Antioxidant and Anticancer Activities of *Buchanania siamensis* Miq. Stem and Leaf Extracts

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Abstract

Buchanania siamensis (BS) is a local plant found in mixed deciduous forests of eastern and north-eastern Thailand. The plant has been used as traditional medicine for treatment of food poisoning, gingivitis and aphthous stomatitis. Its stems (S) and leaves (L) were extracted by using dichloromethane (1) and methanol (2) and the extracts were called BSS1, BSS2, BSL1 and BSL2, respectively. Thin layer chromatography fingerprint, antioxidant activity, total phenolic content and anticancer activity of the extracts were studied. The methanolic extracts contained higher total phenolic content than those of dichloromethane extracts but there was no significant difference between stems and leaves. BSS2 showed higher antioxidant activity (EC_{50} of 7.1±0.1 µg/ml) than BSL2, BSS1 and BSL1, respectively. BSL1 exhibited the highest cytotoxicity against HaCaT, HepG2, MCF-7 and MDA-MB-231 cells by IC_{50} of 119.41±4.80, 196.47±41.36, 264.76±8.50 and 289.81±36.57 µg/ml, respectively. However, the BSL1 showed the lowest selectivity index (SI) against those cancerous cell lines (SI of 0.61, 0.45 and 0.41, respectively). Interestingly, the extracts could significantly inhibit the proliferation of HepG2 human hepatoma cells better than MCF-7 and MDA-MB-231 human breast cancer cells. In addition, the SI between HepG2 and HaCaT cells of BSS2 was 2.25, which was higher than BSL2, BSS1 and BSL1 (SI of 1.58, 0.88 and 0.61, respectively) and correlated to its antioxidant activity. These results suggested that *B. siamensis* extracts have high antioxidant and anticancer activities, especially BSS2. Therefore, *B. siamensis* extracts are a good candidate for further studies in herbal medicine and natural product development.

Keywords: *Buchanania siamensis*, Antioxidant, Anticancer, Phenolic Content

Introduction

Buchanania siamensis Miq. (family: Anacardiaceae) has many local Thai names, such as Si thanon chai, Rang thai (Northeastern) and Phang phuai nok, Thanon chai (Central). It is a large perennial shrub or small tree that is widely distributed in tropical areas, and mixed deciduous forests in eastern and north-eastern Thailand. The young shoots and leaves are locally consumed as fresh vegetables and appetizers [1]. The plant has been used as a Thai traditional medicine and medicinal formulation according to local wisdom. Stems and roots have also been used to relieve food poisoning and fever [1-2]. The stem and bark have been used for the treatment of gingivitis, aphthous stomatitis and herpes simplex virus infection [1]. However, to our knowledge, there is no scientific report of its chemical and pharmacological activities. Therefore, *B. siamensis* stem and leaves were selected in order to study their chemical composition and the cytotoxic effect of extracts on cancerous and non-cancerous cell lines.

Methodology

Extract preparation

The stems and leaves of *B. siamensis* Miq. were collected from a community forest in Sa Kaeo province, eastern part of Thailand in April 2015. The plant was identified by Dr. Chakkrapong Rattamanee, Faculty of Agricultural Technology, Burapha University, Thailand. Its herbarium specimen is C. Rattamanee M273 and it is kept at the Faculty of Agricultural Technology, Burapha University and at the Bangkok Herbarium, Department of Agriculture, Bangkok. The air-dried stems (2.3 kg) of *B. siamensis* were extracted with CH_2Cl_2 and MeOH successively (each 2 × 10 L, for 5 days) at room temperature. The crude extracts were evaporated under reduced pressure to afford brownish CH_2Cl_2 (6.09 g) and MeOH (97.80 g) extracts, respectively. Ground-dried leaves (316.69 g) of *B. siamensis* were extracted with CH_2Cl_2 and MeOH (each 2 × 5 L, for 5 days) successively at room temperature and the solvent was evaporated under reduced pressure to afford CH_2Cl_2 (10.60 g) and MeOH (18.37 g) extracts, respectively. *B. siamensis* leaf extracts from CH_2Cl_2 and

MeOH fractions were called as BSL1 and BSL2, respectively. *B. siamensis* stem extracts from CH_2Cl_2 and MeOH fractions were called as BSS1 and BSS2, respectively.

Thin layer chromatography

The extracts were dissolved in methanol at 1 mg/ml and then spotted onto TLC Silica gel 60 F254 plates (Merck KGaA, Germany). One dimensional TLC analysis was performed with EtoAC:Hex:Acetic acid in volume ratio of 60:3.93:0.07, respectively, as a mobile phase. Lane 1 to 11 were epigallocatechin (EGC), vanillic acid, gallic acid, BSL1, BSL2, BSS1, BSS2, catechin, quercetin, caffeic acid and tannic acid, respectively. Spots were observed under Ultra-Violet light (UV light) at 254 nm and 360 nm. The plates were then sprayed with 30% sulfuric acid, anisaldehyde-sulfuric, DPPH solution and observed (Figure 1).

Study of Antioxidant

DPPH radical scavenging assay [3] was used for determination of antioxidant activity of the extracts. Briefly, 1 mM DPPH (2,2-diphenyl-picryl hydrazine) was freshly prepared by dissolving in methanol. The extracts were diluted to various concentrations by using methanol. The DPPH solution (200 µl) was added to diluted extracts (2800 µl) and then incubated at room temperature for 15 min. The absorbance of bleaching solution was measured at 515 nm. The percent of inhibition was calculated and expressed as 50% effective concentration (EC_{50}). Ascorbic acid and tocopherol were used as positive compounds for comparison.

Study of Total phenolic content

Folin-Ciocalteu method [4] was used for determination of total phenolic content. Briefly, tannic acid (0.5 ml), a standard phenolic compound, was diluted with methanol to various concentrations and Folin reagent (0.25 ml) was added with 20% sodium carbonate solution (0.25 ml). After incubation at room temperature for 40 min, the absorbance of the solution was measured at 725 nm. The standard curve was plotted. The extracts were diluted and then mixed with Folin reagent and 20% sodium carbonate solution as the description above. The sample absorbance was compared to the standard curve and expressed as tannic acid equivalent (mg TAE/g).

Anticancer determination

The extracts and the standard medicine, tamoxifen, were dissolved in dimethyl sulfoxide and filtered by using 0.4 µm syringe filter. The cell lines in this study were non-cancerous cells i.e. human keratinocytes or HaCaT and cancerous cells i.e. human hepatoma (HepG2) and human breast cancer cells i.e. MCF-7 and MDA-MB-231 cells. These cells were cultured in 96-well plate (10,000 cells/well) by using DMEM culture medium supplemented with 10% FBS and 1% Penicillin-Streptomycin and overnight incubated at 37ºC in a humidified atmosphere with 5% CO_2. The cells were added with various concentrations of either extracts or tamoxifen and then incubated for 24 h. Cell viability was measured by using Alamar blue assay [5]. The cytotoxicity was calculated to % cytotoxicity and then expressed as 50% inhibitory concentration (IC_{50}). The selectivity index (SI) was calculated by using the following equation.

SI = IC_{50} from non-cancerous cells / IC_{50} from cancerous cells

Statistical analysis

The results were reported in mean ± S.D. One-Way ANOVA and multiple comparison (LSD) were analyzed by using SPSS version 16.0.

Results

The phytochemical screening (Figure 1) of CH_2Cl_2 leaf extracts (BSL1) revealed the absence of vanillic acid and gallic acid while MeOH leaf extracts (BSL2) revealed the absence of caffeic acid and tannic acid. The CH_2Cl_2 stem extracts (BSS1) revealed the presence of vanillic acid and quercetin while MeOH stem extracts (BSS2) revealed the presence of EGC, vanillic acid, catechin, quercetin and caffeic acid. In addition, all extracts showed positive chemical reaction screening by using DPPH spray reagent (Figure 1E) suggesting that the extracts had antioxidative activity. In Table1, the methanolic extracts (BSL2 and BSS2) can be seen to contain higher total phenolic content than those of dichloromethane extracts (BSL1 and BSS1). However, there was no significant difference between stem and leaf extracts. For the DPPH assay, BSS2 showed stronger antioxidant activity (EC_{50} of 7.1±0.1 µg/ml) than BSL2, BSS1 and BSL1, respectively (Table 1).

The extracts were tested for their inhibitory effect on both cancerous and non-cancerous cell lines to determine the SI (Table 2). Although BSL1 exhibited the highest cytotoxicity against HaCaT, HepG2, MCF-7 and MDA-MB-231 cells with IC_{50} of 119.41±4.80, 196.47±41.36, 264.76±8.50 and 289.81±36.57 µg/ml,

respectively, BSL1 showed the lowest SI compared with the other extracts. BSL2 showed a lower cytotoxic effect than BSL1, especially in MCF-7 and MDA-MB-231 cells where the extract had no cytotoxic effect in the concentration range of 50 to 800 µg/ml. Similarly, BSS2 showed no cytotoxic effect against MCF-7 and low cytotoxicity against MDA-MB-231 cells. These results suggest that the dichloromethane extracts were more cytotoxic than the methanolic extracts. In addition, the extracts from leaves showed higher cytotoxic effect than stem extracts. Moreover, all extracts could significantly inhibit the proliferation of human hepatoma, HepG2 cells, better than human breast cancer cells, MCF-7 and MDA-MB-231 cells. The SI between HepG2 and HaCaT cells of BSS2 was 2.25, which was higher than BSL2, BSS1 and BSL1, respectively. However, there was no significant difference between IC_{50} of extracts against estrogen receptor positive MCF-7 breast cancer cells and estrogen receptor negative MDA-MB-231 breast cancer cells.

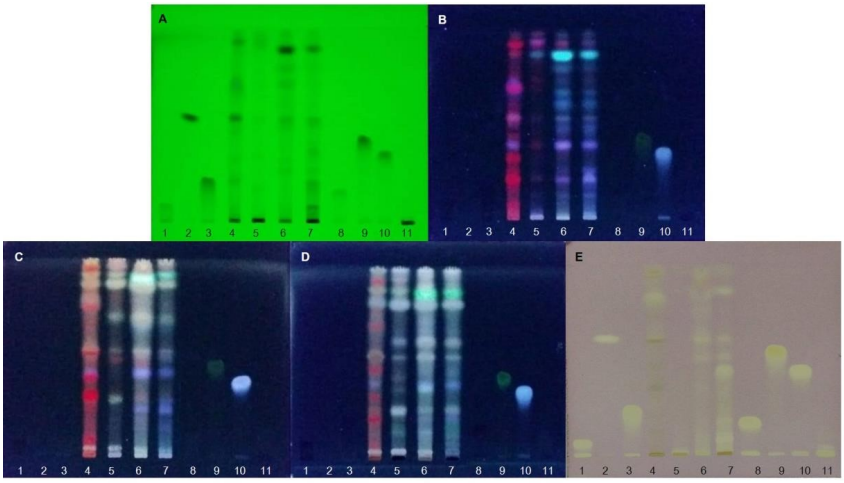

Figure 1. TLC fingerprint of *B. siamensis* extracts. A) 254 nm, B) 360 nm, C) Anisuldehyde-sulfuric under 360 nm, D) 30% sulfuric under 360 nm and E) DPPH spray reagent. Lane 1: EGC, 2: vanillic acid, 3: gallic acid, 4: BSL1, 5: BSL2, 6: BSS1, 7: BSS2, 8: catechin, 9: quecetin, 10: caffeic acid and 11: tannic acid.

Table 1 Antioxidant activity and total phenolic content of *B. siamensis* extracts

Extracts/Standard	Antioxidant	Total phenolic
BSL1	750.1±52.4^{a}	6.2±0.4^{A}
BSL2	10.0±0.2^{b}	206.7±12.8^{B}
BSS1	13.5±1.1^{c}	6.4±0.5^{A}
BSS2	7.1±0.1^{d}	192.3±8.7^{B}
Ascorbic acid	3.5±0.1^{e}	ND
Tocopherol	6.7±0.1^{f}	ND

Note: ND represents no determination.

Discussion and Conclusion

Phenolic compounds are widely dispersed in the plant kingdom with structures ranging from one aromatic ring with one or more hydroxyl groups attached to large and complex phenolic structures [6]. Phenolic substances are better extracted with high polarity solvents such as methanol, ethanol, acetone and ethyl acetate than low polarity solvents [7]. Therefore, the methanolic extracts of *B. siamensis* in this study contained higher amount of phenolics than dichloromethane extracts. Although the extracts were screened for their phenolic composition using HPLC, there was no compatible spectrum peak with the general standards of phenolic compounds (data not shown). However, the TLC mobile phase system and fingerprints from *B. siamensis* in this study can be used for quality control of the extracts.

Table 2 IC_{50} and SI values of *B. siamensis* extracts

Ext. /Cpd	Inhibitory effect						
	HaCaT	HepG2		MCF-7		MDA-MB-231	
	IC_{50} (µg/ml)	IC_{50} (µg/ml)	SI	IC_{50} (µg/ml)	SI	IC_{50} (µg/ml)	SI
BSL1	$119.41\pm4.80^{a,A}$	$196.47\pm41.36^{a,B}$	0.61	$264.76\pm8.50^{a,C}$	0.45	$289.81\pm36.57^{a,C}$	0.41
BSL2	$434.12\pm16.56^{b,A}$	$275.27\pm37.47^{b,c,B}$	1.58	$>800^{b,C}$	0.54	$>800^{b,C}$	0.54
BSS1	$256.34\pm6.63^{c,A}$	$291.25\pm13.75^{c,B}$	0.88	$312.97\pm32.74^{c,B,C}$	0.82	$341.43\pm5.50^{c,C}$	0.75
BSS2	$490.24\pm16.10^{d,A}$	$218.34\pm38.75^{a,b,B}$	2.25	$>800^{b,C}$	0.61	$778.48\pm15.09^{b,C}$	0.63
Tamoxifen	$5.62\pm0.05^{e,A}$	$11.03\pm0.47^{d,B}$	0.51	$4.18\pm0.14^{d,C}$	1.34	$8.42\pm0.05^{d,D}$	0.67

Note: a to e represented significant difference of IC_{50} between extracts in the same cell line (column).

A to D represented significant difference of IC_{50} between cell lines in the same extract (row).

B. siamensis extracts showed anticancer activity *in vitro*, especially BSS2 which showed the highest SI, which represents cytotoxicity against cancerous cells compared to non-cancerous cells. In addition, BSS2 showed higher antioxidant activity than the other extracts which correlated with the high amount of total phenolic content and TLC fingerprint. Plenty of phenolic compounds have been reported to have anticancer activity both *in vitro* and *in vivo* [6-7]. For example, gallic acid was reported to have a cytotoxic effect on several cancer cells and in mouse models via inhibition of cell proliferation, ribonucleotide reductase, cyclooxygenases and angiogenesis and also by apoptotic induction [8-10]. Several flavonoids, e.g. quercetin, kaempferol and catechin, are also well-known non-enzymatic antioxidants which show strong anticancer activity [6-7]. The antioxidants are reported to inhibit mechanisms of cancer progression including inhibition of phase I enzymes, induction of phase II enzymes, stimulation of DNA repair, induction of cell cycle arrest, induction of apoptosis, inhibition of cell proliferation and antiangiogenesis [7]. Therefore, natural phenolics are a great source of antioxidants and anticancer agents.

In conclusion, the extracts from *B. siamensis* stem and leaves are promising candidates for further studies and development of herbal health products, especially the methanolic extract from its stem.

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