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# Antioxidant capacity and phenolic content of some Thai culinary plants

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**Abstract:** The antioxidant capacity of red and green bird chili (*Capsicum frutescens* Linn.), red and green chili spur pepper (*Capsicum annuum* Linn. var. acuminatum Fingerh.), red and white holy basil (*Ocimum sanctum* Linn.), garlic (*Allium sativum* Linn.), and pumpkin (*Cucurbita moschata* Decne.), which have been normally used in Thailand as food ingredients, was estimated by three different methods: ferric reducing antioxidant power (FRAP) assay, improved ABTS radical cation decolorization assay, and DPPH free radical scavenging assay. Additionally, their total phenolic contents were analyzed by Folin-Ciocalteau micro method. The result showed that red holy basil and red bird chili seemed to be better sources of antioxidant compounds, followed by green bird chili and red chili spur pepper, white holy basil, green chili spur pepper, garlic and pumpkin, respectively. For results of total phenolic content analysis, they were significantly related to those of FRAP ($r = 0.825$), ABTS ($r = 0.973$) and DPPH ($r = 0.683$).

**Keywords**: antioxidant capacity, total phenolic content, chili, holy basil, garlic, pumpkin.

## Introduction

Most of culinary plants in Thailand, such as chilies, garlic, holy basil, pumpkin, etc., are also used as medicinal plants. Chilies have been recognized by many cultures around the world for their medicinal qualities. When chilies are eaten, capsaicin stimulates the release of endorphins, which give a pleasurable feeling. Moreover, chilies are believed to increase circulation, relieve rheumatic pain,

treat mouth sores and infected wounds, reduce blood clots and aid digestion by stimulating saliva and gastric juice flow [1]. Garlic has a high concentration of sulfur-containing compounds. The thiosulfinates, including allicin, appear to be the active substances in garlic. Allicin is formed when alliin, a sulfur-containing amino acid, comes into contact with the enzyme alliinase when raw garlic is chopped, crushed, or chewed. The antimicrobial, hypolipidemic, antioxidant, and antithrombotic effects that have been attributed to garlic are thought to be related to allicin and other breakdown products [2].

Holy basil has a strong anise-like, slightly musky and lemony taste with a camphoraceous aroma. The dominant aroma component in holy basil is eugenol. This herb also has been used by Asian people in traditional medicine. It is used for most stomach disorders, cramps, diarrhea, headaches, whooping cough and head colds [1]. Pumpkin is a good source of beta – carotene, an important antioxidant, and it is also a good source of vitamin C, another good antioxidant [3].

The objective of this study was to compare the antioxidant capacity of bird chili (green and red), chili spur pepper (green and red), garlic, holy basil (red and white), and pumpkin available from fresh markets in Chiangmai, Thailand, by three different methods, viz. ferric reducing antioxidant power (FRAP) assay, improved ABTS radical cation decolorization assay, and DPPH free radical scavenging assay. The total phenolic contents of the above culinary plants were also estimated and related to their antioxidant capacity results.

## Materials and Methods

### *Chemicals*

Trolox (6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid) [Aldrich], TPTZ (2,4,6-tripyridyl-s-triazine), DPPH (2,2-diphenyl-1-picrylhydrazyl) [Sigma], ABTS (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)), Folin-Ciocalteau phenol reagent, ferric chloride, ferrous sulphate, gallic acid, glacial acetic acid, hydrochloric acid, sodium acetate, potassium persulphate, sodium carbonate, and vitamin C [Fluka], were all of analytical grade.

### *Sample extraction*

Fresh samples of the studied plants were purchased from fresh markets in Chiangmai. Their moisture contents were analyzed by the method of AOAC [4]. The sample extraction method of Leong and Shui [5] was modified for sample preparation. Edible portion of the fresh sample was homogenized using a blender. A mixture of deionized water and 95% ethanol was used as extraction solvent and 95% ethanol was selected for preliminary studies. Two grams of homogenized sample were added with 10 ml of the selected solvent. The extraction was done by using a vortex mixer for 60 seconds. The mixture was filtered and the filtrate was collected and used for FRAP, ABTS, DPPH, and total phenolic content assays. Milton Roy Spectronic 20D+ spectrophotometer was used for all assays.

*Ferric reducing antioxidant power (FRAP) assay*

The FRAP was assessed according to Benzie and Strain [6]. Briefly, 6 ml of working FRAP reagent prepared daily was mixed with 20 - 100 µl of the extract. The absorbance at 593 nm was recorded after 30-min incubation at 37 °C. Absorbance increases were calculated as FRAP values by comparing with standard curves created by vitamin C (0 - 15 µg), and reported as mg vitamin C equivalent per gram of fresh weight.

*ABTS radical cation decolorization assay*

The ABTS method of Re *et al*. [7] was modified. ABTS radical cation ($ABTS^+$) was produced by reacting 7 mM ABTS stock solution with 2.45 mM postassium persulphate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12 - 16 hours before use. The $ABTS^+$ solution was diluted with deionized water and 95 % ethanol (1:1) to an absorbance of 0.70 ($\pm$ 0.02) at 734 nm. The extract (20-100µl) was mixed with 6 ml of diluted $ABTS^+$ solution. The decrease of absorbance was recorded at 1 min after mixing. Absorbance decreases were calculated as ABTS values by comparing with standard curves created by vitamin C (0 - 20 µg), and the results were reported as mg vitamin C equivalent per gram of fresh weight.

*DPPH free radical scavenging activity*

The method of Brand-Williams *et al.* [8] was used with some modifications. DPPH radical solution (0.8 mM) in 95% ethanol was prepared. The extract (100-1000µl) was diluted to 5.4 ml by deionized water and 95 % ethanol (1:1) before 0.6 ml of the DPPH solution was added and mixed. The decrease of absorbance was recorded at 1 min after mixing. Absorbance decreases were calculated as DPPH values by comparing with standard curves created by vitamin C (0 - 40 µg), and the results were reported as mg vitamin C equivalent per gram of fresh weight.

*Total phenolic content*

The Folin-Ciocalteau micro method of Waterhouse [9] was used to estimate total phenolic content (TPC). The extract (60-300 µl) was diluted with deionized water to 4.8 ml, and 300 µl of Folin-Ciocalteau reagent was added and shaken. After 8 min, 900 µl of 20% sodium carbonate solution was added with mixing. The solution was left at 40°C for 30 min before reading the absorbance at 765 nm. Gallic acid (0 - 50 µg) was used as standard, and the results were reported as mg gallic acid equivalent per gram of fresh weight.

*Calculation and Statistical Analysis*

The values of FRAP, ABTS, DPPH, and TPC (mg standard equivalent per gram of fresh weight) were calculated using the equation below:

$$\text{Values of FRAP, ABTS, DPPH, and TPC (mg standard equivalent per gram of fresh weight)} = \frac{[(SA - BA) / (Slope)]\,[10 / U]}{[2]\,[1{,}000]}$$

where: SA = Sample absorbance for FRAP value and TPC or absorbance decrease of sample for ABTS and DPPH values

BA = Blank (no extract) absorbance for FRAP value and TPC or absorbance decrease of blank for ABTS and DPPH values (extract was substituted by deionized water for blank)

Slope = Slope of standard curve

[10 / U] = Total volume of extract (10 ml) / Used volume of extract (ml)

[2] = Weight of used sample (g)

[1,000] = Factor for changing µg to mg.

Each experiment was performed in triplicate on different purchased samples. A randomized complete block design (RCBD) was used. Plants and market purchases served as treatment and block variables, respectively. Mean comparisons were performed by Duncan's new multiple range test (DMRT). The bivariate correlations between all antioxidant capacity assays and total phenolic contents were analyzed.

## Results and Discussion

The moisture content of each plant is shown in Table 1. From preliminary studies, 60% (v/v) of 95% ethanol was selected as extraction solvent for chilies and holy basil, while deionized water and 95% ethanol were chosen for garlic and pumpkin, respectively.

**Table 1** Moisture content of some Thai culinary plants.

| Thai culinary plant | Moisture content (%) |
|---|---|
| Bird chili, green | 78.46 $\pm$ 2.32 |
| Bird chili, red | 67.09 $\pm$ 1.58 |
| Chili spur pepper, green | 90.92 $\pm$ 1.15 |
| Chili spur pepper, red | 84.28 $\pm$ 2.32 |
| Garlic | 68.66 $\pm$ 2.22 |
| Holy basil, red | 86.44 $\pm$ 0.46 |
| Holy basil, white | 85.90 $\pm$ 0.61 |
| Pumpkin | 86.21 $\pm$ 2.15 |

The results of the antioxidant capacity assessment of the studied plants as determined by FRAP, ABTS, and DPPH assays are shown in Table 2. These differences could be explained by different mechanisms of analytical methods. FRAP assay measures the ability to reduce a ferric tripyridyltriazine ($Fe^{3+}$-TPTZ) to a ferrous form ($Fe^{2+}$-TPTZ) of samples [5]. ABTS and DPPH assays are based on the reduction of ABTS [7] and DPPH free radicals [8] of samples, but values from DPPH assay might be lower than those from ABTS assay. Wang *et al*. [10] showed that some compounds which have ABTS scavenging activity may not show DPPH scavenging activity, and Arts *et al.* [11] found that some products of ABTS scavenging reaction may have a higher antioxidant capacity and can further react with remained ABTS radicals.

Table 2 shows that red holy basil had the highest antioxidant capacity analyzed by FRAP assay, followed by red bird chili and white holy basil, green bird chili and red chili spur pepper, green chili spur pepper, pumpkin and garlic, in that order. In ABTS assay, the plants with the highest antioxidant capacity were bird chili (red and green) and red holy basil, followed by red chili spur pepper and white holy basil, green chili spur pepper, garlic, and pumpkin, respectively. However, results of DPPH assay showed that red chili spur pepper had the highest antioxidant capacity, followed by bird chili (red and green) and holy basil (red and white), green chili spur pepper, garlic and pumpkin, respectively. For overall results, it might be concluded that red holy basil and red bird chili were better sources of antioxidant compounds in this study, followed by green bird chili and red chili spur pepper, white holy basil, green chili spur pepper, garlic and pumpkin, respectively. In case of total phenolic content analysis, it was found that red bird chili contained the highest amount of phenolic compounds, followed by green bird chili and red holy basil, red chili spur pepper and white holy basil, green chili spur pepper, garlic and pumpkin, respectively.

**Table 2.** Results of antioxidant capacity and total phenolic content estimation

| Thai culinary plant | FRAP* | ABTS* | DPPH* | TPC** |
|---|---|---|---|---|
| Bird chili, green | 1.68 ± 0.04 $^{c}$ | 6.48 ± 0.88 $^{a}$ | 0.66 ± 0.09 $^{b}$ | 2.83 ± 0.31 $^{b}$ |
| Bird chili, red | 2.34 ± 0.16 $^{b}$ | 6.68 ± 0.84 $^{a}$ | 0.79 ± 0.07 $^{b}$ | 3.48 ± 0.58 $^{a}$ |
| Chili spur pepper, green | 0.61 ± 0.19 $^{d}$ | 1.32 ± 0.05 $^{c}$ | 0.36 ± 0.04 $^{c}$ | 0.85 ± 0.20 $^{d}$ |
| Chili spur pepper, red | 1.56 ± 0.20 $^{c}$ | 3.82 ± 0.37 $^{b}$ | 1.23 ± 0.31 $^{a}$ | 1.85 ± 0.26 $^{c}$ |
| Garlic | 0.14 ± 0.04 $^{e}$ | 1.06 ± 0.11 $^{d}$ | 0.16 ± 0.03 $^{d}$ | 0.41 ± 0.10 $^{e}$ |
| Holy basil, red | 3.35 ± 0.43 $^{a}$ | 5.91 ± 0.49 $^{a}$ | 0.72 ± 0.02 $^{b}$ | 2.65 ± 0.27 $^{b}$ |
| Holy basil, white | 1.99 ± 0.05 $^{b}$ | 4.23 ± 0.19 $^{b}$ | 0.62 ± 0.01 $^{b}$ | 1.78 ± 0.16 $^{c}$ |
| Pumpkin | 0.19 ± 0.03 $^{e}$ | 0.63 ± 0.24 $^{e}$ | 0.11 ± 0.01 $^{d}$ | 0.24 ± 0.05 $^{e}$ |

* mg Vitamin C equivalent / g fresh weight.
** mg Gallic acid equivalent / g fresh weight.
Means (± S.D.) with different superscripts in each column are significantly different ($p \leq 0.05$). Superscript a indicates the group with the highest value, while e belongs to the lowest value group.

The correlations between results of antioxidant capacity and TPC analysis are highly significant ($p<0.01$) as shown in Table 3. These correlations indicate that the phenolic compounds could be the main cause of antioxidant power of all plant samples, in accordance with the previous findings that many phenolic compounds in plants are good sources of natural antioxidants [12, 13]. However, some

coefficients were not high, which may be due to the fact that some natural antioxidants, eg. vitamin C and carotenoids, are not phenolic compounds, and that each antioxidant compound may show different activities with different assays. In this study, the contribution of the phenolic content to ABTS values was greater than to FRAP and DPPH values. Thus, ABTS assay may preferably be a better method to study the antioxidant power which is mainly caused by phenolic compounds, than FRAP and DPPH methods, in that order.

**Table 3.** Bivariate correlation results of three antioxidant capacity assays and total phenolic content from all extracts.

| Results | Correlation coefficient | Sign (2-tailed) |
|---|---|---|
| TPC | 1.000 | - |
| FRAP | 0.825 | < 0.01 |
| ABTS | 0.973 | < 0.01 |
| DPPH | 0.683 | < 0.01 |

## Conclusion

This work has shown that chilies (bird chilies and chili spur peppers) and holy basils (white and red) could be good sources of natural antioxidants in Thai foods. However, they should be used with care because of their hot and pungent flavours. Garlic and pumpkin may be lower in antioxidant power but it is possible to consume them in higher quantity, especially for pumpkin.

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## References and Notes

1. S. R. Uhl, “Spices, seasonings, & flavorings”, Technomic Publishing Co., Lancaster, **2000.**
2. H. P. Koch and L. D. Lawson, “Garlic: the science and therapeutic application of Allium sativum L. and related species.” 2nd Edn., Williams & Wilkins, Baltimore, **1996.**
3. N. R. Farnsworth and N. Bunyapraphatsara, “Thai medicinal plants recommended for primary health care system”, Medicinal Plant Information Center, Bangkok, **1992**.
4. AOAC (Official method of analysis of AOAC international), 16th Edn, The Association of Analytical Chemists, Arlington, **1997**.
5. L. P. Leong and G. Shui, “An investigation of antioxidant capacity of fruits in Singapore markets”, *Food Chem***., 2002**, *76*, 69-75.
6. I. F. F. Benzie, and J. J. Strain, “Ferric reducing / antioxidative power assay: direct measure of total antioxidant activity of biological fluids and modified version of simultaneous measurement of antioxidant power and ascorbic acid concentration”, *Methods Enzymol.*, **1999**, *299*, 15-27.

7. R. Re, N. Pellegrini, A. Rroteggente, A. Pannala, M. Yang, and C. Rice-Evans, "Antioxidant activity applying an improved ABTS radical cation decolorization assay", *Free Radical Bio. Med.*, **1999**, *26*, 1231-37.
8. W. Brand-William, M. Cuelier, and M. E. Berset, "Use of free radical method to evaluate antioxidant activity", *Lebensm. Wiss. U. Technol.*, **1995**, *28*, 25-30.
9. A. Waterhouse, "Folin-Ciocalteau micro method for total phenol in wine.", (no date), retrieved May 4, 2005, from http://waterhouse.ucdavis.edu/phenol/folinmicro.htm.
10. M. F. Wang, Y. Shao, J. G. Li, N. Q. Zhu, M. Rangarajan, E. J. LaVoie, and C. T. Ho, "Antioxidative phenolic compounds from Sage (*Salvia officinalis*)", *J. Agric. Food Chem.*, **1998**, *46*, 4869-73.
11. M. J. T. J. Arts, G. R. M. M. Haenen, H. P. Voss, and A. Bast, "Antioxidant capacity of reaction products limits the applicability of the Trolox equivalent antioxidant capacity (TEAC) assay", *Food Chem. Toxicol.*, **2004**, *42*, 45-49.
12. C. T. Ho, in "Phenolic compounds in food and their effects on health I: analysis, occurrence, & chemistry" (Eds. C. T. Ho, C. Y. Lee, and M. T. Huang), American Chemical Society, New York, **1992,** Ch. 1.
13. M. J. Amiot, A. Fleuriet, V. Cheynier, and J. Nicolas, in "Phytochemistry of fruits and vegetables" (Eds. F. A. Tomas-Barberan and R. J. Robins), Clarendon Press, Oxford, **1997**, Ch. 4.

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