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Determination of ascorbic acid and total phenolics related to the antioxidant activity of some local tomato (*Solanum lycopersicum*) varieties

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ABSTRACT

The present study was aimed to determine ascorbic acid, total phenolics and antioxidant activity of five local tomato varieties available in fresh markets, namely "Rashinee", "Puang", "Sida", "Tou" and "Cherry", under optimum conditions of 2,6-dichlorophenolindophenol, Folin-Ciocalteu reagent and 2,2-diphenyl-picrylhydrazyl radical methods, respectively. Fresh tomato fruits were homogenized and kept as a freeze-dry powder prior to be extracted with methanol, ethanol and deionized water using ultrasonicator as a green extraction method. It was found that the methanol extracts of these samples gave rather higher antioxidant activities (171.6-197.1 mg BHT/100 g dry weight) and total phenolics (302.3-349.7 mg GAE/100 g dry weight) than those of both ethanol and aqueous extracts. It is evident that the total phenolics contents relate well with its corresponding antioxidant activity for all tomato varieties. However, the ascorbic acid contents (37.6-62.9 mg/100 g dry weight) of the aqueous extract could be cooperated as the synergistically reducing factor.

Key words: antioxidant activity, total phenolics, ascorbic acid, tomato

INTRODUCTION

Tomato (*Solanum lycopersicum*) 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. It contains not only the nutritional antioxidants, but also a great quantity of non-nutritional antioxidants such as carotenoids, flavonoids, flavones and phenolic compounds, etc. [1-7]. Thus, consumption of tomato products has been associated with decreased risk of some cancers, and the tomato antioxidant, lycopene, is thought to be positive to the observed health [8]. Phenolic compounds are one of the main groups of dietary phytochemicals found in fruits, vegetables and grains. They are found in plant tissues, and frequently serve as pigments in plants to attract pollinators, or as plants' chemical defense mechanism against infections caused by microorganisms and injuries by insects [9,10]. A significant role of phenolics that has been under active research in recent years is their possible beneficial health effects for humans. Phenolic compounds have been recognized for their antioxidant activity which has been linked to slow down the ageing process and lowered risks of many prevalent chronic diseases such as cancer and coronary heart disease. Most of these problems are considered to be caused by an imbalance between the oxidative stress and antioxidants in the body [11]. Ascorbic acid, a well-known antioxidant, has been suggested to act synergistically with tocopherol to regenerate the tocopheryl radicals. It may scavenge peroxy radical and inhibit cytotoxicity induced by oxidants. In addition, it can reduce or prevent H₂O₂-induced lipid peroxidation and the formation of OH-deoxyguanosine [12,13]. 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 [14,15].

The aim of the present study was to determine the antioxidant activity of the tomato extracts obtained from five local varieties comparatively using each of three common solvents (methanol, ethanol and deionized water) by 2,2-diphenyl-1-picryl-hydrazyl (DPPH) assay. Total phenolics and ascorbic acid of both kind of alcohols and the aqueous extracts were also quantified.

MATERIALS AND METHODS

2.1. Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Sigma-Aldrich (USA). Gallic acid and 2,6-dichlorophenolindophenol (DCPIP) were obtained from Fluka (Switzerland). Folin-Ciocalteu reagent was purchased from Merck (USA). Metaphosphoric acid and sodium carbonate (Na_2CO_3) were purchased from Carlo Erba (Italy). Ascorbic acid was purchased from Unilab (New Zealand). Butylated hydroxytoluene (BHT) was obtained from Acros Organic (USA). Methanol and ethanol were obtained from QRecTM (New Zealand). All chemicals and solvents used were of analytical grade.

2.2. Tomato fruits

Five kinds of tomatoes used in this study were commercially available in local markets in Khon Kaen, Thailand. Their common Thai names of the tomatoes are "Rashinee", "Puang", "Sida", "Cherry" and "Tou". 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 procedures

2.3.1. Extraction of total phenolics

Each of the freeze-dried tomato samples (0.1 g) was extracted with 10 mL of 1% (v/v) hydrochloric acid in deionized water, methanol and ethanol followed by ultra-sonication (35 Hz) at ambient temperature for 20 min. The mixture was then centrifuged at 4000 rpm for 10 min and the supernatant was used for determination of total phenolic compounds [16].

2.3.2. Extraction of ascorbic acid

Each of the freeze-dried tomato samples (0.5 g) was extracted by 20 mL of 3% (w/v) metaphosphoric acid followed by shaking at 300 rpm for 30 min. The extract was centrifuged at 4000 rpm for 10 min. The supernatant was collected and used for further analysis [17].

2.3.3. Extraction of antioxidants

Each of the freeze-dried tomato samples (0.5 g) in a brown vial (20 mL) was extracted with 10 mL of each of deionized water, methanol and ethanol followed by ultra-sonication (35 Hz) at ambient temperature for 20 min. The mixture was then centrifuged at 4000 rpm for 5 min and the supernatant was used for determination of total phenolic compounds.

2.4. Analytical procedures

2.4.1. Assay for ascorbic acid

Ascorbic acid was quantitatively determined according to the slightly modified method of 2,6-dichlorophenolindophenol (DCPIP) [18]. A standard curve with a series of the known ascorbic acid solutions was prepared in 3% (w/v) metaphosphoric acid. 1 mL of either sample extract or standard compound was added into 3 mL of 0.2 mM DCPIP and measured immediately after mixing for 15 sec at 515 nm. The results were expressed in mg ascorbic acid per 100 g dry weigh (mg/ 100 g DW). The experiment was replicated with three independent assays.

2.4.2. Assay for total phenolics

Total phenolic constituents of the polar and nonpolar subfractions of methanol extracts were determined using Folin-Ciocalteu reagent and gallic acid used as standard compound [19]. The solution of each sample extract (0.2 mL) was taken individually in test tube, 1 mL of 10% Folin-Ciocalteu reagent was added, and the tube was thoroughly shaken. After 3 min, 0.8 mL of 7.5% Na_2CO_3 solution were added and the mixtures were allowed to stand for 30 min at room temperature. The absorbance of the solution was measured spectrophotometrically at 765 nm. The same procedure was repeated for all gallic acid standard solutions (100-800 μM). All tests were carried out in triplicate and phenolic contents were reported as $\mu\text{mol GAE/g DW}$.

2.4.3. Assay of DPPH free radical scavenging activity

Radical scavenging activity of BHT used as a reference standard and tested sample extract was measured by modified DPPH method [20]. 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 μ L of BHT or the 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 blank sample (Agilent 8453 UV-Visible spectroscopy, Germany). The percentage of an inhibition of the DPPH was calculated and plotted as a function of concentration of BHT used as the reference. The final DPPH values were calculated using a regression equation between the BHT concentration and the percentage of DPPH inhibition. 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.

RESULTS AND DISCUSSION

The comparative extraction studies of total antioxidants in different tomato varieties were investigated using three common solvents including deionized water, methanol and ethanol, without any solvent combination, in order to obtain a suitable extraction solvent giving highest antioxidant activity.

3.1. Determination of ascorbic acid

The extraction of ascorbic acid in these tomato samples was performed. Good results were obtained using a mixture of water and 3% metaphosphoric acid assayed by DCPIP method. The calibration equation for ascorbic acid was constructed by plotting the UV response against the ascorbic acid concentrations (in triplicate). The UV response of ascorbic acid over a concentration range of 100-450 μ M was linear ($y = -0.0021x + 1.08$) with a regression coefficient (r^2) of 0.9979. The amounts of ascorbic acid in five varieties of tomatoes are shown in Table 1. The obtained results were found in the range from 37.59 ± 0.08 to 62.90 ± 0.67 mg/ 100 g DW. The ascorbic acid content in Puang variety (62.90 ± 0.67 mg/ 100 g DW) was higher than other varieties, while that of Sida sample gave the lowest (37.59 ± 0.08 mg/ 100 g DW).

Table (1): The contents of ascorbic acid and total phenolics, and antioxidant activity of the aqueous extracts

Sample	Ascorbic acid (mg/100 g DW)	Total phenolics (mg GAE/100 g DW)	Antioxidant activity (mg BHT/100 g DW)
Rashinee	54.20 ± 1.31	263.0 ± 2.7	136.33 ± 4.62
Puang	62.90 ± 0.67	306.9 ± 4.4	166.81 ± 3.08
Sida	37.59 ± 0.08	271.3 ± 4.3	120.17 ± 4.53
Cherry	58.51 ± 2.34	294.1 ± 1.7	150.02 ± 2.55
Tou	52.80 ± 1.74	289.7 ± 2.0	134.53 ± 3.29

Table (2): Total phenolics and antioxidant activity of methanol and ethanol extracts

Sample	Total phenolics*		Antioxidant activity**	
	Methanol	Ethanol	Methanol	Ethanol
Rashinee	302.3 ± 7.2	248.4 ± 5.7	179.29 ± 2.56	167.41 ± 4.72
Puang	349.7 ± 1.3	335.9 ± 1.5	197.14 ± 0.78	193.18 ± 1.74
Sida	331.6 ± 3.3	284.8 ± 8.5	185.03 ± 5.98	145.44 ± 4.47
Cherry	343.3 ± 3.9	313.1 ± 6.6	177.16 ± 3.54	156.05 ± 2.36
Tou	313.1 ± 5.8	304.4 ± 3.0	171.62 ± 1.56	125.32 ± 3.56

*mg GAE/100 g DW

**mg BHT/100 g DW

3.2. Determination of total phenolics

The concentration of total phenolics in the aqueous extracts of five tomato varieties examined ranged from 263.0 to 306.9 mg GAE/ 100 g DW. The total phenolic content was highest (306.9 mgGAE/ 100 g DW) in Puang sample and lowest (263.0 mg GAE/ 100 g DW) in Rashinee sample. For the others, the total extractable phenolic concentrations were found within this range (Table 1). For both methanol and ethanol used as the extraction solvents, similar trends in total phenolics of these samples were also found between 302.3-349.7 mg GAE/ 100 g DW and 248.4-335.9 mg GAE/ 100 g DW, respectively (Table 2). Figure 1 shows the comparative data obtained from five kinds of the tomato extracts, resulted in resemble trends in the extraction efficiency by using these solvents. However, these values are only indicative of the concentration of polyphenols in tomato, since there is no single analytical method that, collectively and accurately, is able to measure the total polyphenol content. Reasons for this include the

structural diversity found amongst phenolic compounds and the large variation in content depending on the nature of food and the plant part from which it derives [16]. Genetic factors and growing conditions may play an important role in the formation of secondary metabolites including phenolic acids [21].

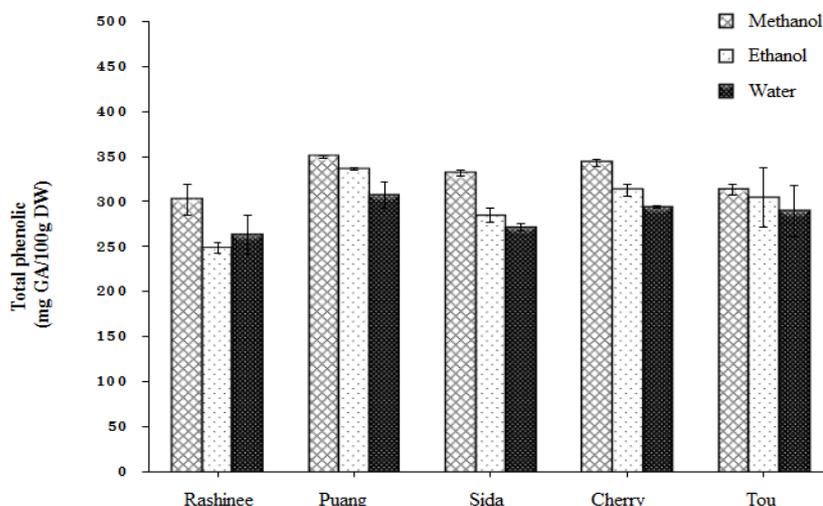


Figure (1): Total phenolics contents of the tomato extracts of five local varieties using methanol, ethanol and water as the extraction solvents

3.3. Determination of antioxidant activity

DPPH assay was used for an evaluation of antioxidant activity of five varieties of tomatoes. The obtained results are shown in Table 1 and Table 2. Their antioxidant activities in the aqueous extracts were ranged from 120.17 to 166.81 mg BHT/ 100 g DW, while those values were found between 171.62-197.14 mg BHT/ 100 g DW and 125.32-193.18 mg BHT/ 100 g DW for methanol and ethanol extracts, respectively. The methanol extracts of Puang sample exhibited the highest antioxidant activity (197.14 mg BHT/ 100 g DW) and followed by Sida sample (185.03 mg BHT/ 100 g DW). Figure 2 also shows the same comparative data of the antioxidant activities of these tomato extracts when using these extraction solvents. Similar trends in their antioxidant activities were also related to their total phenolics as mentioned above. The relatively stable organic radical, DPPH, has been widely used in the determination of antioxidant activity of pure compounds used as reference, as well as of different plant extracts [22]. Tomato lipophilic fraction also contains vitamin E 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 and phenolic compounds [23,24].

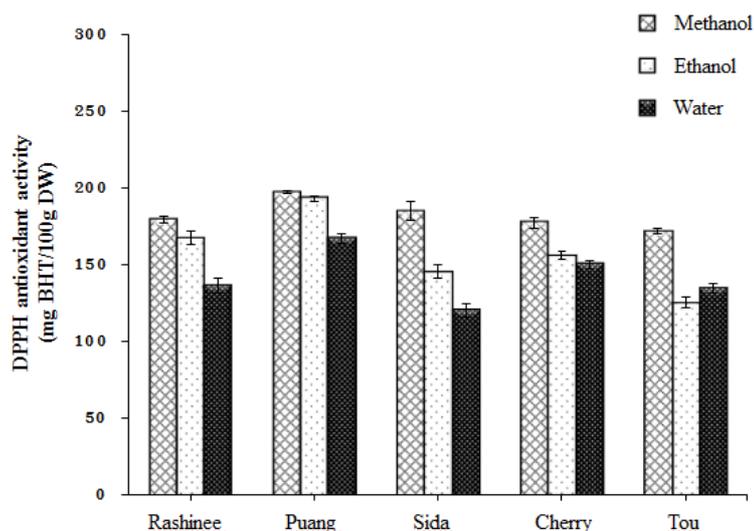


Figure (2): The antioxidant activities of the tomato extracts of five local varieties using methanol, ethanol and water as the extraction solvents

CONCLUSION

Five local varieties of tomatoes were found to be effective antioxidant sources. Quantification of total phenolics and ascorbic acid were helpful in detailed evaluation of their antioxidant activity. However, neither a single compound nor a group of the ones sufficiently defines total antioxidant activity, since other antioxidant nutrients present in fresh tomatoes can produce a synergistic effect on the antioxidant activity.

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