RESEARCH ARTICLE

Screening of antioxidant activity of vegetables in Thailand

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Abstract

The methanolic extracts of ten Thai vegetables from the leaves of *Litsea elliptica* Blume, *Callerya atropurpurea* (Wall.) A. M. Schot, Horaphanam (*Limnophila* sp.), *Sesbania grandiflora* (L.) Desv. Young, *Kaempferia galanga* L., *Cucurbita moshata* Decne*, Allophylus cobbe* (L) Raeusch and *Manihot esculenta* (L) Crantz, rhizomes of *Alpinia oxymitrum* K. Schum. and roots of *Zingiber zerumbet* (L.) Smith were investigated for antioxidant activities and total phenolic contents. The antioxidant activities of these extracts were determined by 1,1-diphenyl-2-picryl hydrazyl radical scavenging (DPPH) and ferric-reducing antioxidant power (FRAP) assays. Total phenolic content was evaluated according to the Folin-Ciocalteu procedure. Among the investigated Thai vegetables, the extract from leaves of *L. elliptica* showed the highest free radical scavenging power with IC_{50} value of 4.2 µg/ml and the most potent reducing power of 2.02 mmol $FeSO_4$ per g of extract as well as the highest amount of phenolic contents of 11.059 g of gallic acid per 100 g of extract. The results indicated that the antioxidant activity was correlated well with the total phenolic contents suggesting that the phenolic compounds might play a major contribution to free radicals scavenging activity of the studied plants.

Introduction

Reactive oxygen species (ROS), highly reactive ions or very small molecules, play a vital role in the pathogenesis of various chronic diseases such as diabetes mellitus, cancer, neurodegenerative and cardiovascular diseases. These diseases are the consequences of the imbalances of oxidative state, usually exceeding levels of ROS in the bodies (Casetta *et al*, 2005, Haidara et al, 2006, Valko *et al*, 2006, Halliwell *et al*, 1992). Antioxidative activities of some compounds from plants are attempted to be discovered as they are shown to have potential effects for treatment and/or prevention of various chronic disorders. Phenolic compounds, exerting antioxidative activities, can be found in plants including vegetables (Sies, 1993, Sies, 1997, Ka¨hko¨nen *et a*l, 1999, Sati *et al*, 2010).

Litsea petiolata Hook. f. (family Lauraceae), a medium-sized plants natively found in the Southern and Northeastern regions of Thailand, is locally known as "Tham-mung". The leaves possess distinctive unique smell. These are used as an ingredient in a mixed chili powder. No previous report on phar-macological activities of this plant, but the related species, i.e. *Litsea japonica* leaf extract*,* showed anti-oxidative and anti-inflammatory acti-vities (Yoon *et al*, 2010).

Allophylus cobbe L. (family Sapindaceae) can be found in the Southern and Northeastern parts of Thailand, locally known as "Tor-sai". In some local Thai dishes, its fresh shoots are eaten raw. Traditional practitioners in some local Bangladesh employed this plant as anti-feedant, oxytocic and antidiarrheal agents (Jayasinghe *et al*, 2003). Pharmacological studies indicated that the alcoholic extract from leaf of this plant exhibited antimicrobial and showed moderate to strong cytotoxic effects (Islam *et al*, 2012).

Manihot esculenta (L) Crantz (family Euphorbiaceae), a plant crop harvesting for edible elongate roots, has been cultivated throughout Thailand. The local name is "Mun Sam Pa Lung", but the cultivated variety used in this study is named as "Mun Ha Na Tee", which referring to the rapid-to-be-cooked roots within 5 min. According to the abundant of cyanide content in both leaf and root parts, it is recommended to boil before eating in Thai traditional cuisine. Aqueous extract of its leaves has been shown to possess antioxidant and antiradical activity (Tsumbu *et al*, 2011).

Callerya atropurpurea (Wall.) Schot. (family Fabaceae) is locally known as "Sae" and commonly found in the Southern part of Thailand. Its fresh leaves are local vegetables eaten with chili paste. Its leaves contain high levels of proteins and possess anti-lipid peroxidation activity (Fadziliah and Hanum, 1999).

Limnophila sp*.* (family Scrophulariaceae) is found throughout Thailand and locally known as "Horapanum". It is eaten fresh as vegetables served with some local chili pastes. The leaves contain essential and volatile oils with a unique pleasant smell. To date, there is no pharmacological report on this plant but a plant in the related species, *Limnophila aromatica*, has been shown to possess antioxidant activity (Tangkanakul *et al*, 2006).

Sesbania grandiflora (L.) Desv, (family Fabaceae) is generally found in most of the regions of the country and used in various Thai dishes. Its local name is "Kae". Cooked flowers and leaves are incorporated in local dishes. Pharmacological studies

showed that its leaves possess antiurolithiatic and antioxidant activities (Doddola *et al*, 2008) with hepatoprotective actions (Pari and Uma, 2003).

Kaempferia galanga L., *Alpinia oxymitrum* K. Schum. and *Zingiber zerumbet* (L.) Smith belong to the ginger family (family Zingiberaceae) which can be generally found in the country. The local names of *K. galanga*, *A. oxymitrum* and *Z. zerumbet* are "Pro-hom", "Raew-hom" and "Kratue", respectively. Leaves of *K. galanga*, as vegetables in hot curry dishes, have been shown to possess anti-inflammatory (Sulaiman *et al*, 2008) and antioxidant (Chan *et al,* 2009, Mustafa *et al,* 2010). Rhizomes and roots of *A. oxymitrum and Z. zerumbet*, used in Thai dishes, are not well pharmacologically documented. However, rhizomes of *Curcuma longa* and *Z. cassumunar* which belong to the same family, were shown to have antioxidant activities (Jitoe, 1994, Nagano, 1997, Miquel *et al,* 2002, Iqbal et al, 2003, Eybl *et al*, 2004).

Cucurbita moschata Decne (family Cucurbitaceae) is commonly known as pumpkin. Inner part of its fruits is generally used in multi-cultural dishes and has been shown to have hepato-protective activity (Shayesteh *et al*, 2012). The leaves and young twigs are favorite in Thai cuisine but neither pharmacological activity nor chemical component was reported.

The purpose of this study was to evaluate antioxidant activities of the selected plants used in Thai dishes, mostly as vegetables. Most of the selected plants have not been well documented or yet been investigated.

Materials and Methods

Materials: Plant samples were collected from local food markets and immediately transported to the laboratory with tender care while carrying. The plants samples included leaves of *L. elliptica* Blume, *C. atro-purpurea* (Wall.) A. M. Schot, Horaphanam (*Limnophila* sp.), *S. grandiflora* (L.) Desv. Young, *K. galanga* L., *C. moshata* Decne*, A.* (L) Raeusch and *Manihot esculenta* (L) Crantz, rhizomes of *A. oxymitrum* K. Schum. and roots of *Z. zerumbet* (L.).

Chemicals used were 2,2-diphenyl-1-picrylhydrazyl (DPPH) from Fluka (U.S.A.), 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) from Aldrich (Germany), ferric chloride hexa-hydrate from Aldrich (Germany), Ascorbic acid (vitamin C) from Fluka (U.S.A.) and Trolox from Fluka (U.S.A.).

Extraction: Each fresh plant part was chopped into small pieces and macerated with 80% methanol for 7 days. After filtering and the filtrates were evaporated with rotary evaporator to obtain the dry crude extracts.

Free radical scavenging by DPPH: The modified method from that described by Brand-Williams *et al* (1995) was performed. The anti-oxidant activity of each extract and standard solutions (vitamin C and trolox) were determined based on the radical scavenging ability in reacting with a stable DPPH free radical. In 96-well microplates, 100 µl each of the extracts or the standards was mixed with 100 µl of 0.2mM of DPPH reagent in absolute methanol solution. After incubation at 37^{o}C for 30 min, the absorbance of the reactants was measured at 595 nm using a microplate reader.

Reducing power assay: The method used in this study was modified from that described by Omidreza *et a*l (2005). In preparing the FRAP solution, 10 ml of 300 mM acetate buffer was mixed with 1 ml of 20 mM ferric chloride hexahydrate and 1 ml

of 10 mM 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ). In 96-well microplates, 25 µl of the sample at 1 mg/ml was placed in triplicate. FRAP solution (175 µl) was added to samples, while the same volume of acetate buffer was added to samples for blank. After incubation at 37^{o}C for 1 min, the absorbance of the reactant was measured at 595 nm by the microplate reader.

Determination of total phenolic compounds: The content of total phenolic compounds was determined using Folin-Ciocalteu procedure as described by Pothitirat *et al (*2009) with slight modification. One fifth ml of each sample at 1 mg/ml was mixed with 0.5 ml of 1:10 Folin-Ciocalteu reagent and 0.8 ml of 7.5% w/v sodium bicarbonate solution. The mixture was kept at room temperature for 30 min with intermittent shaking. The absorbance of the mixture was measured at 765 nm using a UV–visible spectrophotometer. The content of total phenolic compounds was calculated as g of gallic acid equivalence (GAE) per 100 g of the extract.

Results and Discussion

From the DPPH assay, results in Table 1 shows that the extract of *L. elliptica* leaves possessed the highest activity. Its IC_{50} was 4.2 µg/ml which was lower than the IC_{50} of vitamin C (8.0 µg/ml) and trolox (6.5 µg/ml) indicating this extract had higher potency than these two popularly used antioxidants. For the antioxidant potency of other Thai vegetable extracts regarding to the criteria suggested by Cervantes-Cervantes (2005) including *A. cobbe* and *M. esculenta* with high potency*, A. oxymitrum* with moderate potency, and *Limnophila* sp*.* with low potency. The IC_{50} of these extracts was 20.0, 28.3, 78.5 and 160.5 µg/ml, respectively. The IC_{50} of the rest was all over 300 µg/ml.

Table 1 C_{50} of Thai vegetable extracts tested by DPPH Method

Sample	**IC_{50}(µg/ml)**	**Potency ***
Litsea elliptica (Thammung)	4.2	High
Allophylus cobbe (Torsai)	20.0	High
Manihot esculenta (Mun ha natee)	28.3	High
Alpinia oxymitrum (Raewhom)	78.5	Moderate
Limnophila sp. (Horaphanam)	160.5	Low
Kaempferia galanga (Prohom)	321.9	-
Cucurbita moschata (Pumpkin)	386.1	-
Callerya atropurpurea (Sae)	460.5	-
Sesbania grandiflora (Kae)	1520.8	-
Zingiber zerumbet (Kratue)	1659.7	-
Standard VitC	8.0	High
Standard Trolox	6.5	High

***** Cervantes-Cervantes (2005)

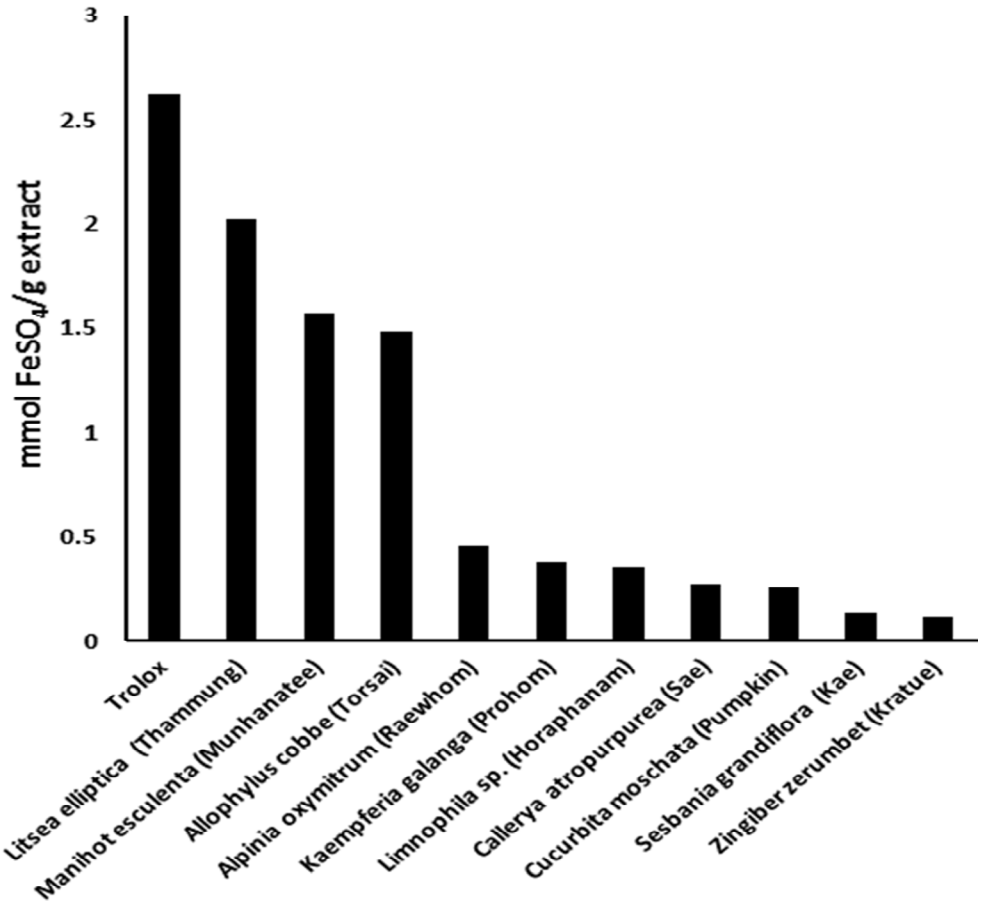

Figure 1 FRAP value (Reducing power assay) of Thai vegetable extracts

The FRAP assay also showed that the extract of L. elliptica leaves provided the highest antioxidant activity (2.02 mmol $FeSO_4$/g of extract) as shown in Figure 1. This extract correlated well with the total phenolic content. It was present with the highest amount of phenolic compounds (11.059 g of gallic acid /100 g of extract) in comparison to other Thai vegetable extracts in this study as seen in Figure 2.

The overall studies herein suggest that the methanolic extract from leaves of *L. elliptica* possessed the highest antioxidant potential. The results from the test on scavenging activity by DPPH assay were in line with the reducing power tested by FRAP assay. Additionally, the total phenolic content of this extract was highest in comparison to other Thai vegetable extracts. Based on the criteria suggested by Cervantes-Cervantes (2005), the extracts from leaves of *A. cobbe and M. esculenta* also showed appreciably high antioxidant potentials as the IC_{50} of these extracts were lower than 50 µg/ml.

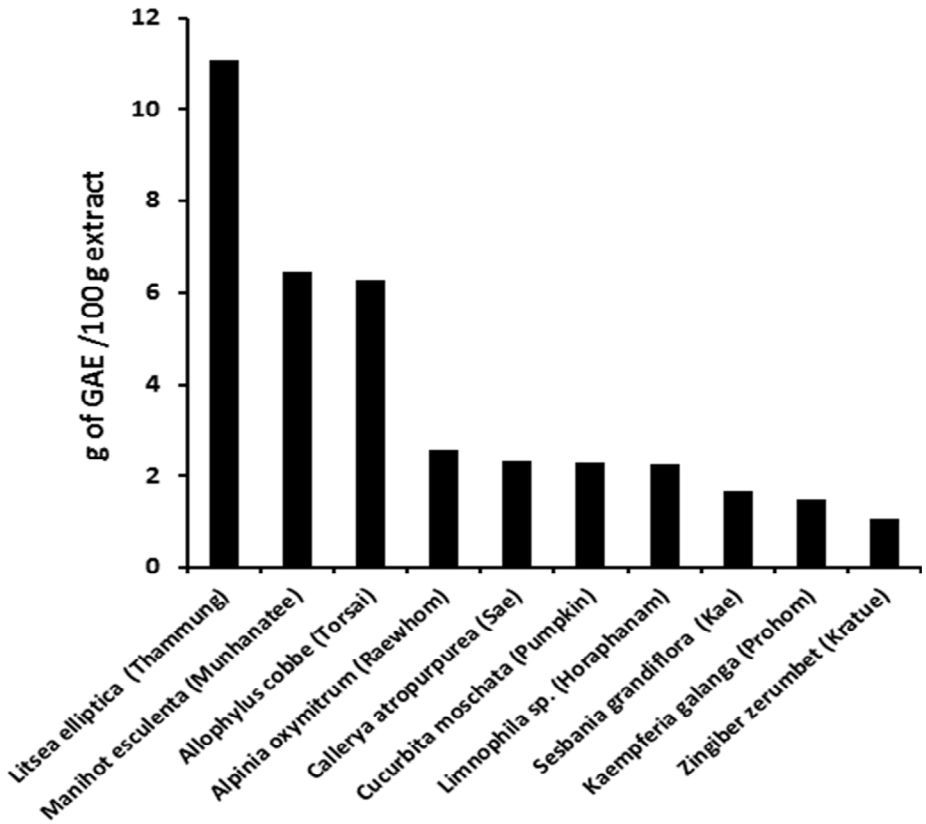

Figure 2 Total phenolic content of the crude extracts by Folin-Ciocalteu assay.

Conclusion

The methanolic extracts of leaves of *L. elliptica*, *A. cobbe and M. esculenta* showed the appreciably high antioxidant potentials. These plants are all traditionally consumed as vegetables in Thai cuisine, some of which having been utilized as herbs in Thai folk medicines. Thus, these Thai vegetables are likely to be further investigated to strongly confirm its benefits for health in preventing any diseases caused by oxidative stress or free radicals.

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