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# Standardization of DPPH, ABTS and FRAP assays with six reference compounds for estimating antioxidant capacity of the tomato extracts using an ultrasound assisted extraction

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## Abstract

Comparative calibrating of the antioxidant power of standard compounds, including gallic acid, catechin, Trolox, -tocopherol, ascorbic acid and BHT were assessed by DPPH, ABTS and FRAP assays to determine total antioxidant activity of ten varieties of tomato extracts using ultrasound assisted extraction. Similar trends in their scavenging activities were found, resulted in the following rank; gallic acid > catechin > trolox > -tocopherol > ascorbic acid > BHT. Both lipophilic and hydrophilic parts of the extracts were given in these assays in association with using hexane and 50% (v/v) methanol as their efficient extraction solvents, respectively. Their averaged values of the ascorbic acid equivalent antioxidant capacities (AEAC, µmol ascorbic acid/100g DW) were relatively confined in the same ranges of 1341.8-1834.5, 1334.2-2194.8 and 930.7-1734.7 for DPPH, ABTS and FRAP, respectively. In addition, both contents of total phenolics and ascorbic acid known as major deals of antioxidants were also determined, indicating highly existing constituents of the natural antioxidants.

**Keywords:** Antioxidant capacity, Total phenolics, Ascorbic acid, Ultrasonication extraction.

## Introduction

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start its chain reactions. When the chain reaction occurs in a cell, it can cause injury or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit further oxidation reactions. They achieve this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid, or polyphenols (Sies 1997). Nowadays there is just 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 free radicals, which are unstable molecules that are linked to 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, spoilage and poisoning of foods by microorganisms are still the problem. The consumer acceptance for preservatives with chemical origin is decreasing; therefore, the producers are looking for natural organic compounds which can be an alternative and supplemented with food products will help to prolong their shelf-life and microbial safety. A significant number of in vitro antioxidant activities have been developed to measure the efficiency of biological 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 (Muselík et al., 2007).

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. 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. (Havsteen, 1983; Hudson and Lewis, 1983; Takahama, 1985; Wang et al.,

1996; Takeoka et al., 2001; Yao et al., 2002; Pernice et al., 2010). Thus, consumption of tomato products has been associated with decreased risk of some cancers, and the tomato antioxidant, lycopene, is thought to be positive for the observed health (Heijnen et al., 2001). Phenolic compounds are one of the main groups of dietary phytochemicals found in fruits, vegetables and grains. They are discovered in plant tissues, and frequently serve as pigments in plants to attract pollinators, or as a plant chemical defense mechanism against infections caused by microorganisms and injuries by insects (Ballard et al., 2010; Rosa et al., 2010). 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 (Karacabey and Mazza, 2010). Ascorbic acid, a well-known antioxidant, has been suggested to act synergistically with tocopherol to regenerate the tocopherol radicals. It may scavenge peroxyl radical and inhibit cytotoxicityinduced by oxidants. In addition, it can reduce or prevent $H_2O_2$-induced lipid peroxidation and the formation of OH-deoxyguanosine (Retsky and Frei, 1995; Tsou et al., 1996). Since the resulting data of antioxidant capacity depend on the method used, a single method can not give an accurate prediction of the antioxidant capacity of antioxidant compounds (Arts et al., 2003; Rebiai and Lanez, 2012). It is recommended to use more than one method to estimate the in vitro antioxidant capacity of substantial materials extracted due to the complex nature of reactive chemical species. There are various methods that differ in terms of their assay's principles and experimental conditions, and particular antioxidants have varying contributions to the total antioxidant potentials (Cao and Prior, 1998).

The aim of the present study was to evaluate the antioxidant properties of tomato extracts related to some relevant standard compounds 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 built on scavenging of reactive nitrogen and oxygen species (Yao et al., 2002). The antioxidant activities of the extracts using these assays were then compared to those of six widely used synthetic antioxidants. All existing methods described in the literature are based on the use of $IC_{50}$ value which is defined as the amount of antioxidant required to scavenge 50% of the free radical of the authentic standards used in that assay. Percentage of radical scavenging activity should be plotted against the corresponding concentration of the antioxidant to obtain $IC_{50}$, and the antioxidant activities of six standards for the FRAP assay were expressed as $EC_1$, concentration of antioxidant that reduced $Fe^{3+}$-TPTZ equal to 1 mm $FeSO_4{\cdot}7H_2O$. On the other hand, in order to evaluate the technological and biological potentials of the tomato varieties, the obtained results of the extracts were expressed as micromoles of ascorbic acid equivalent antioxidant capacity per gram dry weight (µmol AEAC/100 g DW).

## Material and Methods

### Plant Materials

Ten varieties of tomatoes (*Lycopersicon esculentum* Mill) used in this study were collected from local breeding cultivars. 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. Most samples were experimentally cultivated in the practical fields belonging to the Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University.

All tomato, fresh fruits were washed with distilled water, cut into pieces and homogenized. The homogenized sample was moved into the PTFE centrifuge tube and frozen at -20ºC. This frozen puree was freeze-dried (SCANVAC Centrifuge for Vacuum Concentrator Freeze-Dry, China). The sample was enclosed in a container of the laboratory mill and grounded into a fine powder. These materials were later stored in a freezer at -20ºC until analysis.

### Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), Trolox, (+)-catechin and 2,2'-azino-bis(3-ethylbenzothiazolin-6-sulfonic acid (ABTS) were obtained from Sigma-Aldrich (USA). Gallic acid, -tocopherol, and 2,6-dichlorophenolindophenol (DCPIP) were obtained from Fluka (Switzerland). Folin-Ciocalteu reagent was acquired from Merck (USA). Metaphosphoric acid, ferrous sulfate heptahydrates ($FeSO_4.7H_2O$) 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). Potassium persulfate, sodium acetate, ferric chloride ($FeCl_3$), methanol, hexane, acetone, acetic acid and hydrochloric acid were available from QRec™ (New Zealand). All chemicals and solvents used were of analytical grade.

### Extraction of lipophilic and hydrophilic antioxidants

A modified method was used to separate the lipophilic and hydrophilic extracts of tomato (Toor and Savage, 2005; Teow et al., 2007; Kotíková et al., 2011; Riahi and Hdider, 2013). In brief, 1 g of freeze-dried sample powder in 20 mL hexane was ultrasonicated 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 at 50ºC using a vacuum evaporator. Residue was then redissolved in 5 mL acetone and vortexes to get homogenous samples. The lipophilic extract

was achieved for the determination of lipophilic antioxidant activity. The residue after hexane extraction was then extracted with 20 mL of 50% (v/v) methanol in water and ultrasonicated for 20 min. The mixture was taken to a centrifuge tube followed by centrifugation at 4000 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 needed to be measured in triplicates for each extract. Four variables that could potentially affect the extraction efficiency were studied using the same procedure mentioned above. The experiments were carried out at three types of organic solvents for lipophilic fractions (ethyl acetate, hexane, 50% acetone in water) and four types of solvent for hydrophilic fractions (0.1M phosphate buffer pH7.4, acetone: water: acetic acid (70:29.5:0.5), 50% methanol in water, 7% acetic acid in 80% methanol). The amount between solid (sample) and liquid (organic solvents) was carried out at the ratios of 1:10, 1:15, 1:20 and 1:25 g:mL. The ultrasonication times were varied at six extraction time levels (10, 20, 30, 40, 50, and 60 min). Temperatures were altered at 30, 40, 50, 60 and 70ºC.

## Extraction of ascorbic acid

Each of the freeze-dried tomato samples (0.5 g) was extracted with 20 ml of 3% (w/v) metaphosphoric acid followed by ultrasonication for 20 min. The extract was centrifuged at 4000rpm for 10 min. The supernatant was gathered and used for further analysis (Butcher et al., 2013).

## 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 80% (v/v) methanol followed by ultra-sonication 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 (Martínez-Valverde et al., 2002).

## DPPH free radical Sscavenging activity assay

Radical scavenging activity of six common organic compounds used as a reference standard and a tested sample extract was measured by modifying the DPPH method (Vallverdú-Queralt et al., 2012). 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 1000 µL. 100 µL of various concentrations of each single standard or lipophilic or hydrophilic extract was 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 a blank sample (Agilent 8453 UV-Vis spectrophotometer, 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 the ascorbic acid concentration and the percentage of DPPH inhibition, and the results were expressed as micromole of ascorbic acid equivalent antioxidant capacity per gram dry weight (µmol AEAC/100 g DW). The percentage of inhibition of DPPH free radical was calculated using the following equation:

% Inhibition = $[(A_c - A_s) / A_c]$ 100

Where $A_c$ is the absorbance of control reaction which contains all reagents 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.

## ABTS radical cation decolorization assay

Radical cation scavenging capacity of the tomato extracts including a reference standard was examined against $ABTS^+$ with some modifications (Thaipong et al., 2006). The ascorbic acid equivalent antioxidant capacity (AEAC) method is based on the ability of antioxidant to scavenge the performed radical cation $ABTS^+$ as compared with ascorbic acid. The $ABTS^+$ was produced by the reaction of 7.4 mM ABTS in methanol with 2.6 mM $K_2S_2O_8$, stored in the dark at room temperature for 12-16 h. Before use, the $ABTS^+$ solution was diluted with methanol to get the absorption between 0.7 and 0.9 AU at 734 nm. Briefly, 60 µL of the antioxidant extract or reference standard were mixed with 1000 µL of $ABTS^+$ solution and kept 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. Ascorbic acid with concentrations from 500-1000 µM was invoked as a standard curve. The free radical scavenging activity was expressed as µmol AEAC/100 g DW. All determinations were performed in triplicate.

## Ferric ion reducing antioxidant power (FRAP) assay

The ferric ion reducing antioxidant power (FRAP) method was used to measure the decreasing capacity of tomato extracts from different varieties. This method was carried out with slight modifications (Hossaina et al., 2008; Li et al., 2012). The FRAP method measures the ability of antioxidants to reduce ferric–tripyridyl-triazine ($Fe^{3+}$-TPTZ) complex in the blue colored ferrous form 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 FRAP reagent and incubated at 37ºC for the duration of the reaction. Absorbance readings were taken at 593 nm at 30 min. The increasing absorbance of the reaction mixture indicate an increase of reduction capability. Six concentrations of 500, 600, 700, 800, 900 and 1000 µM were used to prepare the standard curve of ascorbic

acid. The antioxidant activities of the tomato extracts were expressed as µmol AEAC/100 g DW while the antioxidant activities of six reference standards were expressed as $EC_1$, the concentration of antioxidant that reduced $Fe^{3+}$-TPTZ equal to 1 mM $FeSO_4 \cdot 7H_2O$.

### Determination of ascorbic acid

Ascorbic acid content was quantitatively determined in accordance with the slightly modified method of 2,6-dichlorophenolindophenol (DCPIP) (Klein and Perry, 1982). A standard curve with a series of known ascorbic acid solutions was prepared in 3% (w/v) metaphosphoric acid. 1 mL of each sample extract or standard was added in 3 mL of 0.2 mM DCPIP and measured immediately after mixing for 15 secs at 515 nm. The results were expressed in µmol of ascorbic acid per g dry weight (µmol/g DW). The experiment was repeated with three independent assays.

### Determination of total phenolics

Total phenolic constituents of polar and non-polar subfractions of methanol extracts were determined using Folin-Ciocalteu reagent and gallic acid used as standard compound (Queiroz et al., 2009). The solutions of each sample extract (0.2 mL) were taken individually in a test tube. 1mL of 10% Folin-Ciocalteu reagent was inserted, and the tube was thoroughly shaken. After 3 min, 0.8 mL of 7.5% $Na_2CO_3$ solution was added and the mixtures were permitted 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 µmol GAE/100 g DW.

### Data analysis

Data results are given as the mean ± Standard deviation (SD) of the three measurements ($n$ = 3). All graphs, linear regression in this paper were analyzed by Microsoft Excel 2013 software. Statistical analysis was determined by Origin 8.1 software for Windows.

## Results and discussion

### Optimal extraction for lipophilic and hydrophilic antioxidants

The extraction of lipophilic and hydrophilic antioxidants in different tomato varieties was conducted using an ultrasound assisted extraction. For the optimal extraction efficiency, some of the experimental parameters including extraction solvent, the amount of solid to liquid ratios, extraction time, and temperature were studied in detail.

### Effect of organic solvents

Various solvents including seven typical organic/water solvents were utilized to test their extracting efficiency for the extraction of tomato sample (Figure 1). For the extraction of lipophilic antioxidants, three kinds of organic solvents (hexane, ethyl acetate and 50% (v/v) acetone/water) were used, while the extraction of hydrophilic ones was performed with four selected aqueous solutions including 0.1M phosphate buffer pH 7.4, acetone: water: acetic acid (70:29.5:0.5, v/v/v), 50% (v/v) methanol/water and 7% (v/v) acetic acid in 80% (v/v) methanol/water. It was found that hexane, ethyl acetate and 50% (v/v) acetone/water were becoming effective solvent for tomato powder, which resulted in the co-extraction of fat soluble compounds, although the lipophilic antioxidant extracts of each organic solvent did not give much difference in the $ABTS^+$ antioxidant activity, that of the hexane extract was relatively higher. While the hydrophilic antioxidant extract with the highest $ABTS^+$ antioxidant activity was obtained from 50% (v/v) methanol/water. The mixtures of alcohol and water have been more efficient in extracting compounds and give a better yield than the corresponding mono-component solvent system. Therefore, suitable solvents for $ABTS^+$ antioxidant assay, in this case, would be hexane and 50% (v/v) methanol/water for lipophilic and hydrophilic extracts, respectively.

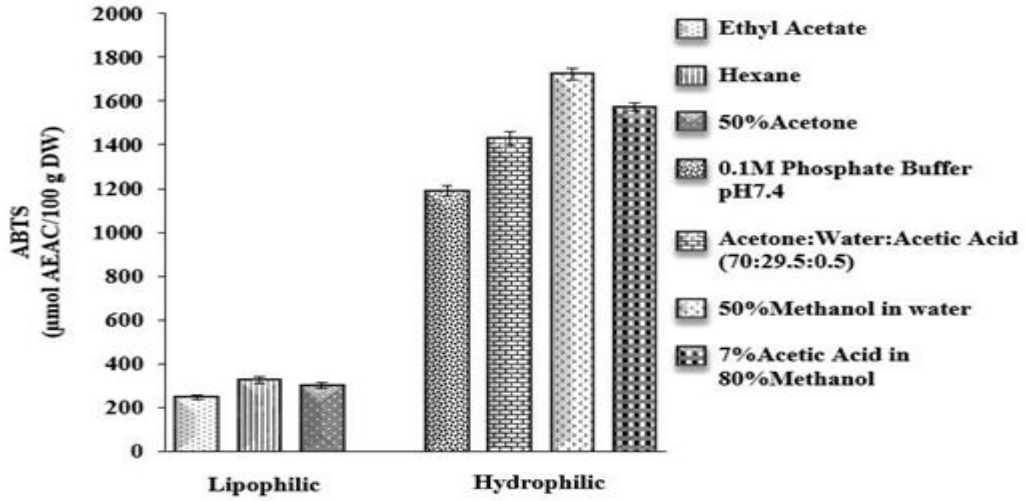


**Figure 1. Effect of extraction solvents for the tomato extracts on the ABTS radical cation scavenging activity.**

## Effect of sample to solvent ratio

The ratios of solid-liquid concerns the contact area of solid and liquid, consequently influence extraction efficiency. Contact area can reach to biggest when solid are saturated with liquid. In this study, the maintaining the sample quantities constant of 1 g of tomato powder while solvent volume of 10, 15, 20, 25 and 30 mL of hexane has been employed. Figure 2 shows that the low ratio of solid-liquid, 1:10 and 1:15, led to extracting incompletely, the ABTS antioxidant activity slightly increased. To the contrary the high ratio of solid-liquid, 1:25 and 1:30, resulted in decreasing of the ABTS antioxidant activity because of dilution solvent. Using 1:20 a higher signal is going to be obtained in the final measurement method. Therefore, a ratio of solid-liquid 1:20 was used in the further optimization experiments.

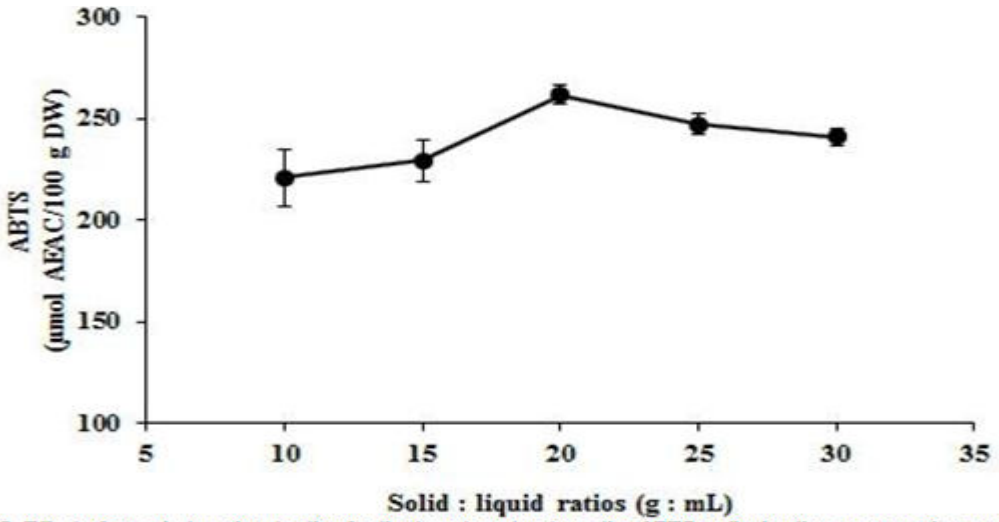


**Figure 2. Effect of sample to solvent ratios for the tomato extracts on the ABTS radical cation scavenging activity.**

## Effect of extraction time

For an effect of ultrasonication time (10 to 60 min) using hexane extraction (Figure 3) of ABTS$^{+}$ assay, antioxidant activity slightly increased with sonication time between 10 and 20 min, the duration of 20 min is enough to completely extract and then kept nearly constant up to 60 min. Further prolongation of the reaction time led to some extra disadvantages in some cases. Thus, optimum ultrasonication time of 20 min was chosen.

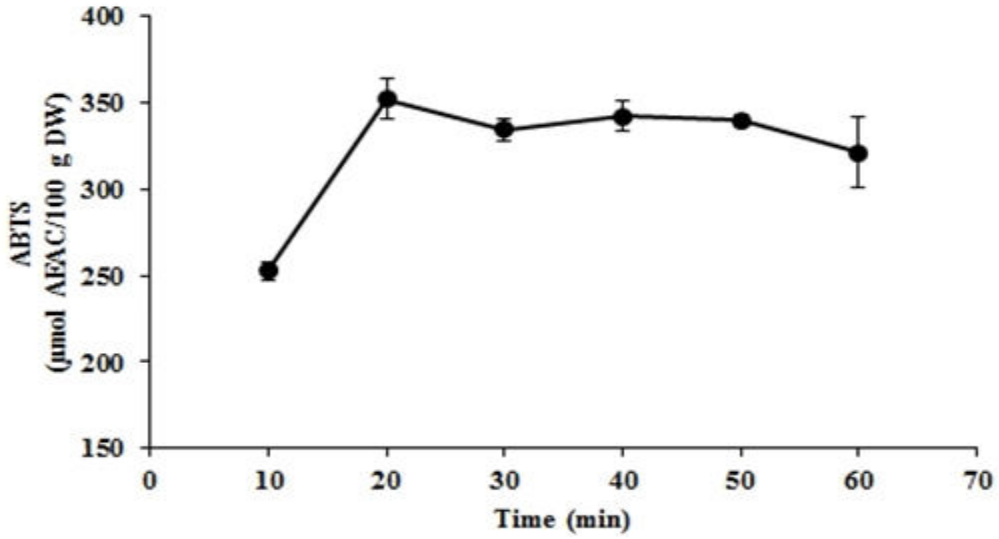


**Figure 3**. Effect of ultra-sonication times using hexane as extraction solvent on the ABTS radical cation scavenging activity.

## Effect of temperature

Effect of extraction temperature was launched at 25 up to 70ºC. Higher temperatures were not checked because the abnormal losses of organic solvent occur changing the solid-liquid ratio, i.e., evaporation of methanol or hexane and thus the increase of product's concentration (effect on absorbance values), then producing low repeatability. The antioxidant activity at the different temperatures on ABTS assay is presented in Figure 4. At 30ºC produced the highest antioxidant activity. The trend was reduced with increasing extraction temperature. However, most likely degradation processes also increased due to elevated temperatures promote the oxidation degradation reaction of antioxidant compounds. Therefore, 30ºC was invoked as the extraction temperature in the experiments.

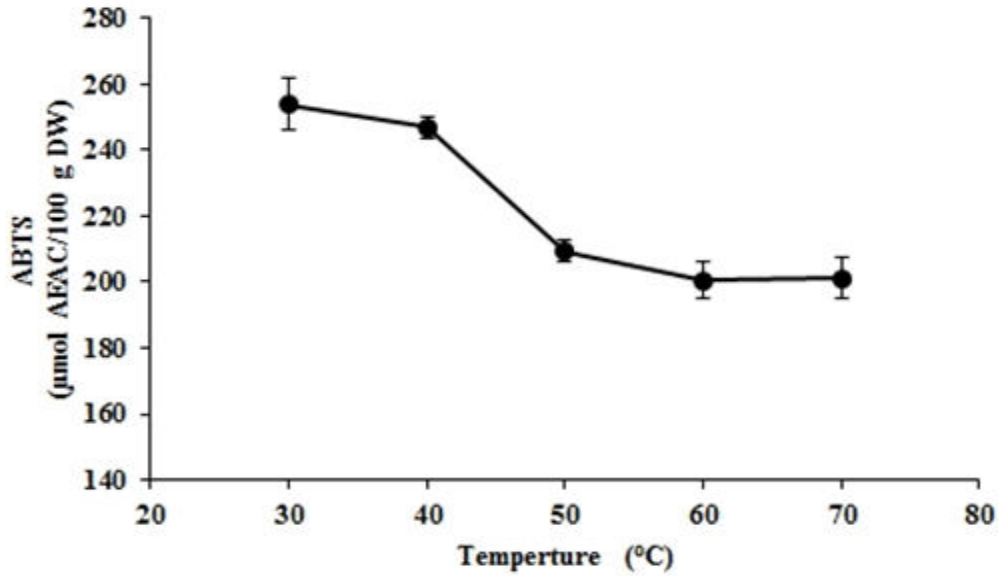


**Figure 4.** Effect of extraction temperatures on the ABTS radical cation scavenging activity

## Antioxidant capacity of the relevant standards

Some relevant organic compounds are used as antioxidant standards, including gallic acid, catechin, Trolox, -tocopherol, ascorbic acid and BHT were comparatively evaluated for their ranking of antioxidant power by DPPH ABTS and FRAP assays. First of all, the results of DPPH and ABTS assays were reported as the concentration providing 50% of the radical scavenging ($IC_{50}$) while that of FRAP assay was reported as the concentration of an antioxidant having a ferric reducing ability equivalent to that of 1 mM ferrous salt ($EC_1$). A lower $IC_{50}$ and $EC_1$ value correspond to a larger scavenging activity. As showed in Table 1, the DPPH radical scavenging activities of these reference compounds were comparatively evaluated. Gallic acid possessed the highest radical scavenging activity, 202 µM as compared with catechin 264 µM, Trolox 568 µM, -tocopherol 574 µM, ascorbic acid 750 µM and BHT with the lowest activity of 1700 µM. The ranks of the scavenging activity were found in similar trends with ABTS and FRAP assays; gallic acid > catechin > Trolox > -tocopherol > ascorbic acid > BHT. However, changes in the scavenging activity rank were found in some order if their unit was expressed in µg/mL; gallic acid > catechin > ascorbic acid > Trolox > -tocopherol > BHT. Gallic acid, naturally occurring plant phenolics, was also found to be a potent antioxidant in emulsion or lipid systems and exhibits anti-mutagenic (Lindberg and Bertelsen 1995). It is much more effective than several water-soluble antioxidants, such as ascorbic acid and fat-soluble, such as -tocopherol (Cholbi et al., 1991). Therefore, these reference standards can be used for directing in vitro antioxidant activity of tomato crude extracts depend on the applied test system and the selection of a suitable, generally applicable standard for all methods enabled us to obtain a set of simple comparable results. It would strongly be prominent and applicable data. The linearity of calibration curves allowed quantification of antioxidant activity using any of the standards listed above. DPPH, ABTS and FRAP values (Table 1) for the antioxidant activity of these standards were used for investigating the correlation coefficients using a 2-tailed test of significance at the 0.05 level. Correlations among antioxidant activity based on DPPH, ABTS, and FRAP assays were positively high and ranged between 0.9722 and 0.9920. The results indicate that when all standard materials were comparatively analyzed by statistics, there was a positive and highly significant relationship for DPPH *vs* ABTS (r = 0.9805). Statistically significant correlations were also noted between DPPH *vs* FRAP value (r = 0.9920) and ABTS *vs* FRAP value (r = 0.9722).

**Table 1 Calibration data of each standard of antioxidant compounds and antioxidant capacity analyzed by DPPH, ABTS and FRAP assays.**

| Compound | DPPH | | | | ABTS | | | | FRAP | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Regression equation ($n$ = 3) | $R^2$ | $IC_{50}$ | | Regression equation ($n$ = 3) | $R^2$ | $IC_{50}$ | | Regression equation ($n$ = 3) | $R^2$ | $EC_1$ | |
| | | | µM | µg $mL^{-1}$ | | | µM | µg $mL^{-1}$ | | | µM | µg $mL^{-1}$ |
| Gallic acid | y = 0.4369x - 30.2985 | 0.9964 | 184 | 35 | y = 0.3447x - 19.5314 | 0.9900 | 202 | 38 | y = 0.0031x - 0.1232 | 0.9926 | 243 | 46 |
| Catechin | y = 0.1505x - 4.5452 | 0.9977 | 362 | 105 | y = 0.2435x - 14.2467 | 0.9953 | 264 | 77 | y = 0.0013x + 0.2110 | 0.9926 | 323 | 94 |
| Trolox | y = 0.1603x - 41.0627 | 0.9951 | 568 | 142 | y = 0.0991x - 19.4572 | 0.9919 | 701 | 264 | y = 0.0014x - 0.2299 | 0.9955 | 615 | 154 |
| -Tocopherol | y = 0.0997x - 7.2035 | 0.9973 | 574 | 247 | y = 0.0738x - 2.8992 | 0.9979 | 717 | 309 | y = 0.0007x + 0.0965 | 0.9953 | 763 | 329 |
| Ascorbic acid | y = 0.1323x - 49.269 | 0.9954 | 750 | 130 | y = 0.1177x - 46.172 | 0.9959 | 817 | 144 | y = 0.0011x - 0.2457 | 0.9958 | 796 | 140 |
| BHT | y = 0.0282x + 2.0473 | 0.9932 | 1700 | 375 | y = 0.0198x + 18.1581 | 0.9958 | 1608 | 354 | y = 0.0002x + 0.1684 | 0.9962 | 2310 | 509 |

## Antioxidant capacity of the tomato extracts

The choice of a suitable common standard for the tested methods, different calibration standards and different ways of expression of concentrations (dry weight, DW; fresh weight, FW; in molar or mass units) have been applied to express the results in the literature. These facts are held for reducing complicated the comparison of the results from one source to another one. Therefore, calibration data of ascorbic acid were used for the evaluation antioxidant activity of ten varieties of tomatoes, the unit was expressed as µmol AEAC/100 g DW. The obtained results are presented in Table 2. Total antioxidant activity, measured by the DPPH method, ranged from 1341.8 to 1834.5 µmol AEAC/100 g DW; e.g. Black Cherry Kham Kaen sample exhibited the highest antioxidant activity, followed by Seeda variety. 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 (Katalinic et al., 2006). Tomato lipophilic fraction also contains vitamin E ( - 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 (Burton et al., 1983; Biacs et al., 1988). The results, Antioxidant activity measured by DPPH showed the same pattern as did by ABTS method, but their AEAC values were slightly higher. Total antioxidant activity, measured by ABTS method, ranged from 1334.2 to 2194.8 µmol AEAC/100 g DW. The highest antioxidant activity of the tomato sample was Black Cherry Kham Kaen while that of Mo Kho 40 sample was the lowest among these samples. Total antioxidant capacity measured by FRAP method was also compared among the tomato varieties. FRAP value was found within the range of 930.7 - 1734.7 µmol AEAC/100 g DW. The

Black Cherry Kham Kaen also gave its highest antioxidant activity. AEAC values are really magnificent, however, on the other hand a recalculation of determined values in different unit has to be considered as well.

**Table 2 Lipophilic, hydrophilic antioxidant activities determined by DPPH, ABTS and FRAP assays, ascorbic acid, total phenolics and its recovery of the tomato extracts. Values represent mean ± standard deviation of three replicates.**

| Sample | Antioxidant activity (µmol AEAC/100 g DW) | | | | | | | | | Ascorbic acid (µmol/100g DW) | % Recovery | Total phenolic (µmol GAE/100 g DW) | % Recovery |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DPPH | | | ABTS | | | FRAP | | | | | | |
| | Lipophilic | Hydrophilic | Total | Lipophilic | Hydrophilic | Total | Lipophilic | Hydrophilic | Total | | | | |
| Black Cherry Kham kaen | 257.2±3.32 | 1577.3±15.91 | 1834.5 | 284.6±7.95 | 1910.3±64.85 | 2194.8 | 175.0±0.71 | 1559.7±9.13 | 1734.7 | 561.1±24.1 | 87.3±3.86 | 6392±162.8 | 73.9±7.7 |
| Lai Ko Red | 259.7±4.14 | 1249.6±25.34 | 1509.3 | 308.3±7.55 | 1471.6±12.42 | 1780.0 | 153.6±0.50 | 890.5±4.40 | 1044.2 | 458.8±7.8 | 74.6±7.23 | 6671±114.9 | 79.5±4.9 |
| Mani Siam | 212.2±9.40 | 1394.4±6.71 | 1606.6 | 231.7±6.31 | 1606.2±16.56 | 1837.8 | 165.8±0.38 | 1189.7±4.44 | 1355.6 | 503.0±30.6 | 85.3±3.66 | 5803±84.9 | 78.1±2.6 |
| Mani Thapthim | 249.9±4.04 | 1405.8±20.77 | 1655.6 | 290.7±7.94 | 1572.4±23.13 | 1863.1 | 160.9±0.24 | 882.7±6.48 | 1043.6 | 645.0±39.5 | 72.5±1.75 | 6504±37.5 | 93.6±4.6 |
| Mo Kho 40 | 208.0±3.52 | 1133.8±39.35 | 1341.8 | 240.3±1.73 | 1093.9±66.04 | 1334.2 | 143.0±1.07 | 787.7±5.34 | 930.7 | 333.2±22.4 | 90.9±8.59 | 5269±61.0 | 102.2±6.7 |
| Phuang Thong80 | 225.1±3.30 | 1294.3±13.83 | 1519.4 | 246.4±8.09 | 1402.8±42.17 | 1649.2 | 161.3±0.35 | 925.7±5.40 | 1087.0 | 535.1±23.2 | 82.4±8.68 | 6294±53.5 | 95.1±4.6 |
| Red Sweet | 198.3±3.39 | 1174.5±17.12 | 1372.7 | 228.6±3.21 | 1190.3±28.11 | 1418.9 | 146.0±0.73 | 803.6±7.98 | 949.7 | 505.8±37.2 | 91.6±9.63 | 5995±93.2 | 82.0±6.3 |
| Seeda | 277.3±3.04 | 1401.3±19.21 | 1678.6 | 331.7±3.33 | 1632.0±17.26 | 1963.7 | 166.2±3.53 | 1332.4±1.94 | 1498.6 | 636.9±43.5 | 94.8±6.64 | 5318±53.4 | 95.5±9.1 |
| Tha-ap-green | 229.8±4.27 | 1268.6±18.76 | 1498.4 | 237.4±9.26 | 1379.5±7.19 | 1616.9 | 148.6±0.35 | 834.3±4.31 | 982.9 | 629.9±50.6 | 78.6±4.79 | 4935±149.2 | 98.0±2.9 |
| ThapthimDaeng | 191.2±4.32 | 1395.9±32.99 | 1587.1 | 211.7±11.81 | 1584.9±6.14 | 1796.6 | 163.1±0.23 | 949.8±10.13 | 1112.9 | 664.6±44.5 | 91.9±4.64 | 6497±37.8 | 100.7±10.6 |

## Ascorbic acid contents

The extraction of ascorbic acid in these tomato samples was also 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 concentration at eight concentration levels (analyzed in triplicate). The UV response of ascorbic acid over a concentration range of 100 - 450 µM was linear (y = - 0.0021x + 1.08) with a regression coefficient ($R^2$) of 0.9979 (data not shown). Table 2 illustrates that the amount of ascorbic acid was founded in the range from 458.8 ±7.8 to 664.6 ±44.5 µmol/100 g DW. Ascorbic acid content in Thapthim Daeng variety was higher than other varieties, while that of the Mo Kho 40 sample gave the lowest. In other were discovered within this range (Table 2). To evaluate the effect of the sample matrix on the accuracy of the analysis, taking into account the fact that there is no appropriate reference material containing ascorbic acid in the sample analyzed, a recovery test was carried out. Standard ascorbic acid was added to the tomato samples at the concentration of 200 µM and analyzed in triplicate using the extraction methods evaluated in this study. The percentage recoveries of ascorbic acid are also set out in Table 2. Their mean recovery values of ten tomato varieties were in the range between 72.5 ±1.75 and 94.8 ±6.64%. The results, this extraction method was acceptable for analyzing the ascorbic acid contents in the tomato samples.

## Total phenolic Contents

A detailed study was conducted on the contents of phenolic compounds determined by the Folin-Ciocalteu reagent. The calibration curve of standard gallic acid over a concentration range of 100-800 µM was linear (y = 0.0010x – 0.0086) with a regression coefficient ($R^2$) of 0.9979 (data not shown). The concentration of total extractable phenolics in tomato samples of ten varieties examined ranged from 4935 ±149.2 to 6671 ±114.9 µmol GAE/100 g DW. The highest total phenolics were obtained for Lai Kho Red variety, substantially lower for Mani Thapthim and the lowest for Tha-ap-green. Percentage recoveries were also set out in Table 2. Their mean recovery values of ten tomato varieties were in the range between 73.9 ±7.7% and 102.2 ±6.7%. The results, this extraction method was acceptable for analyzing the ascorbic acid contents in the tomato samples. However, these values are merely 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 (Martínez-Valverde et al., 2002). Genetic factors and growing conditions may play an important role in the formation of secondary metabolites, including phenolic acid (Howard et al., 2003).

A rapid and simple spectrophotometric method for analysis of antioxidant activity was utilized. According to the data obtained from the present study, tomato was found to be effective antioxidant sources as demonstrated by numerous in vitro assays, including DPPH, ABTS and FRAP.These three used methods for the determination of antioxidant activity applied to the same sets of the extracts using identical calibration procedures and common

standard permitted the better comparison of the results. In association with these natural and synthetic antioxidant powers, it is evident that the rank of their relevant antioxidant power can be used to directly focus on the total antioxidant activity of the crude extracts from tomato varieties. Quantification of total phenolic compounds and ascorbic acid are helpful in a thorough evaluation of their antioxidant activity. 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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