

ANTIOXIDANT AND RADICAL SCAVENGING ACTIVITY OF HERBAL MEDICINE SAMPLES

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Abstract: Herbal medicines compose of natural plant substances to treat and prevent illness. Herbs may be used directly or extracts or they may be used in the production of drugs. The use of herbs to treat disease is almost universal among non-industrialized societies and still popular in Thailand. The knowledge of these medicines are needed to be explored. Hence 5 herbal tonic medicines were evaluated for antioxidant activities by DPPH radical assays. Their total phenolics content was determined by Folin–Ciocalteu’s reagent.

Introduction

Free radical [1] is any atom or molecule that has a single unpaired electron in an outer shell. It is unstable and fast to react with other molecule in the body. For most biological structures, free radical damage is closely associated with oxidative damage. Plants or herbal plants are important source of antioxidant [2-5]. The use of herbs to treat disease is almost universal among non-industrialized societies and still popular in Thailand.

Herbal tonic medicine [4-6], sometimes referred to as herbalism or botanical medicine, is the use of herbs for their therapeutic or medicinal value. An herb is a plant or plant part valued for its medicinal, aromatic or savory qualities. Herb plants produce and contain a variety of chemical substances that act upon the body. Herbal medicine is the oldest form of healthcare known to mankind. Herbs had been used by all cultures throughout history. Many drugs commonly used today are of herbal origin. Some are made from plant extracts; others are synthesized to reproduce a natural plant compound.

Plants (fruits, vegetables, medicinal herbs, etc.) may contain a wide variety of free radical scavenging molecules, such as phenolic compounds (e.g. phenolic acids, flavonoids, quinones, coumarins, lignans, stilbenes, tannins), nitrogen compounds (alkaloids, amines, betalains), vitamins, terpenoids (including carotenoids), and some other endogenous metabolites, which are rich in antioxidant activity [4, 6-10].

Phenolics in vegetables, fruits, spices, and medicinal herbs might prevent cancer through antioxidant action and/or the modulation of several protein functions. Phenolics may inhibit carcinogenesis by affecting the molecular events in the initiation, promotion, and progression stages [7-12].

Cancer prevention and treatment using traditional Chinese medicines have attracted increasing interest [6]. Tea polyphenols and many tannin components were also suggested to be anticarcinogenic [13].

Herbal tonic medicines were contained so many kinds of herb such as the Chinese herb and also Thai herb. Some of the herbal tonic medicines are profit to nurture the body. The knowledge of this medicine needs to be explored. Hence 5 herbal tonic medicines obtained from Chiang Mai markets were evaluated for antioxidant activities by DPPH radical assay. Their total phenolics content by Folin–Ciocalteu’s reagent were also presented. The correlation between antioxidant activity and total phenolic compound were determined using Excel[®].

Materials and Methods

Apparatus and Chemicals: Potassium persulfate was purchased from Ajax Finechem, Australia. 1,1-diphenyl-2-picrylhydrazyl (DPPH), gallic acid, and sodium carbonate (Na₂CO₃) were purchased from Fluka, Germany. Folin–Ciocalteu’s phenol reagent was purchased from Merck, Germany. HPLC grade methanol was purchased from Fisher Scientific UK Limited, UK. Gallic acid (GA) was purchased from Sigma–Aldrich (Steinem, Germany). Ultra-pure water (18 MΩ-cm) obtained from Millipore Milli-Q purification system (Millipore Corp., Bedford, MA, USA). All other chemicals and solvents used were of analytical grade available commercially. The UV–VIS measurements were performed using Hitachi U-2001 spectrophotometer.

Herbal medicine samples: Herbal medicine samples were purchased from Chiang Mai Province, Thailand. Samples were kept refrigerated at 4°C in darkness until ready for analysis. A hundred milliliters of sample was filtered and evaporated under vacuum at 80°C for 15 minutes by a rotary evaporator. Finally, the crude was weighed and reconstructed in 10 mL methanol.

DPPH radical scavenging activity: Radical scavenging activity of sample extracts against stable DPPH (2,2’-diphenyl-2-picrylhydrazyl hydrate, Germany) was determined spectrophotometrically. When DPPH reacts with an antioxidant compound [14], which can donate hydrogen, it is reduced. The changes in color (from deep-violet to light-yellow) were measured at 515 nm on a UV/visible light spectrophotometer. Radical scavenging activity of extracts was measured by slightly modified a method of Brand-Williams, Cuvelier, and Berset. The solution of DPPH in methanol (6x10⁻⁵M) was prepared freshly [15, 16], before UV measurements. Each herbal medicine samples 100 µL was added to 300 µL of

6×10^{-5} mol/L methanolic solution of DPPH. The absorbance [17] at 515 nm was measured using an ultraviolet-visible spectrophotometer after the solution has been allowed to stand in the dark for 30 min at room temperature. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. Absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured daily. Scavenging capacity of the sample was compared to that of control set (methanol, 0% radical scavenging). Radical scavenging activity was calculated by the following equation:

%DPPH radical scavenging activity

$$= 100 \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \quad (1)$$

where: A_{control} = absorption of blank sample (t=0 min);
 A_{sample} = absorption of tested solution (t=15 min).

IC_{50} value, the concentration of sample required for 50% inhibition of DPPH free radical, was determined from the plot between %inhibition and concentration.

Total phenolic content assay: The total phenolic content of the herbal medicine samples was determined according to Folin-Ciocalteu spectrophotometric method [18, 19]. Briefly, 100 μ L of the extraction was mixed with 250 μ L of 10% (v/v) Folin-Ciocalteu's phenol reagent and allowed to react for 10 min. Then, 100 μ L of 7.5% (w/v) Na_2CO_3 solution was added. After incubation for 15 min at 50°C then the absorbance at 760 nm was determined. The measurement was compared to a standard curve of gallic acid (GA) solution and the total phenolic content was expressed as milligrams of gallic acid equivalents (mg GAE/ml). Each sample was done in triplicate.

Results and Discussion

Total antioxidant power of herbal medicine samples measured by DPPH assay: The extracts of the herbal medicine samples were tested for antioxidant activity using the DPPH assay. The IC_{50} values and the total phenolic compound of the antioxidant activity of herbal medicine samples are shown in Table 1. The averaging percentage of radical scavenging and concentration of samples are plotted and shown in Figure 1. The higher concentration is the higher samples percentage inhibition. The IC_{50} of different herbal medicine samples were found in range of $1.00 \times 10^{-3} - 4.77 \times 10^{-3}$ ppm which is narrow distributed antioxidant power. Sample No. 1, the concentration of radical scavenging activity by IC_{50} plotted was found to have the lowest antioxidant activity and sample 4 was found to have the highest antioxidant activity.

Table 1: The IC_{50} value and the total phenolic compound of the antioxidant activity of herbal medicine samples

Sample No.	$IC_{50} \times 10^{-3}$ (ppm)	The total phenolic content (mg GAE/ml)
1	4.77	5.70
2	1.40	2.07
3	1.75	3.80
4	1.00	2.17
5	1.48	3.88

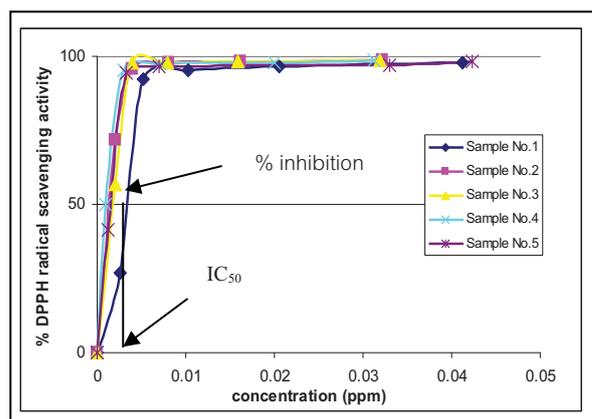


Figure 1. The averaging percentage of DPPH radical scavenging activity of herbal medicine samples

Total phenolic content of the herbal medicine samples: The phenolic contents of methanol extracts were varied from 2.07 to 5.70 mg GAE/ml which is also narrow distributed. For sample No.1, the total phenolic content was found to have the highest value that was the best in powering for antioxidant activity (5.70 mg GAE/ml). The sample No.2, total phenolic content was found to have the lowest value (2.07 mg GAE/ml).

Correlations between total phenolic content and the DPPH radical scavenging activity: The IC_{50} and the total phenolic compound of each samples were shown strongly positive correlation ($r^2 = 0.8795$). Since the IC_{50} was positive correlation to total phenolic content, the antioxidant power of herbal tonic medicines may be caused by the other group of compounds rather than polyphenolic compounds.

Conclusions

The antioxidant activity and total phenolic content maybe used in evaluation of medicine properties. Parameter such as antioxidant power or total phenolic content may be used primarily for testing the quality of herbal medicine. The high and similar results on antioxidant and total phenolic content were observed for all herbal medicine samples. All of the samples have strong antioxidative power on DPPH radicals but low total phenolic content. Hence, the antioxidant power may not cause by only phenolic compounds. Chemical component of herbal medicines have to be

identified and quantified by using the other methods such as HPLC and GC.

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