



## Total antioxidant activity of Thai medicinal plants associated with the treatment of cardiovascular diseases, diabetes and cancers

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### ABSTRACT

Herbs have been used as traditional medicine for centuries and believed to possess antioxidant properties. We screen aqueous extracts from 40 medicinal plants in Thai traditional medicine that believed to reduce the risks of cardiovascular diseases, diabetes and cancers. Total antioxidant activity was measured by Trolox equivalent antioxidant capacity (TEAC) assay. In the plants associated with the treatment of cardiovascular diseases, we found that the total antioxidant activities of *Nelumbo nucifera* Gaertn., *Bixa orellana*, *Michelia alba*, *Mammea siamensis*, and *Michelia champaca* were higher than others. In the plants associated with the treatment of diabetes, we found that total antioxidant activities of *Xylinbaria minutiflora*, *Leptocarpus disjunctus*, *Salacia chinensis*, *Albizia myriophylla* and *Urceola minutiflora* were higher than others. In the plants associated with the treatment of cancers, we found that total antioxidant activities of *Balanophora abbreviata*, *Alpinia allughas*, and *Datura fastuosa* were higher than others. Our results suggest Thai medicinal plants as valuable sources of natural antioxidants.

**Key words:** Antioxidant activity, Thai plants, *Nelumbo nucifera*

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### INTRODUCTION

Plants have played a vital role in maintaining human health and improving the quality of human life for thousands of years. Also there are valuable components of seasonings, beverages, cosmetics, dyes and medicines. The World Health Organization has estimated that 80% of the earth's inhabitants relied on traditional medicine for their primary health care needs, and most of this therapy involved the use of plant extracts or their active components [1]. Self-prescribed herbal preparations are widely used today for a host of common ailments and conditions, such as anxiety, arthritis, cold, cough, constipation, fever, headaches, infection, insomnia, intestinal disorder, premenstrual syndrome, stress, ulcer, and weakness [2].

In Thailand, herbs have been used as food and medicine for centuries. There were believed to possess hypolipidemic, anti-tumor, or immune-stimulating properties. Heart attack and cancer are very common causes of death in Thailand. Recently, phytochemicals in herbs have attracted a great deal of attention mainly concentrated on their role in preventing diseases caused as a result of oxidative stress [3]. It is well accepted that oxidative stress which releases free oxygen radicals in the body is involved in the number of disorders including cardiovascular diseases, diabetes and cancers. Oxidation caused by free radicals sets reduced capabilities to combat cancer, kidney damage, atherosclerosis and heart diseases [4]. Many chemical substances derived from herbs are known to be effective chemo-preventive. Some Thai vegetables and herbs have been shown to induce a chemo-preventive and anti-tumor

action [5-11]. Epidemiological studies have consistently shown that there is a clear significant positive association between intake of fruits, vegetables and herbs. They may reduce the rate of heart diseases mortality, diabetes, cancer and other degenerative diseases [3,12-17].

In this study, forty Thai traditional herbs believed to possess anti-cardiovascular disease, anti-tumor activity, as well as reduce the risk of diabetes were examined for antioxidant activity. Total antioxidant activities of herbal extracts were demonstrated by using improved ABTS radical cation decolorization assay. Antioxidant screening would provide important preliminary data to help select potential plant extracts with antioxidant property for the future study.

## EXPERIMENTAL SECTION

### Sample preparation

Forty dried medicinal plants were purchased from Thai Traditional Pharmacy in Bangkok. Each sample was prepared by boiling in water for 10 minutes with a ratio of herb to water at 1:20 w/v. The mixture was then shaken intermittently. After boiling, the mixture was cooled at room temperature, centrifuged at 2,500 rpm for 15 minutes. The supernatant of the extract was filtered through Whatman no.2 filter paper and then immediately analyzed. Samples, not yet investigated, were stored at -20 °C until analyzed.

### Chemicals

Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (Aldrich chemical, USA), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid diammonium salt (ABTS) (Sigma-Aldrich, USA), potassium persulfate (Sigma-Aldrich, USA). All chemicals were of analytical grade and available locally.

### Total antioxidant activity assay

Total antioxidant activity was measured by using radical cation decolorization assay [18]. This assay based on the inhibition by antioxidants of the absorbance of the free radical cation from ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid diammonium salt). ABTS was incubated with potassium persulfate in order to produce the free radical cation (ABTS<sup>0+</sup>). This had a relatively stable blue-green color, which was measured at 734 nm. Antioxidant compounds will suppress the absorbance of ABTS<sup>0+</sup> to an extent on a time scale dependent on the antioxidant capacity in plasma. This assay was calibrated using Trolox (a water-soluble vitamin E analogue) as standard.

In brief, ABTS was dissolved in deionized water to make a 7 mM concentration solution. ABTS<sup>0+</sup> was produced by mixing ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and the mixture was allowed to stand in the dark at room temperature for 12-16 hours before use. For our study, the ABTS<sup>0+</sup> solution was diluted with PBS, pH 7.4, to an absorbance of 0.70 ( $\pm 0.02$ ) at 734 nm. After addition of 1.0 ml of diluted ABTS<sup>0+</sup> ( $A_{734nm} = 0.700 \pm 0.200$ ) to 10  $\mu$ l of each plasma or Trolox standards (final concentration 0-15  $\mu$ M) in PBS the absorbance reading was taken at 30°C exactly 6 minutes after initial mixing. PBS blank were run in each assay. All determinations were carried out at least in triplicate. The percentage of absorbance inhibition at 734 nm was calculated and plotted as a function of antioxidants of Trolox for the standard reference data.

## RESULTS AND DISCUSSION

The total antioxidant activities of medicinal plant extracts associated with anti-cardiovascular activity are shown in Table 1. We found that the extracts from *Nelumbo nucifera* Gaertn. was the highest activity followed by *Bixa orellana* Linn., *Michelia alba*, *Mammea siamensis*, and *Michelia champaca* respectively. Total antioxidant activities of medicinal plant extracts associated with anti-diabetes activity are shown in Table 2. We found that the extract from *Xylinbaria minutiflora* Pierre. was the highest activity followed by *Leptocarpus disjunctus* Mast., *Salacia chinensis* Linn., *Albizia myriophylla* Benth., *Urceola minutiflora* Pierre, and *Pandanus tectorius*, respectively. The total antioxidant activities of medicinal plant extracts associated with anticancer activity are shown in Table 3. Our data was shown that the extracts from *Balanophora abbreviata* was the highest activity followed by *Alpinia allughas*, *Alpinia allughas* Rosc. and *Datura fastuosa* Linn., respectively. The relation between the total antioxidant activity of all plant extracts was presented in Figure 1.

Nowadays, many researchers have found that the chemical substances in herbs contained a variety of phytochemicals, such as phytochemicals, triterpenes, flavonoids, saponins, and carotenoids, which have been accepted to be chemo-protective [19]. Flavonoids have extensive biological properties that promote human health and help to reduce the risk of diseases. Flavonoids extend the activity of vitamin C, act as antioxidants, protect LDL cholesterol from oxidation, inhibit platelet aggregation, and act as anti-inflammatory and anti-tumor agents as well [20-22]. A variety of phenolic compounds, in addition to the flavonoids, are found in fruits, vegetables, and herbs. Phenolics influence the quality and stability of foods by acting as flavorants, colorants, and antioxidants. The phenolic compounds, such as caffeic, ellagic, and ferulic acids, sesamol, and vanillin, also exhibit anti-carcinogenic activity and inhibit atherosclerosis as well [23]. The antioxidant properties of polyphenols may change as a consequence of their oxidation state. Polyphenols with an intermediate oxidation state can exhibit higher radical scavenging activity than nonoxidized polyphenols [3,24]. The higher antioxidant activity of the partially oxidized polyphenols could be attributed to their increased ability to donate a hydrogen atom from the aromatic hydroxyl group to a free radical and/or to the capacity of their aromatic structure to support unpaired electrons through delocalization around the p-electron system [25].

We investigated the antioxidant activity of Thai medicinal plants, extracted by boiling in the water in order to mimic the preparation of traditional medicine in Thailand. Our data suggest that a seedpod of *Nelumbo nucifera* Gaertn. (sacred lotus) has played the highest activity of all medicinal plant extracts. The seedpod of sacred lotus was reported to possess hepatoprotective and free radical scavenging activity [26], antifertility activity [27] and suppress cell cycle progression, cytokine genes expression, and cell proliferation in human peripheral blood mononuclear cells [28]. The major phytoconstituents present in the seeds are alkaloids like dauricine, lotusine, nuciferine, pronuciferine, liensinine, isoliensinine, roemerine, nelumbine and neferine [29-32]. Both dauricine and neferine isolated from *Nelumbo nucifera* block the Na<sup>+</sup>, K<sup>+</sup> and Ca<sup>2+</sup> transmembrane currents in cardiac cells [32]. Neferine has shown to have anti-arrhythmic action and also significantly inhibits rabbit platelet aggregation [33-34]. Xiao et al. (2005) [35] reported an inhibitory effect of isoliensinine on bleomycin-induced pulmonary fibrosis in mice. Procyanidins were isolated from the seedpods of *Nelumbo nucifera* by Ling and coworkers [36]. They have also reported the antioxidant activity of procyanidins. *Nelumbo nucifera* seeds also contain saponins, phenolics and carbohydrates. Hyun et al. (2006) isolated new isorhamnetin glycosides from the n-butanol fraction of *Nelumbo nucifera* stamens. The isolated isorhamnetin glycosides showed marked antioxidant activities in the assay using DPPH, and ONOO<sup>-</sup> scavenging activities [37].

Table 1 Total antioxidant activity of medicinal plant extracts associated with the treatment of cardiovascular diseases

Scientific name of plant	Part used	Total Antioxidant Activity (mM/g)
<i>Nelumbo nucifera</i> Gaertn.	seedpod	67.317
<i>Bixa orellana</i> Linn.	flower	65.935
<i>Michelia alba</i>	flower	45.635
<i>Mammea siamensis</i>	flower	42.902
<i>Mimusops elengi</i>	flower	28.437
<i>Michelia champaca</i>	flower	23.6
<i>Drosera indica</i> Linn.	stem	21.373
<i>Piper locum</i>	seed	19.708
<i>Mesua ferrea</i> Linn.	flower	19.308
<i>Jasminum sambac</i> Ait.	flower	19.056
<i>Cinnamomum spp.</i>	bark	15.562
<i>Cananga odorata</i>	flower	15.063
<i>Alyxia reinwardtii</i> BL var. <i>lucida</i> Markgr.	root	9.144
<i>Dracaena loureiri</i> Gagnep.	core	5.319
<i>Picrorhiza kurroa</i> Benth	root	4.406
<i>Illicium verum</i> Hooh.f.	flower	3.583
<i>Euphorbia antiquorum</i> Linn.	core	2.028
<i>Fagraea fragrans</i> Roxb.	core	2.022
<i>Aquilaria agallocha</i> Roxb.	flower	1.704
<i>Cassia fistula</i>	flower	1.651
<i>Pogostemon cablin</i> Benth	stem	1.481
<i>Myristica fragrans</i> Houtt.	stem	1.424
<i>Aglaia pyramidata</i> Hance	stem	1.468
<i>Lanthoxylum limonalla</i> Alston.	root	1.347
<i>Artocarpus altissimus</i> (Parkinson) Fosb.	stem	1.328

Table 2 Total antioxidant activity of medicinal plant extracts associated with the treatment of diabetes

Scientific name of plant	Part used	Total Antioxidant Activity (mM/g)
<i>Xylinbaria minutiflora</i> Pierre	creeping stem	14.756
<i>Leptocarpus disjunctus</i> Mast.	creeping stem	10.687
<i>Salacia chinensis</i> Linn.	creeping stem	9.451
<i>Albiziamyriophylla</i> Benth.	stem	7.320
<i>Urceolaminutiflora</i> Pierre D.J. Middleton	creeping stem	7.090
<i>Solanum trilobatum</i> Linn.	root	4.054
<i>Pandanustectorius</i>	air root	3.113
<i>Harrisonia perforata</i> (Blanco) Merr.	stem	3.094

Table 3 Total antioxidant activity of medicinal plant extracts associated with the treatment of cancer

Scientific name of plant	Part used	Total Antioxidant Activity (mM/g)
<i>Balanophora abbreviata</i>	flower	54.879
<i>Alpinia allughas</i> Rosc.	fruit	13.22
<i>Datura fastuosa</i> Linn.	root	4.178
<i>Streblus asper</i>	root	3.19
<i>Suregada multiflorum</i> (Juss.) Baill.	bark	3.046
<i>Acanthus ebracteatus</i> Vahl.	stem	1.731
<i>Hydnocarpus antheilmithicus</i> Pierre	fruit	1.488

### Total Antioxidant Activity of Medicinal Plants

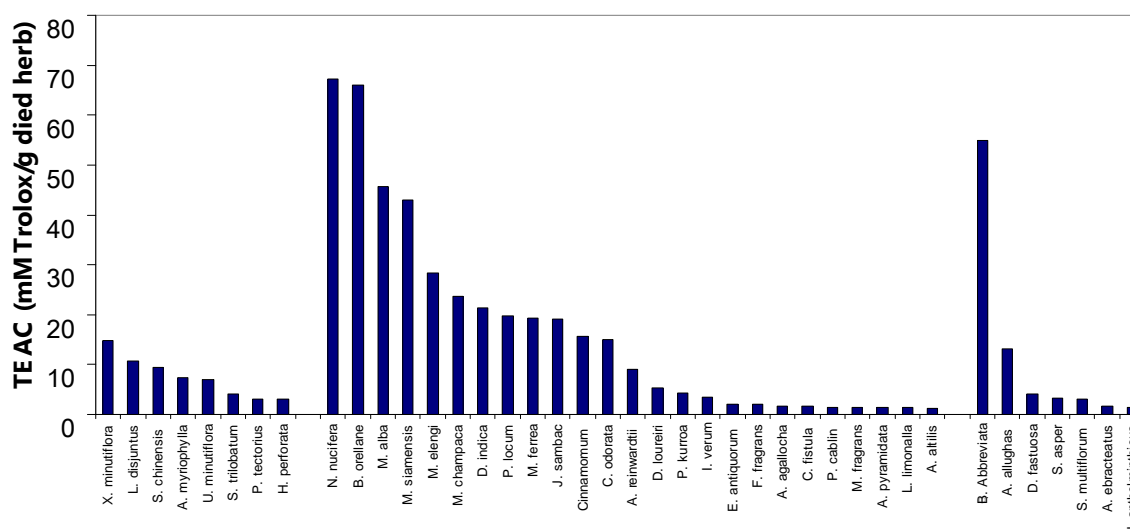


Figure 1 Total antioxidant activities of 40 plant extracts

### CONCLUSION

The present results propose that the extract of *Nelumbo nucifera* Gaertn. and other Thai herbs have antioxidant activity, which indicates its effectiveness in protection from diseases caused by overproduction of free radicals. Further studies would be required to evaluate *in vivo* assay.

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