Medicinal & Aromatic Plants
ISSN: 2167-0412

Itharat et al., Med Aromat Plants 2016, 5:3
DOI: 10.4172/2167-0412.1000243

Research Article Open Access

In Vitro Antioxidant Activities of Extracts of *Bauhinia strychnifolia* Stems and Leaves: Comparison with Activities in Green Tea Extracts

Itharat A[1,2]*, Sayompark S[1], Hansakul P[2,3] and Dechayont B[1,2]

[1]*Department of Applied Thai Traditional Medicine, Faculty of Medicine, Thammasat University, Pathumthani 12120, Thailand*
[2]*Center of Excellence on Applied Thai Traditional Medicine Research (CEATMR), Thammasat University, Pathumthani 12120, Thailand*
[3]*Department of Preclinical Science, Faculty of Medicine, Thammasat University, Pathumthani 12120, Thailand*

Abstract

Dried stems and leaves of Yha-nang dang (*Bauhinia strychnifolia* Craib.) have long been used in Thailand to make tea for promoting health and for detoxification of the body. The objectives of this study were to investigate antioxidant activities of *B. strychnifolia* dried stem and leaf extracts obtained by different extraction methods. The antioxidant power of *B. strychnifolia* extracts and water extracts of green tea leaves (Camellia sinensis) were evaluated using four different methods: DPPH radical scavenging assay, Ferric reducing antioxidant power (FRAP) assay, and Superoxide radical scavenging by NBT dye reduction assay, and measurement of total phenolic contents by the Folin-Ciocalteu colorimetric method. The 95% ethanolic extract of B. strychnifolia stems exhibited the strongest DPPH radical scavenging activity, with an EC_{50} value of 4.2 µg/ml. This was in agreement with results from the Ferric reducing antioxidant power assay, which showed that this extract had the highest FRAP value and TEAC (Trolox equivalent antioxidant capacity) values of 1481.2 mg Fe(II)/g and 421.4 mg Trolox/g, respectively. The 50% ethanolic extracts of *B. strychnifolia* stems showed the highest superoxide radical scavenging activities with an EC_{50} value of 85.7 µg/ml. The total phenolic contents of all extracts ranged from 143.7 to 390.1 mg GAE/g. The 95% ethanolic extract of *B. strychnifolia* stems had the highest total phenolic contents (390.1 mg GAE/g). Green tea water extracts showed less antioxidant activity than 95% ethanol *B. strychnifolia* stem extracts, by all assays, except by the DPPH assay. The results from this study indicate that ethanolic extracts of *B. strychnifolia* stems are rich in phenolic content and demonstrate as good, or better antioxidant activities compared to green tea. This lends scientific support for the use of these extracts for detoxification by Thai traditional medicine practitioners, and suggests that they could be further developed into commercial detoxification products.

Keywords: Antioxidant activity; *Bauhinia strychnifolia*; Green tea; DPPH radical scavenging assay; Herbal plants; NBT assay

Abbreviations: BHT: Butylated hydroxytoluene; DMSO: Dimethyl sulfoxide; DPPH: 1,1-Diphenyl-2-picrylhydrazyl; FRAP: Ferric reducing antioxidant power; GAE: Gallic acid equivalents; MTS: 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; NBT: Nitroblue tetrazolium; TEAC: Trolox equivalent antioxidant capacity; TPTZ: 2,4,6-Tripyridyl-s-triazine; Trolox: 6-hydroxy-2,5,7,8-tetramethyl chlorman-2-carboxylic Acid.

Introduction

Reactive oxygen species (ROS), reactive nitrogen species (RNS), and free radicals are generated in living organisms by a variety of endogenous systems (e.g., respiration, oxidative energy metabolism, immune activity), and after exposure to different physical and chemical factors (e.g., UV radiation, pesticides, pollutants, drugs, food additives) [1]. Oxidative stress leads to a disturbance in the balance of free radicals and antioxidant defenses in living organisms. These reactive species are implicated in mediating various pathological processes in humans, including cancer, aging, atherosclerosis and inflammatory diseases [2,3].

Herbal plants have long been a valuable source of natural antioxidants for maintaining human health. The balance between the production of free radicals and the antioxidant defenses in the body has important health implications [4]. Detoxification is a concept in alternative medicines which regards elimination of any accumulated toxins, and avoiding ingress of new toxins, as essential parts of the healing process and reduction of the risk of acquiring chronic diseases [5,6]. The stems and leaves of *Bauhinia strychnifolia* Craib. (Leguminosea-caesalpiniodeae species) [7], known in Thai as Yha-nang dang, are used in Thai traditional medicine (TTM), to eliminate toxic metals and pesticides, and also used for detoxification. The dried stems and leaves are used to brew a health-promoting herbal tea, and alcoholic and water macerated extracts (ethanol or water) (Thai name Yadong) are prescribed by Thai traditional doctors [8]. Moreover, Yha-nang dang is one of Thai longevity medicine preparations recommended by the Thai national health physicians for improving the quality of life and health.

Although Yha-nang dang is widely used in detoxification products by many Thai traditional medicine practitioners, there have been very few scientific studies that support its folklore historic use. Bunluepuech and Tewtrakul demonstrated that the ethanolic and water extract of its stem showed anti HIV-1 Integrase using the multiplate integration assay with IC_{50} values of 6.40 and 11.20 µg/ml, respectively [9]. Kaewpiboon et al. revealed that the crude hexane extract of its stem exhibited cytotoxic activity using the MTT cytotoxicity assay against the MDA-MB-231 KB3-1 (breast cancer) and SW 480 (colon cancer) cell lines, while the crude dichloromethane extract of the stem exhibited cytotoxic activity against the A549 (lung cancer) and KB3-1 (cervical cancer) cell lines [10].

***Corresponding author:** Itharat A, Department of Applied Thai Traditional Medicine, Faculty of Medicine, Thammasat University, Pathumthani 12120, Thailand, Tel: +6629269749; Fax: +6629269705; E-mail: iarunporn@yahoo.com

Received February 26, 2016; **Accepted** March 21, 2016; **Published** March 29, 2016

Citation: Itharat A, Sayompark S, Hansakul P, Dechayont B (2016) *In Vitro* Antioxidant Activities of Extracts of *Bauhinia strychnifolia* Stems and Leaves: Comparison with Activities in Green Tea Extracts. Med Aromat Plants 5: 243. doi:10.4172/2167-0412.1000243

A recent follow-up study by Tewtrakul et al. has resulted in the isolation of five pure compounds from ethanolic extracts of *B. strychnifolia* stems, two of which showed remarkably potent anti-cancer activities *in vitro* against several cancer cell lines. The activity of one of these compounds was ten times higher than that of Camptothecin, the anti-cancer drug used as a positive control in these in vitro studies [11].

There are several pharmacological reports of Bauhinia species which related detoxification such as *Bauhinia hookeri.* The ethanolic extract of *Bauhinia hookeri* leaves [BHE] showed hepato-protective and antioxidant activity against CCl_4-induced liver injury in mice. It can inhibit increasing of the alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). Values of CCl_4-induced mice liver injury and also inhibit lipid peroxidation or MDA production. Moreover, BHE treatment also increased the antioxidant parameters such as GSH, GPx, GR, GST, and SOD [12]. Its polyphenol-rich fraction which was separated from this plant also showed nepro-protective activity [13]. However, there is no published report to date on antioxidant activities of any parts of *B. strychnifolia* which was used as detoxification. The main purpose of this study was to compare the antioxidant activities of extracts of 7 stems and leaves with green tea (*Camellia sinensis*). The secondary aims were to determine optimum extraction procedures that yield extracts with highest antioxidant activities. It is also hoped to correlate the anti-oxidant efficiency as determined by three different methods to total phenolic content of the plant materials.

Materials and Methods

Chemicals and reagents

Folin-Ciocateu's reagent, 2,2-diphenyl-l-Picrylhydrazyl (DPPH) and 2,6-di-tert-butyl-4-methylphenol (BHT) were purchased from Fluka (MO, USA). Gallic acid, 2,4,6-tripyridyl-s-triazine (TPTZ), ferric chloride, ferrous sulfate, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), RPMI 1640, dimethyl sulfoxide (DMSO), hanks' balanced salt solution (HBSS), nitroblue tetrazolium (NBT), phorbol 12-myristate 13-acetate (PMA) and propyl gallate were purchased from Sigma-Aldrich Inc. (MO, USA). Sodium carbonate and acetic acid were purchased from Merck (Darmstadt, Germany). Absolute ethanol and hydrochloric acid (HCl) were purchased from Labscan Limited (Bangkok, Thailand). *Penicillin-streptomycin* (P/S) and fetal bovine serum (FBS) were purchased from Biochrom (Berlin, Germany). Trypsin-EDTA was purchased from Gibco RBL Life Technologies (NY, USA). Cell Titer 96® aqueous one solution cell proliferation assay and 3-(4,5-dimethylthiazol-2-yl)-5-(3-carbo xymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) were purchased from Promega (WI, USA). Dried green tea leaves were purchased from Doitung (Chiangrai, Thailand).

Stem and leaf sample preparation

The fresh leaves and stems of *B. strychnifolia* were collected from Kanchanaburi Province of Thailand (June, 2012). Voucher specimens (SKP098021901) have been deposited at the herbarium of Southern Center of Thai Medicinal Plants at the faculty of Pharmaceutical Science, Prince of Songkla University, Songkhla Province, Thailand. The two parts of *B. strychnifolia* were washed, sliced thinly, dried in a hot air oven at 50°C (24 hours) and powdered (Mesh size 40). The extraction procedures used were similar to those practiced by Thai traditional doctors. These extracts were obtained by maceration and decoction methods.

Maceration method: Dried powdered plant materials (300 g) were macerated in 95% ethanol (1 L) and 50% ethanol (1 L) for 3 days and filtered. The residue was further macerated with the same volumes of solvent two times. The combined solvent extracts were then evaporated to dryness using a rotary evaporator. The extracts were then dried to constant weight in a vacuum desiccator.

Decoction method: Dried powdered plant materials (300 g) were boiled in distilled water (1 L) for 15 min and filtered. The residue was further boiled again two times. The combined water extracts were then evaporated to dryness using a lyophilizer (Lyolab series, Lyophilization system Incorp., USA). This method also used for extraction of green tea.

The residues after maceration were subjected to extraction by decoction method. The residues after maceration were first dried in a hot air oven at 50°C (1 h) and then boiled in distilled water (1 L) at boiling point for 15 min and filtered. The residue was further boiled again two times. The combined water extracts were then evaporated to dryness using a lyophillizer.

Antioxidant assays

DPPH radical scavenging activity assay: Antioxidant activity was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) [14]. Samples for testing were dissolved in absolute ethanol or distilled water to obtain the highest concentration of 200 µg/ml. Each sample was further diluted to obtain at least 4 solutions at lower concentrations (two-fold dilutions). Each concentration was tested in triplicate. A portion of the sample solution (100 µl) was mixed with an equal volume of 6×10^{-5} M DPPH (in absolute ethanol) and allowed to stand at room temperature for 30 minutes. The absorbance was then measured at 520 nm. BHT (butylated hydroxytoluene), a well-known synthetic antioxidant, was used as a positive standard. The scavenging activity of the samples is the ability to reduce the color intensity of DPPH. Inhibition (%) was calculated using the following equation:

$$\% \text{ inhibition} = [(\text{Abs. control} - \text{Abs. sample})/\text{Abs. control}] \times 100^2 \text{ usn}$$

Abs. control is the absorbance of the control reaction (containing all reagents except the test compound) and Abs. sample was the absorbance of the tested compound. All tests were carried out in triplicate on any one day, and repeated with freshly prepared samples a further two times (n=3). EC_{50} values were calculated from the graph of % inhibition against extract concentration.

Ferric reducing antioxidant power (FRAP) assays: FRAP was evaluated by the measurements of Fe (III)/TPTZ-complex by a colorimetric method using a spectrophotometer [15]. FRAP reagent was prepared using 10 ml of acetate buffer 300 mM, adjusted to pH 3.6 by addition of acetic acid, containing 1 ml of 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl, and 1 ml of 20 mM ferric chloride hexahydrate dissolved in distilled water: chloride solution in proportion of 10:1:1 respectively. The FRAP reagent was prepared fresh daily and was warmed to 37°C for 4 minutes in a water bath. About 10 mg of crude extracts were weighed in centrifuge tubes and diluted to a concentration of 1 mg/ml. Aliquots of sample solutions (20 µl) were added to 180 µl of the FRAP reagent, mixed well and allowed to stand at room temperature for 8 min. The absorbance was measured at 593 nm. Increased absorbance of the reaction mixture indicated increased reducing power of the sample. The results were expressed as mg Fe (II)/g and mg Trolox/g of extract sample.

All tests were carried out in triplicate on any one day, and repeated with freshly prepare samples a further two times (n=3).

Superoxide radical scavenging by NBT dye reduction assay: HL-60 human promyelocytic leukemia cell lines were cultured in RPMI 1640 medium supplement with 10% heated fetal bovine serum, 50 IU/ml penicillin and 50 µg/ml streptomycin. The cell lines were maintained at 37°C in a 5% CO_2 atmosphere with 95% humidity. In this system, intracellular reactive oxygen species generation is detected by a nitroblue tetrazolium (NBT) reduction method [16]. Briefly, HL-60 cells (5×10^5 cells/ml) were incubated in RPMI 1640 medium supplement with 10% heated fetal bovine serum, 50 IU/ml penicillin and 50 µg/ml streptomycin containing 1.3% dimethylsulfoxide (DMSO) for 6 days [17]. Differentiated HL-60 cells (1×10^6) were incubated with 500 µl of various dilutions of the extracts, and then incubated for 15 min. After that, the cells were further incubated with 50 µl PMA (250 ng/ml) and 250 µl (1.25 mg/ml) NBT in HBSS solution for 60 min [18]. After incubation time, 2 ml HCl was added, and the resulting solution mixed by vortex and centrifuged at 4,000 rpm/min for 10 min. The precipitate of insoluble formazan deposit was dissolved in 300 µl DMSO and 100 µl of cells was added to each well in 96-well microplates. Propyl gallate was used as a positive standard.

The absorbance was measured at 572 nm. The inhibition against superoxide formation measured by NBT reduction was calculated as percentage inhibition using the formula below:

% inhibition=[(Abs. Control - Abs. Sample)/ Abs. Control] × 100

Abs. Control is the absorbance of the positive control (stimulated with PMA) minus the absorbance of negative control (without PMA), and Abs. Sample is the absorbance of the sample inhibition minus the absorbance of negative control (without PMA). All tests were carried out in triplicate and EC_{50} value was calculated from the graph plotted of % inhibition against extract concentration.

All test were carried out in triplicate on any one day, and repeated with freshly prepared samples a further two times (n=3).

MTS cytotoxicity assay: HL-60 cells (1×10^6) cells were incubated with the 500 µl of extracts (5 concentrations as 1, 10, 50, 100, 500 µg/ml) in HBSS for 15 min. Then, the cells were incubated with 50 µl PMA (250 ng/ml) and 250 µl HBSS solution for 60 min. A 100 µl of cells was added in 96-well microplates. This assay was established using the Cell Titer 96® Aqueous One Solution Cell Proliferation Assay. Tetrazolium dye (20 µl) was added to each well of the plate and incubated for 4 hours. The amount of formazan product is directly proportional to the number of living cells in culture. The optical density (OD) was then measured at 490 nm. The tested samples were considered cytotoxic when the optical density of the sample-treated group is less than 70% of that in the control.

All test were carried out in triplicate on any one day, and repeated with freshly prepared samples a further two times (n=3).

Total phenolic content

Total phenolic content of all extracts was determined using colorimetric measurement by the Folin-Ciocalteu's reagent [19]. About 10 mg of crude extracts were weighed in centrifuge tubes and diluted with ethanol or water to a concentration of 1 mg/ml. Aliquots of the extracts (20 µl) were mixed thoroughly with 20 µl of the Folin-Ciocalteu's reagent and 80 µl of sodium carbonate in 96-well microplates. The samples in the plates were mixed, and then allowed to react for 30 min. The absorbance was measure at 765 nm using microplate reader spectrophotometers [20]. All test were carried out in triplicate on any one day, and repeated with freshly prepared samples a further two times (n=3). About 1 mg of gallic acid was diluted to a concentration of 1 mg/ml with absolute ethanol. This solution was further diluted by serial dilution to obtain concentrations of 100, 80, 40, 20, 10, 5 µg/ml. The absorbance of different concentration of gallic acid was measured at 750 nm and a calibration curve constructed. Results are expressed as mg GAE/g, which is mg of gallic acid per gram of extract sample.

Statistical analysis

All determinations were carried out on three separate occasions, each time in triplicate. The results are reported as mean ± standard error of mean (SEM). Calculation of EC_{50} and IC_{50} values were done using the GraphPad Prism 4.03. The statistical significance was calculated by one-way analysis of variance (ANOVA).

Results and Discussion

The percentage yield of extracts from *B. strychnifolia* stem and leaf, and green tea are showed in Table 1.

DPPH radical scavenging activity of *B. strychnifolia* stem and leaf extracts

DPPH assay has extensively been used for screening antioxidant activity because it can accommodate many samples in a short period, and is sensitive enough to detect active ingredients at low concentrations [21]. The antioxidant activity of the samples was within a narrow range, from 4.21 to 8.74 µg/ml (Table 2). The 95% ethanolic extract of dried stems (S95) showed the strongest antioxidant activity with an EC_{50} value of 4.21 µg/ml. The 50% ethanolic extracts of dried stems (S50), and water extracts of dried stems (SW) has slightly lower EC_{50} values of 4.70 and 5.14 µg/ml, respectively. These values are comparable to those for green tea (3.86 ± 0.31 µg/ml). Interestingly, all extracts of *B. strychnifolia* exhibited considerably stronger antioxidant power than

Part used (Powdered)	Methods	Solvent	Code	% Yield (w/w)
Dried leaves (DL)	Maceration	50% EtOH	LE50	10.89
	Maceration	95% EtOH	LE95	10.86
	Decoction	Water (W)	LW	5.18
	Decoction of residue (R) after maceration in 50% ethanol	Water	LWRE50	6.50
	Decoction of residue after maceration in 95% ethanol	Water	LWRE95	4.22
Dried stems (DS)	Maceration	50% EtOH	S50	12.70
	Maceration	95% EtOH	S95	12.21
	Decoction	Water (W)	SW	7.49
	Decoction of residue after maceration in 50% ethanol	Water	SWRE50	2.90
	Decoction of residue after maceration in 95% ethanol	Water	SWRE95	5.77
Green Tea (GT)	Decoction	Water	GTW	15.74

Table 1: Percent yields (w/w) of extracts from stems and leaves of B. strychnifolia and green tea using a variety of extraction procedures.

Part used	Methods	Code	Percent inhibition at various concentration (µg/ml)				EC_{50} (µg/ml)[a]
			1	10	50	100	
Dried leaves	Maceration in 50% ethanol	LE50	22.61 ± 4.41[:]	62.23 ± 2.98[*,:]	70.65 ± 1.32[*,:]	81.83 ± 1.28[*,:]	6.98 ± 0.91[*,:]
	Maceration in 95% ethanol	LE95	13.32 ± 3.12[:]	55.03 ± 1.69[*,:]	83.72 ± 0.99[*]	94.16 ± 0.61[:]	8.74 ± 0.67[*,:]
	Decoction	LW	16.86 ± 5.31[*]	61.33 ± 2.36[*,:]	71.05 ± 4.01[*,:]	85.39 ± 1.71[*,:]	7.15 ± 0.32[*,:]
	Decoction of residue after macerated in 50% EtOH	LWRE50	22.12 ± 2.26[*,:]	62.93 ± 1.61 [*,:]	78.15 ± 0.94[*]	84.83 ± 0.53[*,:]	6.73 ± 1.05[*,:]
	Decoction of residue after macerated in 95% EtOH	LWRE95	23.90 ± 4.31[:]	67.20 ± 2.98[*,:]	75.73 ± 1.20[*]	86.11 ± 2.37[*]	6.50 ± 0.59[*,:]
Dried stems	Maceration in 50% ethanol	S50	32.41 ± 2.31[:]	71.67 ± 2.78[*,:]	80.95 ± 1.05[*]	82.99 ± 3.37[*,:]	4.70 ± 0.84[:]
	Maceration in 95% ethanol	S95	34.78 ± 5.14[:]	72.67 ± 3.61[*,:]	90.11 ± 0.98[:]	93.18 ± 0.35[:]	4.21 ± 0.77[:]
	Decoction	SW	14.74 ± 3.12[*]	64.32 ± 2.44[*,:]	83.58 ± 2.89[*]	85.45 ± 1.70[*,:]	5.14 ± 0.48[:]
	Decoction of residue after macerated in 50% EtOH	SWRE50	16.54 ± 6.28[*]	62.43 ± 2.09[*,:]	85.76 ± 1.96[*,:]	88.29 ± 0.98[*]	7.18 ± 0.49[*,:]
	Decoction of residue after macerated in 95% EtOH	SWRE95	23.92 ± 2.86[:]	69.03 ± 3.17[*,:]	74.22 ± 1.31[*]	75.86 ± 0.68[*,:]	5.51 ± 0.78[:]
Green Tea	Decoction	GT	30.12 ± 4.33	91.18 ± 2.64	93.31 ± 2.71	95.32 ± 1.74	3.86 ± 0.31
BHT	-	-	12.78 ± 2.09	40.02 ± 2.16	81.47 ± 4.11	89.54 ± 0.98	14.87 ± 0.31

[a]The values are expressed as the mean ± SEM (n=3); BHT=Butylated hydroxyl toluene; GT=Water extract of Camellia sinensis leaves; p-value<0.05, compared with C. sinensis (GT) extract; [:] p-value<0.05, compared with BHT, used as positive control.

Table 2: DPPH radical scavenging antioxidant activities of B. strychnifolia stems and leaves, and green tea, obtained using different extraction methods.

BHT (EC_{50}=14.87 µg/ml), which is a positive control normally used in this test method. Antioxidant activities of some other medicinal plants within the *Bauhinia Genus (B. monandra, B. racemosa, B. rutescens, B. purpurea, B. galpinii)* have been reported, and EC_{50} values ranged from 5.50 to 2320.00 µg/ml [22-29]. The another report which studied in mice found that the ethanolic of *B. hookeri* leaves inhibited antioxidant values such as Melondiadehyde (MDA) and Superoxide dismutase (SOD) values in Carbon Tetrachloride-Induced Hepatotoxicity in Mice [11,12]. Previous reports of phytochemical studies on the Bauhinia plants have indicated the presence of flavonoid and phenolic compounds, such as Kaempferitrin in *B. forficata*, Quercetin-3-O-galactopyranoside and Myricetin-3-O-galactopyranoside in *B. galpinii* [29,30]. Phenolic compounds possess ideal structural chemistries for free radical scavenging activity due to (a) their high reactivity as hydrogen or electron donors, (b) the ability of the polyphenol-derived radicals to stabilize and delocalize unpaired electrons (chain-breaking function), and (c) their ability to chelate transition metal ions (termination of the Fenton reaction) [31]. Since these flavonoid and phenolic compounds in other plants within the *Bauhinia spp.* have also been known to have antioxidant properties, their presences in *B. strychnifolia* could be the basis for the observed antioxidant activities.

Ferric reducing antioxidant power (FRAP) assay of *B. strychnifolia* stem and leaf extracts

FRAP assay is an electron transfer (ET) reaction, and was evaluated by the measurements of Fe (II) /TPTZ-complex by a colorimetric method. This method utilizes the measurement of the ferric reducing ability of plasma (FRAP) [13]. The FRAP value was calculated from a graph of different concentrations of standard ferrous sulfate solutions versus absorbance. The representative regression coefficient (R^2) was 0.9999 and the linear regression equation was y=0.002x-0.008. The FRAP values of the extracts ranged from 1481.21 (highest activity) to 627.58 (lowest activity) mg Fe (II)/g sample (Table 3). The 95% ethanolic extract of dried stems (S95) again showed the highest antioxidant activity with FRAP value of 1481.21 mg Fe (II)/g, followed by S50, and SWRE50, with FRAP values of 1159.63 and 1087.96 mg Fe (II)/g, respectively. BHT was used as positive control in this method, and its FRAP value of 710.83 mg Fe (II)/g much lower than those for stem extracts. Trolox equivalent antioxidant capacity (TEAC) was calculated from the equation obtained using standard Trolox, and plotting a graph of Trolox concentration versus absorbance. The representative regression coefficient (R^2) was 0.9999 and the linear regression equation was y=0.007x+0.004. The extracts of *B. strychnifolia* showed a capacity for reducing ferric ion, within a range of 209.78 to 421.44 mg Trolox/g. The 95% ethanolic extract of dried stem (S95) again showed the highest ability to reduce ferric ion, with TEAC values of 421.44 mg Trolox/g, followed by SW and S50, with TEAC values of 382.57 and 369.93 mg Trolox/g, respectively. All the extracts of *B. strychnifolia* exhibited a higher ability in reducing ferric ion to ferrous ion than BHT (204.71 mg Trolox/g). The stem and leaf extracts from *B. strychnifolia* by different

Part used (Powdered)	Methods	Code	FRAP value[a] mg (Fe II)/g	TEAC value[b] mg trolox/g
Dried leaves	Macerationin 50% ethanol	LE50	742.44 ± 2.51[*,:]	220.65 ± 4.03[*,:]
	Macerationin 95% ethanol	LE95	886.01 ± 1.03[*,:]	262.49 ± 3.84[*,:]
	Decoction	LW	627.58 ± 1.70[*,:]	215.86 ± 2.74[*]
	Decoction of residue after macerated in 50% ethanol	LWRE50	1027.00 ± 3.06[*,:]	271.18 ± 2.18[*,:]
	Decoction of residue after macerated in 95% ethanol	LWRE95	1038.74 ± 3.43[*,:]	295.26 ± 1.80[*,:]
Dried stems	Maceration in 50% ethanol	S50	1159.63 ± 1.63[*,:]	369.93 ± 1.25[*,:]
	Maceration in 95% ethanol	S95	1481.21 ± 0.76[*,:]	421.44 ± 1.87[*,:]
	Decoction	SW	1037.62 ± 2.86[*,:]	382.57 ± 4.08[*,:]
	Decoction of residue after macerated in 50% ethanol	SWRE50	1087.96 ± 3.70[*,:]	209.78 ± 2.59[*]
	Decoction of residue after macerated in 95% ethanol	SWRE95	1053.17 ± 2.38[*,:]	299.19 ± 1.75[*,:]
Green tea Decoction		GT	387.40 ± 1.94	108.97 ± 0.56
BHT	-	-	710.83 ± 2.97	204.71 ± 4.07

[a]FRAP value was defined as the concentration of antioxidant having a ferric-TPTZ reducing ability equivalent to mg $FeSO_4.7H_2O$ to 1 g of sample [mg(Fe II)/g]; [b]Trolox Equivalent Antioxidant Capacity (TEAC) values is defined as mg of standard Trolox with the equivalent antioxidant capacity to 1 g of sample; BHT=Butylated hydroxyl toluene; GT=Water extract of Camellia sinensis leaves; [*]p-value<0.05, compared with C. sinensis (GT) extract; [:] p-value<0.05, compared with BHT, used as positive control.

Table 3: Antioxidant activities expressed as the FRAP values and TEAC values using the FRAP assay of B. strychnifolia stem and leaf, and green tea, obtained using different extraction methods.

methods possessed higher ability in reducing ferric ion to ferrous ion than the water extract of *C. sinensis* leaves (p-value <0.05), FRAP and TEAC for green tea being 387.40 ± 1.94 mg Fe (II)/g and 108.97 ± 0.56 mg Trolox/g, respectively.

Superoxide radical scavenging by NBT dye reduction assay of *B. strychnifolia* stem and leaf extracts

Morphologically differentiated HL-60 cells were seen after 6 day of stimulation with 1.3% DMSO. These differentiated HL-60 cells are responsive to PMA (phorbol-12-myristate-13-acetate) and generate oxygen radical through the multi-complex NADPH oxidase system. The phorbol-12-myristate-13-acetate stimulated formation of superoxide ions is a typical characteristic of mature granulocytes. The superoxide scavenging activity of samples was measured using the NBT reduction assay [14]. In the assay, HL-60 cells were treated with non-cytotoxic dilution of sample extracts, and assessed for oxygen radical production. Three stem extracts, the 50% ethanolic extract of dried stem (S50), the water extract of dried stems (SW), and the 95% ethanolic extract of dried stems (S95) showed antioxidant activity, with an EC_{50} values of 85.69, 92.66 and 93.04 µg/ml, respectively whereas EC_{50} value of the positive control propyl gallate was 21.25 µg/ml (Table 4). These activities are significantly higher than those for green tea (EC_{50}=152.77 ± 1.50 µg/ml).

The effect of all extracts on cell lethality were determined using the MTS assay, which confirm that the inhibition of oxygen radical generation by HL-60 cells was not simply due to cytotoxic effects of the plant extracts. The result revealed that at the concentrations used, cell viability was more than 70%. However, this study related the previous report which showed increase SOD in mice which were induced by carbon tetrachloride as damage liver tissues [11]. Our studies do not correlate with published data for *B. racemosa*, which reported that the methanolic extract of *B. racemosa* stems and barks scavenged the superoxide generated by the PMS/NADH-NBT system with an EC_{50} value more than 1000.00 µg/ml [24]. The significance of our current study is the new discovery that the stems extracts of *B. strychnifolia* suppress superoxide generation by differentiated-PMA stimulated HL-60 cells.

Determination of the total phenolic content of *B. strychnifolia* stem and leaf extracts

Total phenolic content was estimated by the Folin-Ciocalteu colorimetric method. The absorbance at 765 nm was plotted against concentration of standard gallic acid solution. The representative regression coefficient (R^2) was 0.9999 and the linear regression equation was y=0.005x + 0.002. The results showed that the stem extracts contained higher total phenolic content than leaf extracts (Table 5). The 95% ethanolic extract of dried stem (S95) showed the highest value of total phenolic content of 390.10 mg GAE/g, followed by SW and SRE95 obtained total phenolic content of 380.57 and 329.68, mg GAE/g, respectively. Interestingly, all extracts of B. strychnifolia stems exhibited higher total phenolic content than the water extract from *C. sinensis* leaves (GT) (275.08 mg GAE/g). For leaf extracts, the 50% ethanolic extract of dried leaves (LE50) showed the highest value of total phenolic content of 199.58 mg GAE/g, followed by LWRE50 and LWRE95, with total phenolic content of 161.53 and 154.16 mg GAE/g, respectively. Previous published data on total phenolic content of some medicinal plants within the *Bauhinia spp*. have reported total phenolic contents within the range of 6.50 to 1310.00 mg/GAE g [25,26]. The phenolic compounds are very important plant constituents because of their scavenging ability on free radicals due to their phenolic groups. Therefore, the phenolic content of plants may contribute directly to their antioxidant action [32]. Moreover, they are thought to play important roles in long term health and reduction in the risk of chronic and degenerative diseases [33]. This suggests that methods normally used by traditional medicine practitioner to prepare healthy drinks such as tea (water extracts) and Yadong in Thai (alcoholic macerated extracts) are most appropriate for use for extracting phenolics compounds from *B.*

Part used	Code	% Inhibition (µg/ml)/ (percentage of viable cells) at various concentration					EC_{50} (µg/ml)[a]
		10	50	100	250	500	
Dried leaves	LE50	-	-	-	-	45.85 ± 3.15*,‡ (78.82 ± 2.56)	>500
	LE95	19.53 ± 4.76‡	21.03 ± 2.44*	52.94 ± 3.02*,‡	81.39 ± 1.61*,‡	94.31 ± 1.91* (86.21 ± 1.30)	95.30 ± 0.27*,‡
	LW	-	-	-	-	26.91 ± 3.46*,‡ (98.07 ± 1.76)	>500
	LWRE50	-	-	-	-	13.66 ± 1.67 *,‡ (98.32 ± 2.01)	>500
	LWRE95	10.42 ± 7.12‡	26.02 ± 2.36‡	61.31 ± 4.52*,‡	83.32 ± 3.13*	22.85 ± 1.87*,‡ (87.91 ± 1.82)	>500
Dried stems	S50	13.42 ± 5.71‡	28.02 ± 3.41‡	52.37 ± 4.09*,‡	80.71 ± 3.20*,‡	95.72 ± 2.02* (92.58 ± 1.15)	85.69 ± 0.37*,‡
	S95	17.24 ± 2.24‡	31.78 ± 7.16‡	53.03 ± 5.13*,‡	86.14 ± 4.61*	96.91 ± 3.43* (86.12 ± 2.90)	93.04 ± 0.86*,‡
	SW	-	-	-	-	92.37 ± 1.18* (95.49 ± 3.88)	92.66 ± 0.72*,‡
	SWRE50	-	-	-	-	36.50 ± 2.28*,‡ (96.73 ± 2.35)	>500
	SWRE95	-	-	-	-	40.57 ± 1.93*,‡ (94.60 ± 2.14)	>500
Green tea	-	7.40 ± 6.91	19.09 ± 7.10	35.15 ± 5.95	53.98 ± 4.17	80.65 ± 3.62 (92.40 ± 2.57)	152.77 ± 3.78
Propyl gallate	-	41.22 ± 3.71	62.00 ± 3.68	78.06 ± 5.43	89.23 ± 4.08	91.34 ± 3.93 (72.13 ± 3.48)	21.25 ± 1.50

[a]The values are expressed as the mean ± SEM (n=3); Differentiated HL-60 cells exposed with 250 ng/ml of PMA for 60 minutes, used as control; GT=Water extract of C. sinensis leaves; *p-value < 0.05, compared with C. sinensis (GT) extract. ‡ p-value < 0.05, compared with Propyl gallate, used as positive control.

Table 4: Percent inhibition at various concentrations, and EC_{50} (µg/ml), of stem and leaf extracts of B. strychnifolia and green tea obtained using different extraction methods, against PMA-induced superoxide radicals in DMSO-differentiated HL-60 cells using the NBT assay.

Part used	Methods	Code	Total phenolic content[a] (mg GAE/g dry materials)
Dried leaves	Maceration in 50% ethanol	LE50	199.58 ± 2.56*
	Maceration in 95% ethanol	LE95	149.32 ± 2.89*
	Decoction	LW	143.65 ± 0.63 *
	Decoction of residue after macerated in 50% ethanol	LWRE50	161.53 ± 0.85*
	Decoction of residue after macerated in 95% ethanol	LWRE95	154.16 ± 1.35*
Dried stems	Maceration in 50% ethanol	S50	309.81 ± 0.58*
	Maceration in 95% ethanol	S95	390.10 ± 1.25*
	Decoction	SW	380.57 ± 0.98*
	Decoction of residue after macerated in 50% ethanol	SWRE50	281.64 ± 0.39*
	Decoction of residue after macerated in 95% ethanol	SWRE95	329.68 ± 0.91 *
Green tea	Decoction	GT	275.08 ± 1.00

[a]The phenolic contents were expressed as gallic acid equivalent (GAE) in milligrams per gram dry material; GT=Water extract of Camellia sinensis leaves; Significant different between B. strychnifolia extracts and C. sinensis (GT) extract, *p-value<0.05.

Table 5: Total phenolic contents of stem and leaf extracts of B. strychnifolia, and green tea, obtained using different extraction methods.

strychnifolia stems and leaves. This result related with *B. hookeri* because it showed high procyanidin, apicatechin and flavonoid, so the future work should be continued. It is interestingly to note that *B. strychnifolia* leaves and stems have two types of antioxidant compounds; those that are extractable in 50% and 95% ethanol, and those that are extractable in water. When the residues left over from exhaustive extraction (3 × 1 L) of the leaves and stems with ethanol (RE50, RE95) are subsequently subjected to decoction with water, the resulting extracts (LWRE50, LWRE95, SWRE50, SWRE95) have remarkably high antioxidant properties. This suggests that the powdered dried samples of these leaves and stems may have higher antioxidant power than the ethanolic and water extracts. Phytochemical studies are required to isolate the different active compounds present in the ethanolic and water extracts.

Conclusion

To our best knowledge, this is the first report on antioxidant activities of stem and leaf extracts of *B. strychnifolia*. The results obtained demonstrate that the 95% ethanolic extracts of *B. strychnifolia* stems exhibit relatively strong anti-radical activity toward the DPPH and FRAP assays, as well as a significant decrease of the oxygen radical generation, and had the highest value of total phenolic content. This extract showed higher antioxidant power than green tea extract in all methods used to access antioxidant activities. These results support the folklore knowledge for the use of *B. strychnifolia* stems and leaves for detoxification, or prevention of chronic diseases, as recommended for centuries by Thai traditional medicine practitioners. These results should be continued to study in mice and determine antioxidant value such MDA, SOD, glutathione because *B. strychnifolia* is commonly used to be detoxification. In addition, the investigation on the hepato-protective and naphro-protective activity of this plant should be also studied in the same manner with *B. hookuri*. The data suggests that further studies are warranted that might lead to the development of health-promoting commercial products from this plant. We are currently attempting to isolate active antioxidant compounds from the ethanolic extracts by bioassay guided isolation methods. These active antioxidant compounds will be markers for analysis and determination of stability of any developed health products from *B. strychnifolia* stems extracts.

Acknowledgements

The authors wish to thank the National Research University Project of Thailand Office of Higher Education Commission, Center of Excellence on Applied Thai Traditional Medicine Research (CEATMR) and Thammasat University, Thailand, for financial support, and Professor LA Damani, Visiting Professor at Thammasat University for his critical comments and careful scientific editing of this manuscript.

References

1. Lobo V, Patil A, Phatak A, Chandra N (2010) Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev 4: 118-126.
2. Wiseman H, Halliwell B (1996) Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer. Biochem J 313: 17-29.
3. Pala FS, Gürkan H (2008) The role of free radicals in ethiopathogenesis of diseases. Advances in Molecular Biology 1: 1-9.
4. Dröge W (2002) Free radicals in the physiological control of cell function. Physiol Rev 82: 47-95.
5. Harrison R (2014) Detoxification and Our Toxic World. Nature.
6. Kris-Etherton PM, Hecker KD, Bonanome A, Coval SM, Binkoski AE, et al. (2002) Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. The American journal of medicine 113: 71-88.
7. Hao H, Zhang DX, Zhang MY, Guo LX, Li SJ (2003) Phylogenetics of Bauhinia subgenus Phanera (Leguminosae: Caesalpinioideae) based on ITS sequences of nuclear ribosomal DNA. Botanical Bulletin of Academia Sinica 44: 223-228.
8. Chamratpan S, Homchuen SA (2005) Ethnobotany in Upper Northeastern Thailand. ISHS Acta Horticulturae, 675: 67-74.
9. Bunluepuech K, Tewtrakul S (2011) Anti-HIV-1 integrase activity of Thai medicinal plants in longevity preparations. Songklanakarin Journal of Science & Technology 33: 693-697.
10. Kaewpiboon C, Lirdprapamongkol K, Srisomsap C, Winayanuwattikun P, Yongvanich T (2012) Studies of the in vitro cytotoxic, antioxidant, lipase inhibitory and antimicrobial activities of selected Thai medicinal plants. BMC complementary and alternative medicine 12: 217.
11. Sayed EA, Martiskainen O, Seif el-Din SH, Sabra ANA, Hammam OA, et al. (2014) Hepatoprotective and antioxidant effect of Bauhinia hookeri extract against carbon tetrachloride-induced hepatotoxicity in mice and characterization of its bioactive compounds by HPLC-PDA-ESI-MS/MS. Biomed Research International 2014: 1-9.
12. Sayed EA, Abdel-Daim MM, Kilany OE, Karonen M, Sinkkonen M (2015) Protective role of polyphenols from Bauhinia hookeri against carbon tetrachloride-induced hepato- and nephrotoxicity in mice. Renal Failure 37: 1198-1207.
13. Yuenyongsawad S, Bunluepuech K, Wattanapiromsakul C, Tewtrakul S (2013) Anti-cancer activity of compounds from Bauhinia strychnifolia stem. J Ethnopharmacol 150: 765-769.
14. Koleva II, van Beek TA, Linssen JP, Groot AD, Evstatieva LN (2002) Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochemical analysis 13: 8-17.
15. Benzie IF, Strain JJ (1999) Ferric reducing/ antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods in enzymology 299: 15-27.

16. Murakami A, Takahashi D, Koshimizu K, Ohigashi H (2003) Synergistic suppression of superoxide and nitric oxide generation from inflammatory cells by combined food factors. Mutat Res 523-524: 151-61.

17. Murakami A, Ishida H, Kobo K, Furukawa I, Ikeda Y, et al. (2005) Suppressive effects of Okinawan food items on free radical generation from stimulated leukocytes and identification of some active constituents: implications for the prevention of inflammation-associated carcinogenesis. Asian Pacific Journal of Cancer Prevention 6: 437-448.

18. Dechayont B (2011) Biological activities of Antidesma Thwaitesianum Mull. Arg. fruits and leaves. Unpublished Master of Science's thesis, Thammasat University, Faculty of Medicine, p: 656.

19. Folin O, Ciocalteu V (1927) On tyrosine and tryptophan determination in proteins. Journal of biological Chemistry 73: 627-650.

20. Miliauskas G, Venskutonis PR, Van Beek TA (2004) Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chemistry 85: 231-237.

21. Sanchez-Moreno C (2002) Methods used to evaluate the free radical scavenging activity in foods and biological systems. Food Science and Technology International 8: 121-137.

22. Argolo AC, Sant'Ana AE, Pletsch M, Coelho LC (2004) Antioxidant activity of leaf extracts from Bauhinia monandra. Bioresour Technol 95: 229-233.

23. Menezes FDS, Minto ABM, Ruela HS, Kuster RM, Sheridan H, et al. (2007) Hypoglycemic activity of two Brazilian Bauhinia species: Bauhinia forficata L. and Bauhinia monandra Kurz. Revista Brasileira de Farmacognosia, 17: 8-13.

24. Kumar RS, Sivakumar T, Sunderam RS, Gupta M, Mazumdar UK, et al. (2005) Antioxidant and antimicrobial activities of Bauhinia racemosa L. stem bark. Braz J Med Biol Res 38: 1015-1024.

25. Aliyu A, Ibrahim M, Musa A, Ibrahim H, Abdulkadir I, et al. (2009) Evaluation of antioxidant activity of leave extract of Bauhinia rufescens Lam. (Caesalpiniaceae). Journal of Medicinal Plants Research, 3: 563-567.

26. Chew YL, Chan EWL, Tan PL, Lim YY, Stanslas J, et al. (2011) Assessment of phytochemical content, polyphenolic composition, antioxidant and antibacterial activities of Leguminosae medicinal plants in Peninsular Malaysia. BMC complementary and alternative medicine. 11: 12.

27. Zakaria ZA, Wen LY, Abdul Rahman NI, Abdul Ayub AH, Sulaiman MR, et al. (2007) Antinociceptive, anti-inflammatory and antipyretic properties of the aqueous extract of Bauhinia purpurea leaves in experimental animals. Medical Principles and Practice 16: 443-449.

28. Ferreres F, Llorach R, Gil-Izquierdo A (2004) Characterization of the interglycosidic linkage in di-, tri-, tetra- and pentaglycosylated flavonoids and differentiation of positional isomers by liquid chromatography/electrospray ionization tandem mass spectrometry. Journal of Mass Spectrometry 39: 312-321.

29. Aderogba M, McGaw L, Ogundaini A, Eloff J (2007) Antioxidant activity and cytotoxicity study of the flavonol glycosides from Bauhinia galpinii. Natural product research 21: 591-599.

30. de Sousa E, Zanatta L, Seifriz I (2004) Hypoglycemic effect and antioxidant potential of kaempferol-3,7-O-(alpha)-dirhamnoside from Bauhinia forficata leaves. Journal of Natural Products, 67: 829-832.

31. Rice-Evans C, Miller N, Paganga G (1997) Antioxidant properties of phenolic compounds. Trends in Plant Science 2: 152-159.

32. Hatano T, Edamatsu R, Hiramatsu M, Okuda T (1989) Effects of the interaction of tannins with co-existing substances. VI: Effects of tannins and related polyphenols on superoxide anion radical, and on 1,1-diphenyl-2-picrylhydrazyl radical. Chemical and Pharmaceutical Bulletin 37: 2016-2021.

33. Apak R, Guclu K, Demirata B, Ozyurek M, Celik SE, et al. (2007) Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC assay. Molecules, 12: 1496-1547.