Comparative study on antioxidant capacities of twenty Thai plant extracts using conjugated autoxidizable triene (CAT) assay and conventional methods

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Abstract

Most of the methods for determining antioxidant capacity such as total phenolic content (TPC), ABTS radical decolorization (ABTS), ferric reducing antioxidant power (FRAP) and oxygen radical absorbance capacity (ORAC) assays are usually performed in homogeneous systems. However, the complexity of food matrices is the important factor influencing on the antioxidative performance of substances. Therefore, the conjugated autoxidizable triene (CAT) assay has been developed to investigate the antioxidant capacity in heterogeneous systems e.g. oil-in-water (O/W) emulsions, which could be more relevant to food systems. In this study, antioxidant capacities of twenty ethanolic Thai plant extracts were compared using conventional methods (TPC, ABTS, FRAP and ORAC) and the CAT assay. The results showed that green tea (*Camellia sinensis)* extract exhibited the highest TPC, ABTS and FRAP values, while Sappan *(Caesalpinia sappan)* extract showed the predominant values of ORAC and CAT. The relationships among all tested methods were studied by principle component analysis (PCA). Our study showed that the PC1 was strongly correlated to TPC, ABTS, and FRAP, while PC2 was mainly linked to ORAC and CAT. However, our result suggested that the CAT assay was not strongly related to other methods. In summary, compared to other conventional methods, antioxidants may perform differently in the CAT assay, probably due to complexity of the test accounting not only for antioxidant capacity, but also distribution and/or location of antioxidants.

Keywords: conjugated autoxidizable triene, heterogeneous system, antioxidant capacities

Introduction

Antioxidative performance of substances was generally reported as their ability to scavenge free radicals. The methods relating to free radical scavenging activity such as total phenolic content (TPC), ABTS radical cation decolorization (ABTS), ferric reducing antioxidant power (FRAP) and oxygen radical absorbance capacity (ORAC) have been developed and widely used due to their simplicity. However, these methods performed in homogeneous solution might not be able to predict the antioxidant ability of compounds in food systems (Alamed, Chaiyasit et al. 2009). The conjugated autoxidizable triene (CAT) assay has been developed by Laguerre and co-workers (Laguerre et al. 2008). The conjugated triene triacylglycerols (TAGs) in tung oil were used as a UV probe, and the absorbance of TAGs was monitored to follow their oxidative degradation in oil-in-water emulsions. However, little is known about the performance of the CAT assay. Therefore, this study was 1.) To evaluate antioxidant capacities of twenty ethanolic Thai plant extracts using conventional methods (TPC, ABTS, FRAP and ORAC) and the CAT assay and 2.) To study relationship between the CAT assay and other conventional methods.

Methodology

Twenty Thai plants were purchased from local market in Thailand, washed with distilled water and dried at 45 °C for 24-48 h. After that, dried plants were ground by milling machine with 1 mm diameter sieve size and kept in vacuum foiled bag until used.

Table 1 Twenty Thai plants

Plant	Part	Sciencetific names
Chiang Da (CD)	Leaf	*Gymnema inodorum*
Horse radish tree (HR)	Leaf	*Moringa oleifera*
Longan (LG)	Leaf	*Dimocarpus longan*
Mango (MG)	Leaf	*Mangifera indica*
Green tea (GT)	Leaf	*Camellia sinensis*
Lime (L)	Leaf	*Citrus aurantiifolia*
Star Gooseberry (SG)	Leaf	*Phyllanthus acidus*
Spiny Bitter Gourd (SBG)	shell	*Momordica cochinchinensis*
Blackboard tree (BB)	leaf	*Alstonia scholaris*
Sappan Tree (SP)	wood	*Caesalpinia sappan*
Lychee (LC)	leaf	*Litchi chinensis*
White mugwort (WM)	leaf	*Artemisia lactiflora*
Roselle (RS)	flower	*Hibiscus sabdariffa*
Kaffir lime (KL)	leaf	*Citrus x hystrix*
Madan (MD)	leaf	*Garcinia schomburgkiana*
Mulberry (MB)	leaf	*Morus alba*
Stevia (SV)	leaf	*Stevia rebaudiana*
Jackfriuit (JF)	leaf	*Artocarpus heterophyllus*
Cassia Tree (CS)	leaf	*Senna siamea*
Safflower (SF)	flower	*Carthamus tinctorius*

Extraction method

Ground dried plant materials as shown in Table 1 were extracted with absolute ethanol according to the method described by Vuong et al. (2013).

Measurement of antioxidant capacities

Total phenolic content (TPC) of plants extracted was determined by the method described by Deetae et al. (2012) with some modifications. The reaction was performed using a 96-well microplate. Sample solution was mixed with Folin-Ciocalteau's reagent (10-time dilution) and sodium carbonate (7.5% w/v). The absorbance of mixture was read at 765 nm. The ABTS assay was performed according to the method described by Deetae et al. (2012). ABTS radical cation ($ABTS^{\bullet+}$) was generated by the reaction between ABTS and potassium persulfate. The $ABTS^{\bullet+}$ solution was mixed with samples, and the absorbance of mixture was then read at 734 nm. The FRAP assay was carried out using a slightly modified method described by Deetae et al. (2012). Shortly, FRAP reagent was freshly prepared. Then, sample was added to FRAP reagent. The absorbance changes in the reaction mixture were recorded at 595 nm. The ORAC assay was conducted according to the method of Nkhili and Brat (2011). Fluorescence intensity of the mixture was read by a fluorescence microplate reader

with an excitation wavelength of 485 nm and an emission wavelength of 520 in kinetic mode at 37℃ for 3 h. The CAT assay was performed as the method reported by Laguerre et al. (2008) with some modification. Tung oil containing conjugated triene triacylglycerol was used as the UV probe for monitoring the oxidation of O/W emulsions. All of the reagents and samples were dissolved and diluted in PBS solution. The data of absorbance at 273 nm was recorded in kinetic mode at 37℃ for 2 h.

Data analysis

All experiments were carried out in triplicate. Relationship among the assays was studied using principle component analysis (PCA).

Results

Antioxidant capacities of twenty Thai plant extracts were determined by using different assays. As illustrated in Table 2, green tea (GT) exhibited the highest TPC, ABTS and FRAP values of 1037.78 mg gallic acid/g extract, 370.11 mg ascorbic acid/g extract and 2014.91 mg ascorbic acid/g extract, respectively, while sappan tree (SP) extract showed the highest ORAC and CAT values of 3591.93 and 405.22 mg trolox/g extract, respectively. The relationship between the CAT assay and other conventional methods to understand the relationship among the tested methods, principle component analysis (PCA) was applied. From five parameters, they could be reduced into 2 components (PC1 and PC2) explaining 95.76% of the total variance. Each component was responsible for 63.39% and 32.37%, respectively (Figure 1). The PC1 was correlated to TPC, ABTS and FRAP values and the PC2 was linked to ORAC and CAT values (Figure 1B).

Table 2 Total phenolic content (TPC), antioxidant activities determined by ABTS, FRAP, ORAC and CAT assays of twenty Thai plant extracts [a]

Plants	mg GA[b] /g extract	mg AA[c]/g extract		mg Trolox/g extract	
	TPC	ABTS	FRAP	ORAC	CAT
Chiang Da (CD)	137.49	28.12	157.27	264.64	42.81
Horse radish tree (HR)	302.74	44.86	252.64	245.56	55.01
Longan (LG)	816.92	330.21	1199.25	544.69	238.43
Mango (MG)	154.76	57.62	283.07	619.51	119.87
Green tea (GT)	1037.78	370.11	2014.91	763.74	341.40
Lime (L)	44.08	16.11	74.15	326.46	41.69
Star Gooseberry (SG)	27.93	10.52	66.67	191.08	22.17
Spiny Bitter Gourd (SBG)	12.40	4.58	58.76	232.51	4.82
Blackboard tree (BB)	51.57	19.10	90.48	302.86	81.38
Sappan Tree (SP)	386.33	142.22	279.88	3591.93	405.22
Lychee (LC)	249.55	92.42	547.77	658.40	395.99
White mugwort (WM)	33.46	12.44	74.65	212.28	62.32
Roselle (RS)	19.18	7.15	42.72	395.92	41.03
Kaffir lime (KL)	41.40	15.39	92.48	411.13	72.44
Madan (MD)	26.14	9.72	58.20	127.17	24.95
Mulberry (MB)	37.92	14.09	85.18	55.71	27.95
Stevia (SV)	92.34	36.74	220.24	1019.43	107.53
Jackfruit (JF)	40.24	14.85	89.46	618.28	61.31
Cassia Tree (CS)	74.05	27.36	164.61	849.79	138.38
Safflower (SF)	76.90	28.83	171.71	165.22	35.86

[a] Values represent the average of triplicate (N=3)
[b] Gallic acid, [c] Ascorbic acid

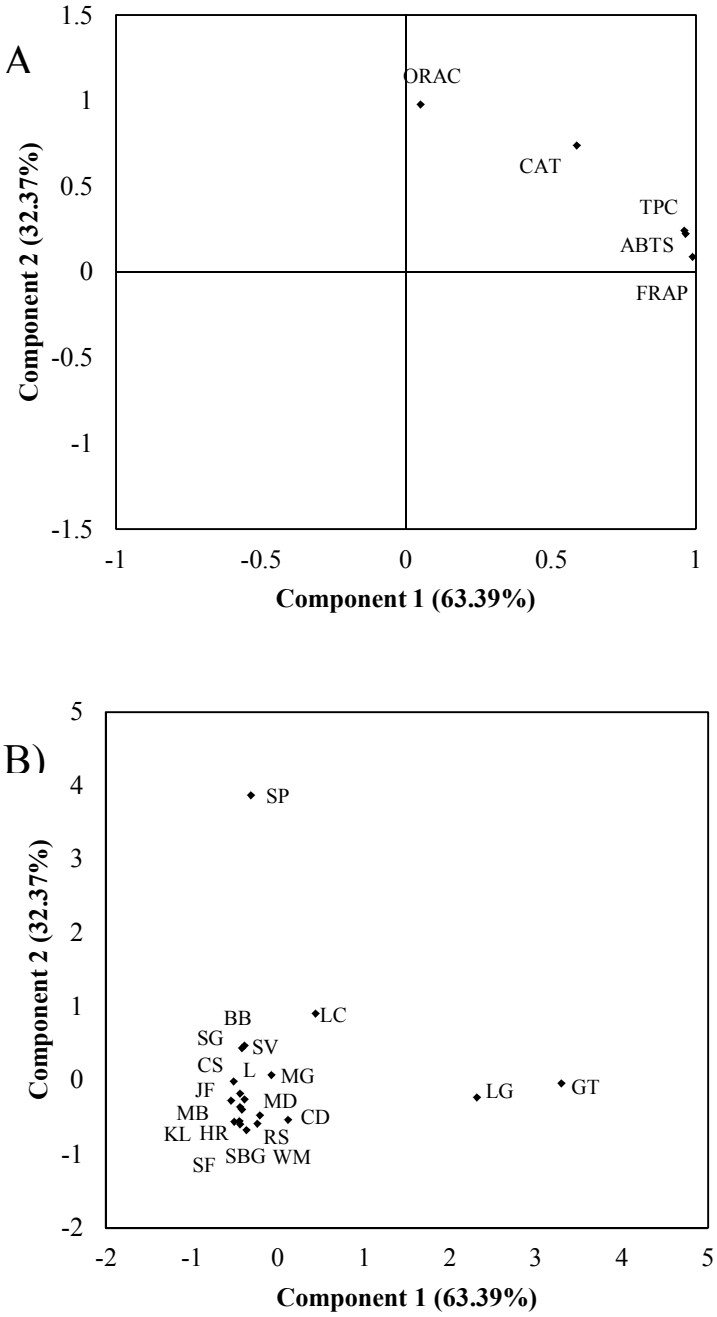

Figure 1 PCA loading plot (A.) and PCA score plot (B.) obtained from total phenolic content (TPC), antioxidant capacities (ABTS, FRAP, ORAC and CAT) of twenty Thai plant extracts.

Discussion

Our results showed that green tea extract exhibited the highest TPC, ABTS and FRAP values. Roedig-Penman and Gordon (1997) reported that green tea extract was a highly effective antioxidant in oil-in-water emulsions at pH 5.5 during prolonged storage (40 days). The main compounds responsible for its antioxidant activity are epigallocatechin-3-gallate (EGCG) and epicatechin-3-gallate (ECG) (Namal Senanayake 2013). Interestingly, sappan extract exhibited the highest ORAC and CAT values in this research. Generally, Sappan tree, distributed and cultivated in Southeast Asia has been used as an herbal medicine for its anti-

inflammatory, anti-influenza, anti-allergic, and neuroprotective activities Cuong et al. 2012. Its antioxidant capacity was also studied by Deetae et al. (2012). It was reported that brazilein is the compound in sappan tree, responsible for high antioxidant activity (Hu et al. 2008). The relationship between all methods was reported as a PCA loading plot (Figure 1A). The PC1 was correlated to TPC, ABTS and FRAP values, while the PC2 was linked to ORAC and CAT values. Our result was consistent with a previous report that the ORAC assay was not strongly related to TPC, ABTS and FRAP assays (Dudonné et al. 2009). However, it should be noted that the CAT assay was not categorized into the same group as other tested methods. It might be due to the complexity of the test accounting for the distribution and location of antioxidant in O/W emulsions, which strongly impacted on antioxidant performance as explained by the polar paradox hypothesis. The polar paradox hypothesis stated that polar antioxidants are more effective in less polar media, such as bulk oils, while nonpolar antioxidants are more effective in relatively more polar media, such as oil-in-water emulsions or liposomes (Shahidi and Zhong 2011). Therefore, the factors could influence on the antioxidants activity are polarity of system media and antioxidants.

Conclusion

Food is a complex system containing various types of organic compound such as carbohydrate, protein, water and lipid. However, most antioxidant activity assays were performed in homogeneous systems, and may not be related to food complex systems. Our results showed that the CAT assay performed differently compared to other conventional methods. These results provided a first insight which the complexity of the CAT assay could differentiate antioxidant performance of plant extracts from other methods. However, to validate the CAT assay link to actual food systems, more experiments on oxidation in oil-in-water emulsions should be further conducted without addition of free radical initiators.

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