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Antioxidant Activity of a Thai Traditional Formula for Longevity

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Abstract

Antioxidant activity of a Thai traditional formula for longevity was studied. The formula comprised six herbs, as follows: *Albizia procera*, *Cyperus rotundus*, *Diospyros rhodocalyx*, *Piper nigrum*, *Tinospora crispa* and *Streblus asper*. Each components and the formula were separately extracted by 95% ethanol and concentrated using a reduced-pressure evaporator. The antioxidant properties were detected by DPPH method using vitamin C and Trolox as reference standards. *In vitro* oxidative hemolysis of sheep red blood cells was used as a model to study the free radical-induced damage of biological membranes by AAPH. From the DPPH method, *Albizia procera* extract demontrated the most potent antioxidant properties (IC_{50} 44.34 µg/ml), while vitamin C and Trolox had IC_{50} of 17.47 µg/ml and 22.75 µg/ml, respectively. From the AAPH hemolysis method, *Albizia procera*, the formula for longevity, *Cyperus rotundus*, *Diospyros rhodocalyx*, *Piper nigrum*, *Tinospora crispa* and *Streblus asper* extracts at a concentration of 5 mg/ml could prolong the time of 50% hemolysis from 78 min to 157, 142, 126, 114, 108, 101 and 100 min, respectively, while the time of 50% hemolysis of Trolox at a concentration of 0.5 mg/ml was 160 min. Phytochemical screening tests showed the presence of phenolic compounds, tannins and flavonoids in the formula *Cyperus rotundus* and *Albizia procera*; and phenolic compounds and flavonoids in *Piper nigrum*, *Diospyros rhodocalyx* and *Streblus asper*. *Tinospora crispa* extract contained only phenolic compounds.

Keyword: *Albizia procera*, Antioxidant activities, *Cyperus rotundus*, *Diospyros rhodocalyx*, DPPH method, *Piper nigrum*, *Streblus asper*, Thai traditional formula for longevity, *Tinospora crispa*, 50% hemolysis

INTRODUCTION

There are many approaches to living long, healthy lives, such as eating healthy food, getting regular exercise, maintaining a good mood, and supplementing one's diet with herbal medicines. A "longevity formula" is an herbal preparation that is claimed to prevent and/or cure disease. Most of these formulas are composed of plant products rich in antioxidants, whose activity can promote cardiovascular health and beneficial inflammatory response, as well as neutralize free radicals. In Thailand, there are many traditional formulas for longevity. The most popular recipe is composed of six kinds of herbs: *Albizia procera* bark, *Cyperus rotundus* underground stem, *Diospyros rhodocalyx* bark, *Piper nigrum* fruit, *Tinospora crispa* stem and *Streblus asper* seed[1].

Reactive oxygen species (ROS) are believed to be the main biological compounds that activate the aging process; ROS from

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both endogenous and exogenous sources also play an important role in the pathogenesis of neurodegenerative and cardiovascular diseases, diabetes mellitus, and cancer. ROS arise due to an imbalance between antioxidants and free radicals in the body [2-5]. Thus, efforts are being expended in the search for substances that can prevent and inhibit the activity of free radicals. The free radical theory of aging implies that antioxidants such as vitamin A, vitamin C, vitamin E and beta carotene will slow the process of aging by reducing the formation of free radicals or by preventing free radicals from oxidizing sensitive biological molecules [6-8].

The aim of this study was to investigate the antioxidant properties of a well-known Thai longevity formula and its components.

MATERIALS AND METHODS

Materials

The components of the formula for longevity – *Albizia procera* bark, *Cyperus rotundus* underground stem, *Diospyros rhodocalyx* bark, *Piper nigrum* fruit, *Tinospora crispa* stem and *Streblus asper* seed – were obtained from Jao-Krom-Pur store, Bangkok; 2,2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid (vitamin C) and Trolox from Fluka (Switzerland), and 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH) from Sigma-Aldrich (USA).

Experiment 1. Extraction sheep red blood cell were obtained from the Salaya Animal center, Mahidol University

Each medicinal plant part was dried in a hot-air oven at 50 °C for 24 h and then pulverized. The powder was extracted with 95% ethanol using a percolator until exhausted; the extracts were then filtered and evaporated under reduced pressure using a rotary evaporator to obtain the dry crude extracts.

Experiment 2. Phytochemical screening[9]

Each medicinal plant part was screened for the presence of flavonoids by Shinoda test and ammonia test, tested for phenolic compounds using 1% ferric chloride solution, and tested for tannins using gelatin solution and gelatin/salt solution.

Experiment 3. Free radical scavenging activity, determined by DPPH method[10]

Antioxidant activities of the formula, its component extracts, and standard solutions (vitamin C and Trolox) were determined based on their radical-scavenging ability in reacting with a stable DPPH free radical. A 100 µl sample of each extract (at concentrations of 50 to 200 µg/ml) or standard was mixed with 200 ml of DPPH in absolute methanol solution (0.2 mM). After incubation at 37 °C for 30 min, the absorbance of each solution was determined at 515 nm using a spectrophotometer; corresponding blank readings were also taken, and % inhibition was then calculated:

The antioxidant activity of the extracts was expressed as IC_{50}, defined as the concentration of the extract required to inhibit DPPH radicals by 50%, using an exponential curve. Ascorbic acid and Trolox was used as standard antioxidants. Every experiment was done in triplicate.

Experiment 4. Assay for hemolysis[11,12]

In the hemolysis assay, 100 ml of 20% packed sheep red blood cells (RBC) was mixed with 100 ml of 5 mg/ml extract in PBS (pH 7.4) and 200 ml of 200 mM AAPH. Every 30 min during incubation at 37 °C for 3 h, 4 ml 0.15 M NaCl was added to each reaction mixture and then centrifuged. The absorbance of the supernatant was spectrophotometrically measured at 540 nm. The anti-hemolytic effect of each extract was determined by 50% hemolysis, and was compared with 0.5 mg/ml Trolox in PBS. Every experiment was done in triplicate.

RESULTS AND DISCUSSION

Phytochemical screening tests revealed the presence of phenolic compounds, tannins and flavonoids in the formula for longevity, *Cyperus rotundus* and *Albizia*

procera; phenolic compounds and flavonoids were found in *Piper nigrum, Diospyros rhodocalyx* and *Streblus asper*; and phenolic compounds, but neither flavonoids nor tannins, were found in *Tinospora crispa* extract (Table 1). Based on the results of the DPPH method, *Albizia procera* possessed the most potent antioxidant properties: IC_{50} of *Albizia procera* extract and the formula for longevity were 44.34 and 187.63 µg/ml, respectively, while vitamin C and Trolox had IC_{50} of 17.47 and 22.75 µg/ml, respectively (Table 2). Based on the results of the AAPH hemolysis method, *Albizia procera*, the formula for longevity, *Cyperus rotundus*, *Diospyros rhodocalyx*, *Piper nigrum, Tinospora crispa* and *Streblus asper* extracts at a concentration of 5 mg/ml could prolong the time of 50% hemolysis from 78 min to 157, 142, 126, 114, 108, 101 and 100 min, respectively, while the time of 50% hemolysis of Trolox at a concentration of 0.5 mg/ml was 160 min (Table 3).

Table 1. %Yield of each extract and results of phytochemical screening

Crude extract	%Yield	Phytochemicals
Albizia procera	9.43	Flavonoids Tannins Phenolic compounds
Cyperus rotundus	5.48	Flavonoids Tannins Phenolic compounds
Piper nigrum	5.65	Flavonoids Phenolic compounds
Diospyros rhodocalyx	2.52	Flavonoids Phenolic compounds
Tinospora crispa	6.35	Phenolic compounds
Streblus asper	5.02	Flavonoids Phenolic compounds
The formula for longevity	9.04	Flavonoids Tannins Phenolic compounds

Table 2. IC_{50} of each standard/extract (µg/ml)

Standard/extract	Concentration (µg/ml)
Vitamin C	17.47
Trolox	22.75
Albizia procera	44.34
Cyperus rotundus	235.91
Piper nigrum	1,111.39
Diospyros rhodocalyx	1,285.09
Tinospora crispa	1,324.25
Streblus asper	8,603.57
The formula for longevity	187.62

Table 3. The time of 50% hemolysis of each extract (5 mg/ml) and Trolox (0.5 mg/ml) expression

Standard/extract	Time (min)
Control	78
Trolox	160
Albizia procera	157
Cyperus rotundus	126
Piper nigrum	108
Diospyros rhodocalyx	114
Tinospora crispa	101
Streblus asper	100
The formula for longevity	142

The overall results indicate that *Albizia procera* possessed highly potent antioxidant activity; although its potency was lower than those of the standard agents vitamin C and Trolox, its median inhibitory concentration (IC_{50}) was less than 50 µg/ml. *Albizia procera*, the formula for longevity, *Cyperus rotundus*, *Diospyros rhodocalyx*, *Piper nigrum*, *Tinospora crispa* and *Streblus asper* extracts all showed anti-hemolytic properties. Even though the time of 50% hemolysis of these extracts appeared to be the same or less than that of Trolox, their doses were 10 times higher than the standard. The ability of the formula for longevity and its component extracts to extend the AAPH-induced RBC hemolytic time suggested its contribution in protecting erythrocyte membranes, which are rich in polyunsaturated fatty acids, from peroxidation[13]. Oxidative damage of erythrocyte membranes (lipid and protein peroxidation) may be implicated in hemolysis associated with some hemoglobinopathies, oxidative drugs, transition metal excess, radiation, and deficiencies in some erythrocyte antioxidant systems[14]. AAPH, a water-soluble free radical generator, was used to imitate the *in vivo* condition of oxidative stress. Peroxyl radicals are generated by thermal decomposition of an azo compound in oxygen, and cause lipid peroxidation of red blood cell membranes, resulting in cell lysis. In addition, oxidants may impair erythrocyte formation, leading to decreased survival of erythrocytes and circulatory impairment[15]. The protective effect of the formula for longevity and its components against red blood cell lysis induced by AAPH may be due to its antioxidant activity.

Based on Cervantes-Cervantes (2005), it might be concluded that the bark of *Albizia procera* possessed potent antioxidant activity while the formula for longevity exhibited only mild activity since the other components did not show potent free radical scavenging ability[16]. Nevertheless, although all of the extracts showed the ability to protect erythrocyte membranes, the extracts of the whole formula and the bark of *Albizia procera* showed the most potent activity. Therefore, their anti-lipid peroxidation activity should be studied to confirm these findings. Furthermore, stimulation of enzyme activity by superoxide dismutase (SOD) or catalase warrants further investigation.

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