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Simple extraction for the scanning of antioxidant activity of vegetables and fruits in Buriram, Thailand by DPPH, ABTS and FRAP assays

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Abstract

This research examines the process to extract antioxidants from 12 plants in Buriram, Thailand via ethanol solution and scanning antioxidant activity by using DPPH, ABTS and FRAP assays. The suitable conditions for simple extraction are: 2 grams of dried sample and 30 mL of 75% ethanol at room temperature. Antioxidant activity showed that the highest inhibition percentage of about 90% was obtained from Makok, Matum Khaek, Ta Khro and Som Kung. The inhibition percentage about 60-80% were obtained in Phak Chi Farang, Saranae, Mara Khee Nok, Phak Plang, Phak Khom, A-ngun Pa and Phak Paew, respectively. Pro Hom presented the lowest antioxidant activity of 23% as indicated by the DPPH and ABTS assays. The FRAP assay presented similar patterns of antioxidant activities as the DPPH and ABTS assays.

Keywords: Antioxidant activity; Edible plant; Vegetables; Fruits

1. Introduction

Thailand is located in a tropical rainforest zone and is home to a great diversity of naturally grown plants produced without chemical substances, i.e., organically grown foods. Local plants traditionally have been an important food source for indigenous people living in and around forested areas [1]. In every Thai cuisine includes a variety of naturally grown fruits and vegetables as required by the menu. Thai fruits and vegetables are cooked in various ways such as frying, boiling and broiling [2]. Furthermore, a healthy diet should include vegetables and fruits that provide sources of dietary fiber, minerals, vitamins and bioactive compounds, which are major sources of antioxidants [1 – 3]. Moreover, epidemiological data suggests that consuming diets rich in fruits and vegetables can reduce the risk of various chronic diseases in humans [4 – 6].

Dietary antioxidants play a crucial role in delaying or preventing the oxidation of lipids or other cellular compounds by inhibiting the initiation or perpetuation of oxidative chain reactions which can prevent, or repair damage done to the body's cells by oxygen [7 – 8]. Thus, there has been increased interest in finding natural antioxidants from plant materials to replace synthetic compounds [9]. Nevertheless, the antioxidant phytochemicals from plants have been reported to inhibit the propagation of free radical reactions to protect the human body from disease [7].

Extraction methods have been widely studied, being predominantly obtained by several solvent extraction methods using water, methanol, ethyl acetate and hexane under different conditions of time and temperature [10]. Various novel extraction techniques, such as ultrasonic-assisted extraction (UAE), enzyme-assisted extraction (EAE) and ultrasonic-enzyme-assisted extraction (UEAE) currently have been used to extract antioxidants from plants. However, when energy consumption,

technical requirements and extraction costs are considered, each extraction technology possesses advantages and disadvantages [11].

Natural antioxidants occur in all higher plants and in all parts of the plant (wood, bark, stems, pods, leaves, fruit, roots, flowers, pollen and seeds) [9]. Since, plants are prolific in nature. Consequently, easy and convenient methods of scanning antioxidants in plants are important. Furthermore, when studying several plant cases at the same time, the extraction methods and technical requires have proved to be expensive while incurring increased energy consumption with high costs. In the present study, 12 edible plant species of vegetables and fruits were collected in Buriram, Thailand. The simple extraction from relevant parts such as leaves, stems, fruit pulp and fruit peels were evaluated for scanning and comparison of their antioxidant activities by DPPH, ABTS and FRAP assays. Simple extraction is a low-cost method and reduces energy consumption during extraction with advanced instruments.

2. Materials and Methods

Chemicals and Instrument

All chemicals and reagents used were of analytical or HPLC grade. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was obtained from Aldrich (Germany). 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) was obtained from Aldrich (USA). 6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox) was obtained from Aldrich (Switzerland). Ethanol and hydrochloric acid were obtained from RCI Labscan Limited (Thailand). 2,4,6-Tri (2-pyridyl)-s-triazine (TPTZ) was obtained from Aldrich (Switzerland). Iron (II) sulfate was purchased from Ajax Finechem (Australia). Iron (III) chloride hexahydrate was purchased from Riedel-deHaën (Germany). UV-Vis Spectrometer instrument was an Lambda 12 (Perkin-Elmer, USA)

Plant material

Twelve plant species (9 vegetables: *Kaempferia galanga* L., *Spondias pinnata*, *Polygonum odoratum* Lour., *Schinus terebinthifolius* Raddi, *Eryngium foetidum* L., *Mentha cordifolia* Opiz., *Basella rubra* L., *Momordica charantia* L. and *Amaranthus viridis* L. 3 fruits: *Schleichera oleosa* (Lour.) Oken., *Ampelocissus martinii* Planch. and *Ampelocissus arachnoidea* (Hassk.) Planch.) were collected between April and August (2017) from Buriram, Thailand (Fig. 1). Certain plants are not indigenous to the area but grow wildly in Thailand. The plant collection samples were different. Certain plants were harvested from gardens and forests; others were purchased from local markets, as shown in Table 1. The relevant parts of plants were washed thoroughly with tap water to exclude contamination from surfaces prior to cutting into small pieces. They were dried at 60 °C in a hot air oven, ground into fine powder and stored at 4 °C prior to extraction.

Table 1 The detail of 12 plant species used in this study.

General name	Scientific name	Edible portion	Part used
Pro Hom	*Kaempferia galanga* L.	leaf, shoot	Leaf
Makok	*Spondias pinnata*	leaf, fruit	Leaf
Phak Paew	*Polygonum odoratum* Lour.	leaf, young stem	Leaf
Matum Khaek	*Schinus terebinthifolius* Raddi	Leaf	Leaf
Phak Chi Farang	*Eryngium foetidum* L.	Leaf	Leaf
Saranae	*Mentha cordifolia* Opiz.	leaf, young stem	Leaf

Table 1 (Cont.)

General name	Scientific name	Edible portion	Part used
Phak Plang	*Basella rubra* L.	leaf, young stem	Leaf
Mara Khee Nok	*Momordica charantia* L.	leaf, fruit, young stem	Leaf
Phak Khom	*Amaranthus viridis* L.	leaf, young stem	Leaf
Ta Khro	*Schleichera oleosa* (Lour.) Oken.	Fruit	Fruit
A-ngun Pa	*Ampelocissus martinii* Planch.	Fruit	Fruit
Som Kung	*Ampelocissus arachnoidea* (Hassk.) Planch.	Fruit	Fruit

Fig. 1 Scientific names [12] and picture of nine vegetables and three fruits from Buriram, Thailand.

Preparation of plant extracts

Dried vegetables and fruits were ground to a fine powder. Ethanolic extract was obtained by soaking exact weights of 1, 2, 3 and 5 grams with different volumes (of 10, 20, 30 and 50 mL) of 75% ethanol solution at room temperature for 3 hours. Each sample was extracted twice with the same volume of solvents. The mixture was filtered and stored in a refrigerator at 4 °C. These ethanol extracts were subjected to DPPH, ABTS and FRAP assays for comparison of their antioxidant activities.

DPPH radical scavenging assay

The DPPH assay was adapted from Tantiphaipunwong and Jaikeandee [13]. The antioxidant activities of plant extracts and Trolox standard solution were measured in terms of hydrogen donating or radical scavenging ability. Then, 4,500 µl of 80 µM DPPH ethanol solution was added to 500 µl of plant extracts. The reaction mixture was thoroughly mixed and kept in the dark at room temperature for 30 minutes. After that, the absorbance was recorded at 517 nm by UV-Vis spectrometer. The percentage of DPPH radical scavenging activity (%inhibition) of each plant extract was calculated using the (1) formula:

$$\% \text{ inhibition} = [\text{Abs control} - \text{Abs sample}/\text{Abs control}] \times 100 \quad (1)$$

ABTS radical decolorization assay

The ABTS assay was adapted from Tachakittirungrod [7]. Briefly, the ABTS free radical cation (a positively charge ion) solution was prepared by reacting ABTS solution (7 mM) with 2.45 mM potassium persulfate ($K_2S_2O_8$). The mixture was allowed to stand for 16 hours in the dark at room temperature. The solution was diluted with a phosphate buffer (pH 7.4) to obtain an absorbance value of 0.70 ± 0.02 units at 734 nm. Then, 4,500 µl of ethanolic extract from each sample was added to 10 mL of ABTS free radical cation solution. The absorbance, monitored for 6 minutes, was measured UV-Vis spectrometer at 734 nm. The free radical scavenging activity of each sample was expressed as an inhibition percentage, which was obtained by comparing the absorbance change at 734 nm in the reaction mixture containing plant extracts and Trolox.

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was adapted from Tachakittirungrod [7]. Briefly, the FRAP reagent contained 10 mL of 10 mM TPTZ solution in 40 mM HCl plus 10 mL of 20 mM $FeCl_3.6H_2O$ and 100 mL of 0.3 M acetate buffer (pH 3.6) was freshly prepared. Then, 4,500 µl of ethanolic extract of each sample was added to 5.4 mL of FRAP reagent and kept in the dark at room temperature for 30 minutes. The absorption of the reaction mixture was measured at 596 nm by a UV-Vis spectrophotometer. Ethanolic solutions of Fe (II) concentration, in the range of 1 – 25 µM ($FeSO_4$), were used as a calibration curve. The reducing power was expressed as equivalent concentration (EC).

3. Results and Discussion

Twelve plant species of selected plants that people consume from local markets were identified. Certain plants might grow during all seasons and some plants are found in some regions of Thailand. However, most of the plants may be purchased for meal preparation year-round. Table 1 showed the general names, scientific names, edible portions and plant parts used from selected local plants. In addition, certain plants that local people consumed in fresh form are (*Spondias pinnata*, *Polygonum odoratum* Lour., *Schinus terebinthifolius* Raddi, *Eryngium foetidum* L., *Mentha cordifolia* Opiz., *Ampelocissus martinii* Planch. and *Ampelocissus arachnoidea* (Hassk.) Planch.) and certain plants that local people consumed in boiling form are (*Kaempferia galanga* L., *Basella rubra* L., *Momordica charantia* L. and *Amaranthus viridis* L.). The objective for boiling some vegetables in Thai cuisine seeks to diminish some smells or increasing a soft texture. Moreover, in traditional boiling vegetables

used less time and salt was used to preserve their color [2].

Some plants do not require a complicated extraction process to investigate antioxidant activity. The results of this study can be used as an alternative to reduce time and cost of basic plant scanning with antioxidants. The optimal conditions for dried powder from each plant sample showed that 2 grams of dried sample were extracted with 30 mL of 75% ethanol solution at room temperature. Each sample was extracted twice with the same volume of solvents. The mixture was filtered and combined for antioxidant activity methods. The antioxidant activity obtained by the simple extraction method is presented in Table 2. Furthermore, the accurate weight of 1 gram with different volumes of 10, 20, 30 and 50 mL and 2 grams in volumes of 10, 20 and 50 mL of 75% ethanol solution at room temperature for 3 hours, resulted in decreased antioxidant activity. However, when the extraction process using 3 and 5 grams with different volumes of 10, 20, 30 and 50 mL of 75% ethanol solution at room temperature for 3 hours, antioxidant activity was not different from the extraction with 2 grams with 30 mL.

Several methods have been used for evaluation of the antioxidant activity of plants. Antioxidant capacities are influenced by many factors; and therefore, cannot be fully described by a single method [13 – 14]: for example, the ferric thiocyanate (FTC) method, total phenolic content, total flavonoid content, determination of lion binding capacity, determination of lipid peroxidation and DNA damage test by comet assay. Such methods require multiple steps or require the use of highly skilled testers to obtain accurate test results. However, the most commonly used for antioxidant activity are DPPH radical scavenging [1, 2, 14 – 16], ABTS decolorization [7, 16, 17] and FRAP assays [1, 2, 5, 7, 14, 16, 18]. DPPH radical scavenging is suitable for solvent extracts and as a rapid analysis which can be applied for monitoring the activity of numerous samples over a limited period of time. Moreover, it is reproducible and strongly correlated with phenolic compounds [15]. ABTS decolorization is an excellent method for determining the antioxidant activity of a broad diversity of substances, such as hydrogen-donating antioxidants or scavengers of aqueous phase radicals and of chain-breaking antioxidants or scavengers of lipid peroxyl radicals. FRAP assay indicates that the antioxidant compounds are electron donors and can reduce the oxidized intermediates of the lipid peroxidation process [7].

In this study, the antioxidant activity of 12 plant species (9 vegetables and 3 fruits) were determined by DPPH radical scavenging, ABTS radical decolorization and Ferric reducing antioxidant power (FRAP) assays. The corresponding increase or decrease of the absorbance at a given wavelength is related to the concentration of antioxidant in the 12 plant species. The results are summarized in Table 2. Ethanolic extracts of all plants showed antioxidant activity in conjunction with inhibition percentages displayed by DPPH radical scavenging and ABTS radical decolorization analyses. The highest inhibition percentages about 90% were obtained in ethanolic extracts of *Spondias pinnata*, *Schinus terebinthifolius* Raddi, *Schleichera oleosa* (Lour.) Oken. and *Ampelocissus arachnoidea* (Hassk.) Planch. In addition, the inhibition percentage of about 60-80% was obtained in *Eryngium foetidum* L., *Mentha cordifolia* Opiz., *Momordica charantia* L., *Basella rubra* L., *Amaranthus viridis* L., *Ampelocissus martinii* Planch. and *Polygonum odoratum* Lour., respectively. The lowest antioxidant activity was presented in *Kaempferia galanga* L. (23%inhibition). Furthermore, the total antioxidant power was measured using the Ferric reducing antioxidant power (FPAP) assay. *Schleichera oleosa* (Lour.) Oken. contained the highest antioxidant activity with 1.86 ± 0.06 mM $FeSO_4$ by FRAP assay, while the antioxidant activity using the FRAP assay in *Kaempferia galanga* L. was the lowest (0.12 ± 0.03 mM $FeSO_4$). In addition, the results showed that during the first 5 minutes, the reactions of DPPH radical with ethanolic extracts of the tested antioxidants sample compared with reference antioxidants (Trolox) occurred rapidly and reached a steady state within 10 minutes (Fig. 2). The results of this study are consistent with many research studies on antioxidants in plants consumed by Thai people, and demonstrates that local fruits or vegetables are popular for cooking and many plants are high in antioxidants [2, 7, 15, 19]. However, the results indicated that

vegetables and fruits have an excellent antioxidant activity compared to the Trolox standard solution (95.67±1.06%inhibition by DPPH assay). Therefore, fruits and vegetables which are naturally found in Thailand can and should be consumed without the need to rely on chemicals. This another excellent method to assimilate antioxidants into the body. These findings are consistent with several research reports showing that many Thai fruits and vegetables have beneficial nutrients and antioxidant activity [1, 2, 5, 7, 9, 14, 15]. Furthermore, recent research indicates that several plants can offer alternative sources of dietary ingredients to promote health and might open promising opportunities for the treatment of a wide range of troublesome diseases, where reactive oxygen species (ROS) are involved. ROS, such as hydroxyl radical (OH), superoxide ion (O_2^-) and hydrogen peroxide (H_2O_2), have been often reported to induce DNA damage, protein carbonylation and lipid peroxidation, causing various chronic health troubles and diseases [20]. With the results of this study, it's possible that these 12 samples may have the ability to act as antioxidants in the above.

Table 2 Antioxidant activity (DPPH, ABTS and FRAP assays) in vegetables and fruits, Buriram, Thailand

Plants	**DPPH (%Inhibition)**	**ABTS (%Inhibition)**	**FRAP (mM $FeSO_4$)**
Kaempferia galanga L.	23.14±2.76	19.79±1.47	0.12±0.03
Spondias pinnata	91.76±0.85	89.28±1.07	1.79±0.07
Polygonum odoratum Lour.	82.79±2.36	82.92±2.00	0.99±0.03
Schinus terebinthifolius Raddi	88.71±1.44	84.87±2.63	1.03±0.04
Eryngium foetidum L.	61.51±1.56	54.19±3.34	0.78±0.03
Mentha cordifolia Opiz.	66.88±2.62	69.47±0.97	0.60±0.04
Basella rubra L.	68.97±1.19	65.04±3.89	0.77±0.03
Momordica charantia L.	67.10±4.57	72.70±2.42	1.01±0.04
Amaranthus viridis L.	71.19±3.51	72.15±2.22	0.87±0.04
Schleichera oleosa (Lour.) Oken.	91.86±2.51	91.77±1.45	1.86±0.06
Ampelocissus martinii Planch.	71.30±6.82	72.78±3.30	0.75±0.09
Ampelocissus arachnoidea (Hassk.) Planch.	88.98±1.75	90.17±0.93	1.15±0.08
Trolox		95.67±1.06	

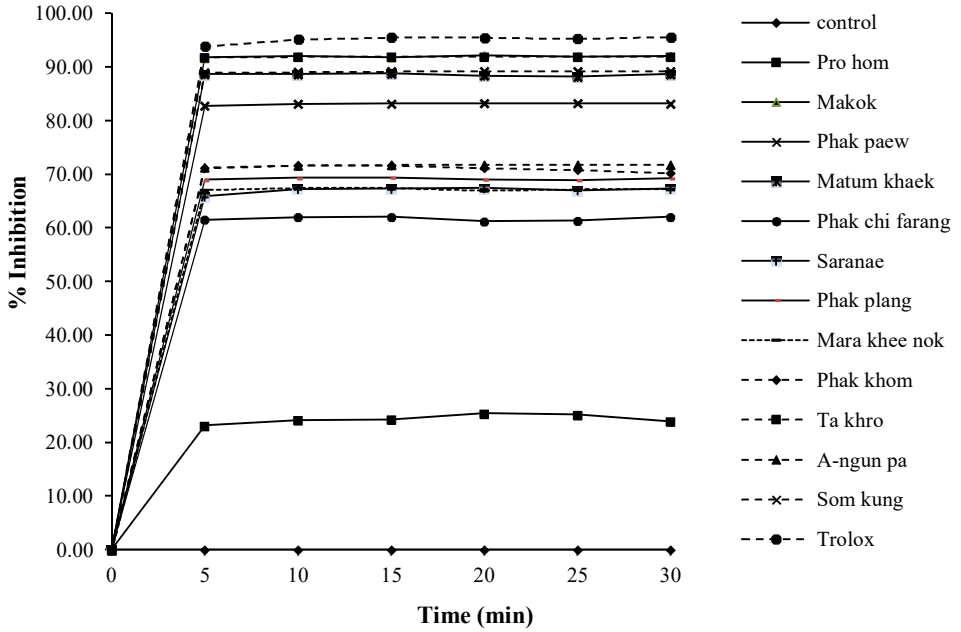

Fig. 2 Kinetic behaviors of radical antioxidant activity of vegetables, fruits, reference antioxidants trolox and control.

4. Conclusion

The result of this study demonstrated that the 9 vegetables and 3 fruits in Buriram, Thailand were extracted with simple extraction which produced a difference of antioxidant activity. *Schleichera oleosa* (Lour.) Oken. indicated the highest antioxidant activity. The antioxidant activity of plant extracts were attributed to radical scavenging and reducing mechanisms by DPPH, ABTS and FRAP assays. However, it was suggested that the 12 plant species may become new important sources of natural antioxidants. Moreover, local Thai people eat most fruits and vegetables in the natural state or blanched, so antioxidant activity is not destroyed during the cooking process. This study can be used expand the knowledge base for Thai people to choose to consume more local fruits and vegetables in their natural state and may use extracts from these plants to study medicinal in the further.

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