acceptance for preservatives with chemical origin is decreasing; therefore, the producers are looking for natural compounds which can be an alternative and supplemented to food products will help to prolong their shelf-life and microbial safety. A great number of *in vitro* antioxidant activity assays have been developed to measure the efficiency of natural antioxidants either as pure compounds or as plant extracts. Mainly,

they may differ concerning the species scavenged by the antioxidants, the reaction conditions and the detection method. These methods involve different mechanisms of determination of antioxidant activity [1]. Tomato (*Lycopersicon esculentum* Mill.) is one of the most widely consumed fresh and processed vegetables in the world for its nutritional and bioactive antioxidants such as vitamin A, C, and E. Tomato contains not only the nutritional antioxidants, but also a great quantity of non-nutritional antioxidants, such as carotenoids, flavonoids, flavones and phenolic compounds, etc. [2-8]. Since the resulting data of antioxidant capacity depends on the method used, a single method cannot give an accurate prediction of the antioxidant capacity of antioxidant compounds [9-10]. The aim of the present study was to evaluate the antioxidant capacity of tomato extracts using three common antioxidant activity assays, namely 2,2-diphenyl-1-picryl-hydrazyl (DPPH), 2,2'-azinobis(3-ethylebenzothiaziline-6-sulfonate) (ABTS) and ferric reducing antioxidant power (FRAP). These chemical methods are based on scavenging of reactive nitrogen and oxygen species [7].

2. Materials and methods

2.1. Chemicals

All chemicals and solvents used were of analytical reagent (AR) grade. Deionize water used for the preparation of all solutions was purified by Milli-Q purification system (Millipore), FRANCE. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), Trolox, 2,2'-azino-bis(3-ethylbenzothiazolin-6-sulfonic acid (ABTS) were obtained from Sigma-Aldrich (USA). Ferrous sulfate heptahydrate ($FeSO_4.7H_2O$) was purchased from Carlo Erba (Italy). Sodium acetate, ferric chloride ($FeCl_3$), methanol, and hydrochloric acid were obtained from QRec™ (New Zealand).

2.2. Plant samples

All samples were experimentally cultivated in the practical fields belonging to the Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University. Ten kinds of tomatoes used in this study were collected from the university breeding varieties. Their common Thai names of the tomatoes are "Black Cherry Kham Kaen", "Lai Kho Red", "Mani Siam", "Mani Thapthim", "Mo Kho 40", "Phuang Thong 80", "Red Sweet", "Seeda", "Tha-ap-green" and "Thapthim Daeng". All tomato fresh fruits were washed with distilled water, cut into pieces and homogenized. The homogenized sample was transferred into PTFE centrifuge tube and frozen at -20°C. This frozen puree was freeze-dried (SCANVAC Centrifuge for Vacuum Concentrator Freeze-Dry, China). The sample was placed in a container of the laboratory mill and grounded into fine powder. These materials were then stored in a freezer at-20°C until analysis.

2.3. Extraction of lipophilic and hydrophilic antioxidant

The amount of 1 g freeze-dried sample powder in 20 mL ethyl acetate was sonicated by ultrasound-assisted extraction (Ultrasonic Sonicator, RF103H, Bandelin Sonorex, Germany) for 20 min, and the mixture was transferred to centrifuge tube and centrifuged at 4000 rpm for 10 min. The supernatant was filtered through Whatman filter paper No. 42. The filtrate was evaporated to dryness using a vacuum evaporator. The residue was then

redissolved in 5 mL acetone and vortexed to get homogenous samples. The lipophilic extract was obtained for the determination of lipophilic antioxidant activity. The residue after ethyl acetate extraction was then extracted with 20 mL of 7 % acetic acid in 80 % methanol and ultra-sonicated for 20 min. The mixture was transferred to centrifuge tube followed by centrifugation at 4,000 rpm for 10 min. The supernatant was filtered once and transferred to another tube prior to the determination of hydrophilic antioxidant activity. The lipophilic and hydrophilic antioxidant activity was measured in triplicates for each extract.

2.4 Analytical procedures

2.4.1. DPPH free radical scavenging activity

Radical scavenging activity of test sample extracts were measured by modified DPPH method [11]. DPPH in methanol or ethanol are stable radical, dark purple in color. The compounds, against hydrogen atom or electron donating ability, are measured by bleaching of a purple colored solution of DPPH. The final concentration of DPPH in methanol was 0.2 mM and the reaction volume was 1.0 mL. 100 µL of various concentrations of standard or lipophilic and hydrophilic extracts were added. These solutions were vortexed thoroughly and then incubated for 30 min in the dark at room temperature and measured spectrophotometrically at 517 nm against methanol as blank sample (spectrophotometer, model Agilent 8453 UV-Vis spectroscopy System, Germany). The percentage of an inhibition of the DPPH was calculated and plotted as a function of concentration of an ascorbic acid used as the reference. The final DPPH values were calculated using a regression equation between Trolox concentration and the percentage of DPPH inhibition, and the results were expressed as micromole Trolox equivalent antioxidant capacity per 100 gram dry weight (µmol TEAC/100 g DW). The percentage of the inhibition of DPPH free radical was calculated using the following equation:

$$\% \text{ inhibition} = [(A_c - A_s) / A_c] \times 100$$

Where A_c is the absorbance of control reaction which contains all reagent except standard or sample and A_s is the absorbance in the presence of standard or sample. IC_{50} which denotes the amount of a single standard required to reduce an initial concentration of DPPH free radical by 50% was also calculated.

2.4.2. ABTS radical cation decolorization

Radical cation scavenging capacity of the tomato extracts including a reference standard was examined against $ABTS^{\cdot+}$ with some modifications [12]. The trolox equivalent antioxidant capacity (TEAC) method is based on the ability of antioxidant to scavenge the performed radical cation $ABTS^{\cdot+}$ as compared with ascorbic acid. The $ABTS^{\cdot+}$ was produced by the reaction of 7.4 mM ABTS in methanol with 2.6 mM potassium persulfate ($K_2S_2O_8$), stored in the dark at room temperature for 12-16 h. Before use, the $ABTS^{\cdot+}$ solution was diluted with methanol to get the absorption between 0.7 and 0.9 absorbance unit at 734 nm. Sixty microliters of the antioxidant extract or reference standard were mixed with 1.0 mL of $ABTS^{\cdot+}$ solution and stored in the dark at room temperature. The absorbance at 734 nm was read after 30 min, and the percentage inhibition of ABTS was calculated, in the same manner

as mentioned in the DPPH assay, for each concentration relative to a blank absorbance. Trolox was used as a standard curve. The free radical scavenging activity was expressed as μmol TEAC/100 g DW.

2.4.3. Ferric ion reducing antioxidant power (FRAP)

The ferric ion (Fe^{3+}) reducing antioxidant power (FRAP) method was used to measure the reducing capacity of tomato extracts from different varieties. This method was carried out with slight modifications [13-14]. The FRAP method measures the ability of the antioxidants to reduce ferric–tripyridyl-triazine (Fe^{3+}–TPTZ) complex to the blue colored ferrous form (Fe^{2+}) which absorbs light at 593 nm. The ferric–TPTZ reagent was prepared by mixing 300 mM acetate buffer, pH 3.6, 10 mM TPTZ in 40 mM HCl and 20 mM $FeCl_3 \cdot 6H_2O$ in the ratio of 10:1:1 (v/v/v). The FRAP reagent was freshly prepared before each experiment. Briefly, 60 μL of different concentrations of the reference standard or the sample extract were mixed with 1000 μL of FRAP reagent and incubated at 37° C for the duration of the reaction. The absorbance readings were taken at 593 nm at 30 min. Increasing absorbance of the reaction mixture indicates an increase of reduction capability. The antioxidant activities of the tomato extracts were expressed as μmol TE/ 100 g DW.

2.5. Statistical analysis

Data results are given as the mean ± standard deviation (SD) of three measurements. All graphs and linear regression in this paper was analyzed by Microsoft Excel 2013 software.

3. Results and discussion

DPPH, ABTS and FRAP assays, expressed as μmol TEAC/100 g DW, were used for evaluation of lipophilic and hydrophilic antioxidant capacity of ten varieties of tomatoes. The result from DPPH method, calibration curve of Trolox over a concentration range of 300-700 μM was linear ($y = 0.1603x - 41.0627$) with a regression coefficient (R^2) of 0.9951. The obtained results of lipophilic and hydrophilic antioxidant activities were in the range of 111.8 ± 1.75 to 140.1 ± 6.45 μmol TEAC/100 g DW and 551.9 ± 5.63 to 1332.5 ± 1.56 μmol TEAC/ 100 g DW, recpectively. Antioxidant activity of Black Cherry Kham Kaen variety was higher than other varieties, while that of Mo Kho 40 sample gave the lowest value (Table 1). The results of ABTS assay showed linear calibration curve of Trolox over a concentration range of 300-800 μM ($y = 0.0991x - 19.4572$) with a regression coefficient (R^2) of 0.9919. Similar pattern of antioxidant activity as mentioned in DPPH assay was obtained, but their TEAC values were slightly higher. Lipophilic and hydrophilic antioxidant activites were in the range of 103.5 ± 1.24to 145.5 ± 9.87 μmol TEAC/100 g DW and 571.1±8.61 to 1505.4 ± 21.04 μmol TEAC/100 g DW, respectively. Antioxidant activity of Black Cherry Kham Kaen variety was higher than other varieties, while that of Mo Kho 40 sample gave the lowest value (Table 2). The result from FRAP method showed a linear calibration curve of Trolox over a concentration range of 300-700 μM ($y = 0.0014x - 0.2299$) with a regression coefficient (R^2) of 0.9955. The FRAP values were within the range of 143.0 ± 1.07

to175.0 ± 0.71 and 571.1 ± 8.61 to 1505.4 ± 21.04 µmol TEAC/100 g DW for lypophilic and hydrophilic antioxidant, respectively. The highest antioxidant activity of the tomato sample was found in Black Cherry Kham Kaen while that of Mo Kho 40 sample had the lowest antioxidant activity (Table 3). For overview, total antioxidant activity (both lipophilic and hydrophilic antioxidant) in value of averaged TEAC were 910.2, 989.4, and 1174 as determined by the ABTS, DPPH, FRAP, respectively. Tomato lipophilic fraction also contains vitamin E (α- and γ-tocopherol) as well, which is one of the most important lipid-soluble radical scavenging antioxidant in membranes and in plasma while the major antioxidants present in the tomato hydrophilic fraction are vitamin C (ascorbic acid) and phenolic compounds [15-16].

Table 1. Lipophilic and hydrophilic antioxidant activities of the tomato extracts determined by DPPH assay

Sample	DPPH (µmol TEAC/100 g DW)		
	Lipophilic	Hydrophilic	Total
Black Cherry Kham Kaen	140.1±6.45	1332.5±1.56	1472.7
Lai Kho Red	115.8±1.31	619.4±4.36	735.2
Mani Siam	114.2±0.44	1091.7±12.86	1205.9
Mani Thapthim	123.0±0.41	617.0±6.47	740.1
Mo Kho 40	112.3±0.81	551.9±5.63	664.1
Phuang Thong 80	119.8±0.77	672.1±5.49	791.9
Red Sweet	111.8±1.75	553.8±8.19	665.5
Seeda	125.5±0.42	1171.3±6.68	1296.8
Tha-ap-green	119.0±0.45	599.4±4.53	718.4
Thapthim Daeng	122.2±0.45	689.3±10.18	811.5

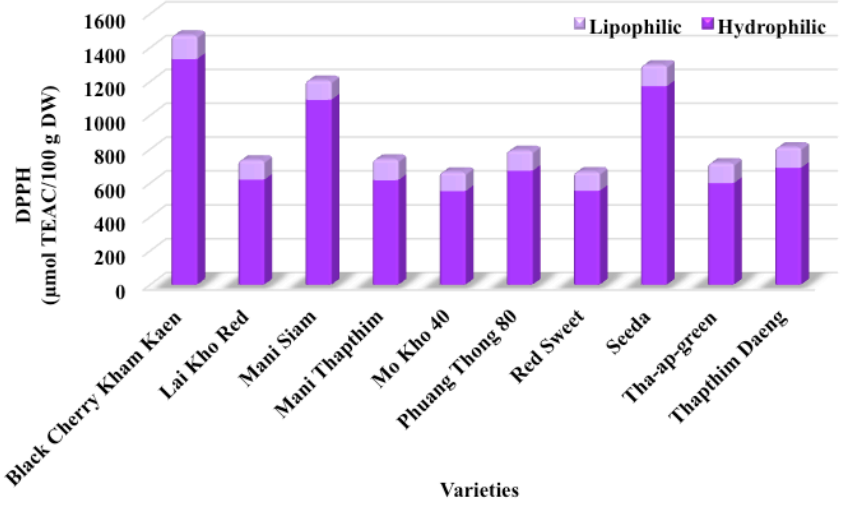

Figure 1. DPPH free radical scavenging activity for the lipophilic and hydrophilic extracts in ten varieties of tomatoes

Table 2. Lipophilic and hydrophilic antioxidant activitis of the tomato extracts determined by ABTS assay

Sample	ABTS (µmol TEAC/100 g DW)		
	Lipophilic	Hydrophilic	Total
Black Cherry Kham Kaen	145.5±9.87	1505.4 ±21.04	1650.9
Lai Kho Red	113.1±2.00	691.3 ±6.66	804.3
Mani Siam	110.1±0.68	1153.1±6.77	1263.1
Mani Thapthim	123.6±0.62	685.4±9.89	809.0
Mo Kho 40	103.5±1.24	571.1±8.61	674.6
Phuang Thong 80	117.5±1.17	764.8±8.39	882.2
Red Sweet	104.5±2.68	581.2±12.52	685.6
Seeda	126.5±0.63	1323.4±2.90	1449.9
Tha-ap-green	114.0±0.69	644.4±6.93	758.4
Thapthim Daeng	121.9±0.69	793.8±15.56	915.7

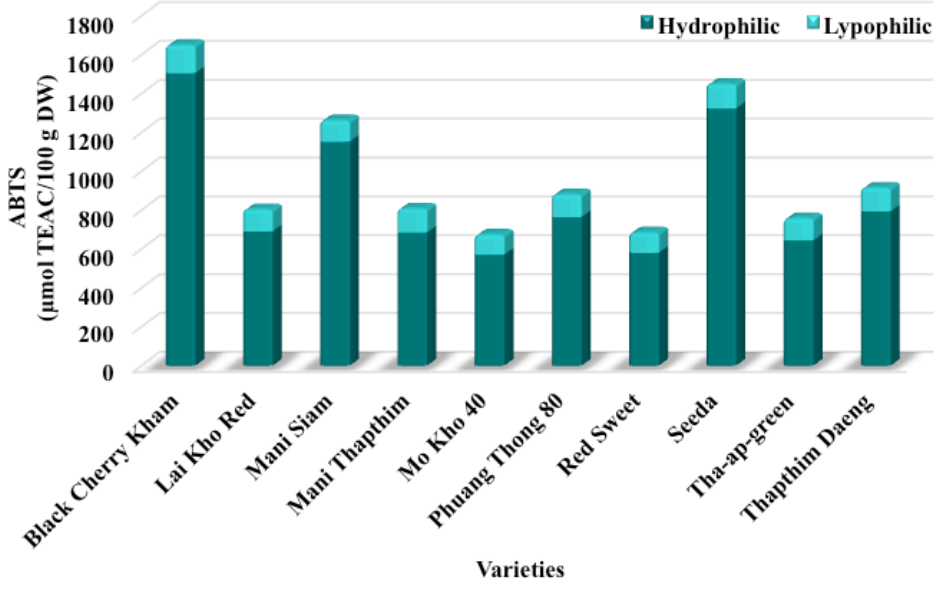

Figure 2. ABTS radical cation scavenging activity for the lipophilic and hydrophilic extracts in ten varieties of tomatoes

Table 3. Lipophilic and hydrophilic antioxidant activitis of the tomato extracts determined by FRAP assay

Sample	FRAP (µmol TEAC/g DW)		
	Lipophilic	Hydrophilic	Total
Black Cherry Kham Kaen	175.0±0.71	1559.7±9.13	1734.7
Lai Kho Red	153.6±0.50	890.5±4.40	1044.2
Mani Siam	165.8±0.38	1189.7±4.44	1355.6
Mani Thapthim	160.9±0.24	882.7±6.48	1043.6
Mo Kho 40	143.0±1.07	787.7±5.34	930.7
Phuang Thong 80	161.3±0.35	925.7±5.40	1087.0
Red Sweet	146.0±0.73	803.6±7.98	949.7
Seeda	166.2±3.53	1332.4±1.94	1498.6
Tha-ap-green	148.6±0.35	834.3±4.31	982.9
Thapthim Daeng	163.1±0.23	949.8±10.13	1112.9

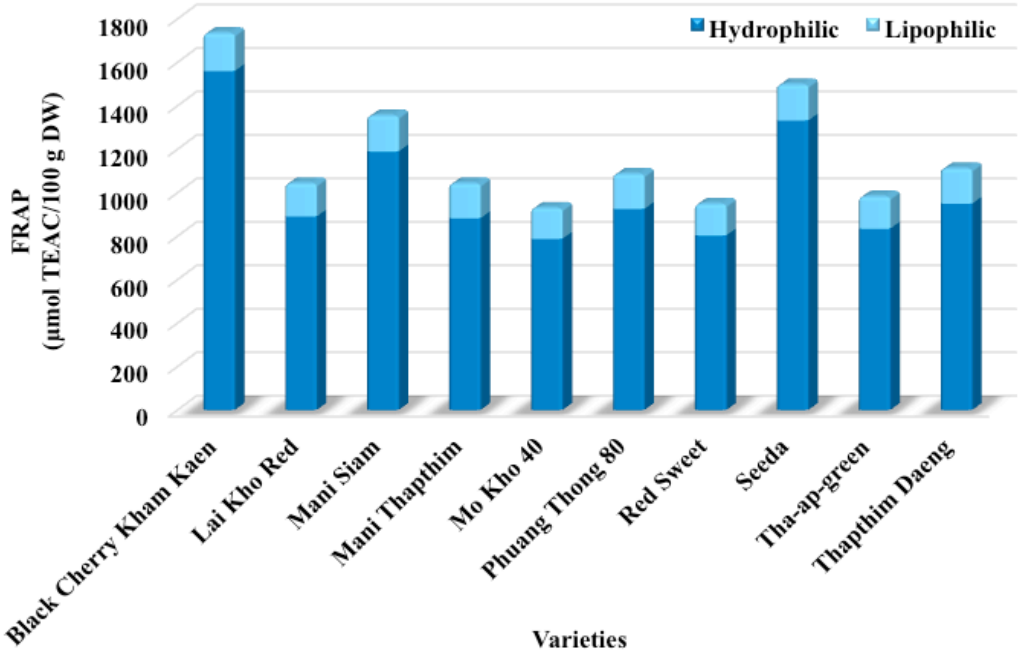

Figure 3. FRAP values for the lipophilic and hydrophilic extracts in ten varieties of tomatoes

4. Conclusion

Three rapid and simple spectrophotometric method for analysis of antioxidant activity were used. According to the data obtained from the present study, 10 varieties of tomato were found to be effective antioxidant sources as demonstrated by DPPH, ABTS and FRAP assays. It is evident that lipophilic and hydrophilic antioxidants were used to directly focus on total antioxidant activity of the crude extracts from tomato varieties. However, neither single compound nor group of compounds sufficiently defines the total antioxidant capacity, since other antioxidant nutrients present in fresh tomatoes can produce a synergistic effect on the total antioxidant activity.

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