

## RESEARCH ARTICLE

# Antioxidant Effect of Berberine and its Phenolic Derivatives Against Human Fibrosarcoma Cells

Veerachai Pongkittiphan<sup>1</sup>, Warinthorn Chavasiri<sup>1</sup>, Roongtawan Supabphol<sup>2\*</sup>

### Abstract

Berberine (B1), isolated from stems of *Coscinium fenestratum* (Goetgh.) Colebr, was used as a principle structure to synthesize three phenolic derivatives: berberrubine (B2) with a single phenolic group, berberrubine chloride (B3) as a chloride counter ion derivative, and 2,3,9,10-tetra-hydroxyberberine chloride (B4) with four phenolic groups, to investigate their direct and indirect antioxidant activities. For DPPH assay, compounds B4, B3, and B2 showed good direct antioxidant activity ( $IC_{50}$  values=10.7±1.76, 55.2±2.24, and 87.4±6.65  $\mu$ M, respectively) whereas the  $IC_{50}$  value of berberine was higher than 500  $\mu$ M. Moreover, compound B4 exhibited a better DPPH scavenging activity than BHT as a standard antioxidant ( $IC_{50}$ =72.7±7.22  $\mu$ M) due to the *ortho* position of hydroxyl groups and its capacity to undergo intramolecular hydrogen bonding. For cytotoxicity assay against human fibrosarcoma cells (HT1080) using MTT reagent, the sequence of  $IC_{50}$  value at 7-day treatment stated that B1 < B4 < B2 (0.44±0.03, 2.88±0.23, and 6.05±0.64  $\mu$ M, respectively). Berberine derivatives, B2 and B4, showed approximately the same level of CAT expression and significant up-regulation of SOD expression in a dose-dependent manner compared to berberine treatment for 7-day exposure using reverse transcription-polymerase chain reaction (RT-PCR) assays. Our findings show a better direct-antioxidant activity of the derivatives containing phenolic groups than berberine in a cell-free system. For cell-based system, berberine was able to exert better cytotoxic activity than its derivatives. Berberine derivatives containing a single and four phenolic groups showed improved up-regulation of SOD gene expression. Cytotoxic action might not be the main effect of berberine derivatives. Other pharmacological targets of these derivatives should be further investigated to confirm the medical benefit of phenolic groups introduced into the berberine molecule.

**Keywords:** Berberine - berberine derivatives - antioxidant gene expression - human fibrosarcoma cells

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### Introduction

Reactive oxygen species (ROS) are oxygen-centered free radicals produced in mitochondria *via* aerobic metabolism or received from the external sources including infections, dietary intake, pollution, and cigarette smoking (Hwang et al., 2002). A low level of ROS affects several cellular processes, i.e. cell proliferation, signal transduction, development of cells, and necrotic or apoptotic cell death (Finkel and Holbrook, 2000). A moderate to high level of ROS in oxidative stress can interfere the intracellular antioxidant system and damage cytoplasmic biomolecules such as protein, lipid, DNA, and RNA (Park et al., 2003). Cells can normally maintain an optimal level of ROS by reducing oxidative stress *via* enzymatic and non-enzymatic systems. Major enzymatic antioxidants frequently mentioned are superoxide dismutase (SOD), catalase (CAT), glutathione peroxidases (GPx), and glutathione reductase (GR) ('t Hoen et al., 2003). ROS are key molecules causing several diseases including Alzheimer, cancer, inflammation, rheumatoid arthritis, diabetes, etc (Pohanka, 2013). In

cancer, ROS can influence mutations and alter gene functions resulting in carcinogenesis through oxidative process (Tangjitjaroenkun et al., 2012).

Healthy individuals have higher plasma levels of antioxidant enzymes (SOD, CAT, and GPx) than patients with oral cancer. In contrast, up-regulation or enhancement of antioxidant enzyme activities including SOD, CAT, and GPx has been proposed as an effective strategy for both cancer prevention and therapy (Khan et al., 2013). For example, progestin induction for catalase activity has been proven to be effective against breast cancer (Petit et al., 2009). Taurine, an abundant free amino acid, was reported to decrease ROS levels and increase the expression of antioxidant enzymes: SOD, GPx, and CAT in the B16F10 melanoma cell line (Yu and Kim, 2009).

Recently, numerous scientific research has shown new antioxidants from natural products for chemoprevention and cancer therapy. Many secondary metabolites from plants, such as alkaloids, are interesting for its potential use in a variety of diseases especially cancer. Berberine, an isoquinoline alkaloid, has been used for traditional medical systems in eastern countries. It possesses several

<sup>1</sup>Natural Products Research Unit, Department of Chemistry, Chulalongkorn University, Pathumwan, <sup>2</sup>Department of Physiology, Faculty of Medicine, Srinakharinwirot University, Bangkok, Thailand \*For correspondence: roongs@swu.ac.th

biological activities including anti-inflammatory (Chu et al., 2014), anticancer (Yip and Ho, 2013; Cai et al., 2014), anti-diabetic (Rajendran et al., 2014), antibacterial (Wojtyczka et al., 2014), and antifungal functions (Zhu et al., 2014). The antioxidant capacity of berberine has demonstrated both direct (Jang et al., 2009; Bashir and Gilani, 2011) and indirect activities (Hwang et al., 2002; Tan et al., 2007). Recently, berberine has been structurally modified to yield new derivatives which are able to improve their biological functions: antibacterial (Zuo et al., 2012; Zhang et al., 2013), antifungal (Liu et al., 2014), antiprotozoal (Bahar et al., 2011), and anticancer activities (Zhang et al., 2012; Lo et al., 2013).

Our study aims to modify berberine structure to improve its antioxidant activity. Phenolic derivatives of berberine were synthesized and investigated both direct and indirect antioxidant activities. Scavenging capacity was determined using DPPH assay. Cytotoxic effect against human fibrosarcoma cells (HT1080) was performed including gene expression of antioxidant genes, SOD and CAT.

## Materials and Methods

### Chemical and general procedure

All commercially available reagents were purchased from Sigma-Aldrich. Structures of all compounds were characterized by  $^1\text{H}$  NMR (400 MHz) and  $^{13}\text{C}$  NMR (100 MHz). Chemical shifts are reported in ppm relative to  $\delta$  values for  $^1\text{H}$  and  $^{13}\text{C}$  NMR in ppm relative to  $\text{CDCl}_3$ ,  $\text{DMSO}-d_6$ , or  $\text{CD}_3\text{OD}$  while coupling constants ( $J$ ) are reported in Hertz (Hz). Thin layer chromatography was also performed using 60 Å silica gel F-254.

### Extraction of berberine (B1)

Stems of *Cosciniium fenestratum* (Goetgh.) Colebr were purchased from local drug store in Bangkok during March 2011. Then they were ground and soaked in MeOH for 5 days (3 times). The methanolic extract was acidified, adjusted to pH=2 by conc. HCl, filtered to collect a yellow precipitate, and finally recrystallized in MeOH to obtain berberine chloride as a yellow powder with 4.8% yield (Nair et al., 1992).

Berberine chloride (B1) showed  $R_f=0.34$  on TLC (10% MeOH: $\text{CH}_2\text{Cl}_2$ ) and  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ ) displayed signal peaks at  $\delta$  9.65 (s, 1H), 8.54 (s, 1H), 7.99 (d,  $J=9.0$  Hz, 1H), 7.88 (d,  $J=9.0$  Hz, 1H), 7.50 (s, 1H), 6.84 (s, 1H), 5.99 (s, 2H), 4.83 (t,  $J=6.4$  Hz, 2H), 4.09 (s, 3H), 3.99 (s, 3H), and 3.13 (t,  $J=6.2$  Hz, 2H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CD}_3\text{OD}$ ) found signal peaks at  $\delta$  152.2, 152.0, 149.9, 146.3, 145.8, 139.6, 135.2, 131.9, 128.1, 124.6, 123.3, 121.8, 121.5, 109.4, 106.5, 103.7, 62.6, 57.7, 57.2, and 28.2.

### Synthesis of berberine derivatives (B2-B4)

Berberrubine (B2) was synthesized from berberine chloride (B1) (1.0 g, 2.730 mmol). Compound B1 was stirred and heated to 190-200°C under high vacuum atmosphere for 1h. The reaction then was cooled down to room temperature. As a result, the desired product (657.9 mg, 75%) was obtained as a red solid. Compound B2

showed  $R_f=0.20$  on TLC (10% MeOH: $\text{CH}_2\text{Cl}_2$ ) and  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) observed signal peaks at  $\delta$  9.18 (s, 1H), 7.56 (s, 1H), 7.23 (d,  $J=7.3$  Hz, 2H), 6.74 (s, 1H), 6.48 (d,  $J=7.9$  Hz, 1H), 6.05 (s, 2H), 4.40 (s, 2H), 3.89 (s, 3H), and 3.08 (s, 2H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ) found signal peaks at  $\delta$  167.8, 150.8, 149.0, 148.1, 145.6, 132.9, 131.3, 128.1, 122.2, 120.5, 120.1, 117.4, 108.3, 104.5, 102.9, 101.7, 56.1, 53.2, and 28.6 (Kim et al., 2002).

Berberrubine chloride (B3) was prepared from berberrubine (B2) (300 mg, 0.8385 mmol). Compound B2 was dissolved in water (1 mL) before 1 N HCl (1 mL) was added. The yellow precipitate was soon filtered and dried under high vacuum to receive the desired product (270 mg, 75% yields) as a yellow solid. Compound B3 displayed  $R_f=0.25$  on TLC (10% MeOH: $\text{CH}_2\text{Cl}_2$ ).  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ ) found signal peaks at  $\delta$  9.83 (s, 1H), 8.77 (s, 1H), 8.05 (d,  $J=9.0$  Hz, 1H), 7.79-7.65 (m, 2H), 7.05 (s, 1H), 6.14 (s, 2H), 4.87 (t,  $J=6.1$  Hz, 2H), 4.02 (s, 3H), and 3.29-3.04 (m, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO}-d_6$ ) observed signal peaks at  $\delta$  149.57, 147.59, 145.59, 145.33, 143.67, 136.53, 132.36, 130.41, 125.45, 120.55, 119.75, 118.13, 117.54, 108.36, 105.28, 101.95, 57.01, 54.97, and 26.43.

2,3,9,10-tetra-hydroxyberberine chloride (B4) was synthesized from berberine chloride (B1) (2.50 g, 6.723 mmol). Compound B1 was dissolved in xylene (100 mL) and anhydrous  $\text{AlCl}_3$  (12.5 g, 9.375 mmol) was added. After stirring at 150 °C for 1 h, 5% HCl (250 mL) was added and heated for 15 min in order to dissolve it. The mixture from this reaction was kept cool in ice bath, filtered to collect precipitate, and purified by recrystallization in MeOH to get desired product (1.4943 g, 67%) as a pale yellow solid. Compound B4 found  $R_f=0.11$  on TLC (10% MeOH: $\text{CH}_2\text{Cl}_2$ ) and  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ ) observed signal peaks at  $\delta$  9.50 (s, 1H), 8.28 (s, 1H), 7.58 (d,  $J=8.7$  Hz, 1H), 7.47 (d,  $J=8.7$  Hz, 1H), 7.33 (s, 1H), 6.68 (s, 1H), 3.21 (dt,  $J=3.2, 1.6$  Hz, 2H), and 3.04 (t,  $J=6.2$  Hz, 2H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CD}_3\text{OD}$ ) showed signal peaks at  $\delta$  150.4, 147.0, 145.3, 144.8, 143.1, 138.7, 134.3, 130.2, 128.4, 120.4, 119.8, 119.8, 119.5, 115.7, 113.2, 57.3, and 27.8 (Kim et al., 2001).

### DPPH assay

Berberine and derivative B2-B4 were dissolved with 1% DMSO in EtOH and serial dilutions of all compounds were carried out to give a suitable concentration ( $\mu\text{M}$ ). A serial dilution of 3,5-di-*tert*-4-butylhydroxytoluene (BHT) was used as a positive control. All diluted compounds, 250  $\mu\text{L}$ , were added to 250  $\mu\text{L}$  of 33  $\mu\text{M}$  DPPH in EtOH solution. After incubation at room temperature for 20 min, the absorbance was detected at 520 nm by UV-spectrophotometer (Biotex-synergy-HT). The percentage of scavenged DPPH was measured as % inhibition from the following equation (Tangjitjaroenkun et al., 2012)

$$\% \text{ inhibition} = [(A_{\text{blank}} - A_{\text{compound}}) / A_{\text{blank}}] \times 100$$

$$A_{\text{blank}} = \text{absorbance of blank and } A_{\text{compound}} = \text{absorbance of compound}$$

The concentration of compound exhibited 50% inhibition ( $\text{IC}_{50}$ ) obtained from dose response curve was calculated and used to compare the scavenging ability of

each compounds. All assays were done in triplicate.

#### Cell cultures

Human fibrosarcoma cells (HT1080), received from Professor Thompson EW, Department of Surgery, St. Vincent's Hospital, the University of Melbourne, Australia, were treated in Dulbecco's Modified Eagle Medium (DMEM) (Gibco BRL) supplemented with 10% fetal bovine serum (FBS) (Gibco BRL), 100 U/mL penicillin (Gibco BRL), and 100 µg/mL streptomycin (Gibco BRL). The cells were kept in a CO<sub>2</sub> incubator at 37 °C with humidified air containing 5% CO<sub>2</sub> until 80% confluence and then subcultured twice a week (Yahayo et al., 2013).

#### Cytotoxic assay

The cytotoxic effect to human fibrosarcoma cells (HT1080) of berberine and its derivatives was explored via the proliferation assay using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent as reported previously with some modifications (Lobner, 2000; Yahayo et al., 2013). Briefly, the cells were seeded in 48-well tissue culture plates at a density of 60,000, 30,000, and 20,000 cells/well for 1-, 4-, and 7-day treatments, respectively and grown to 80% confluency. After that, they were treated with several concentrations of berberine, 0.05 to 1.0 µM, and its derivatives, 1 to 12 µM, for 1, 4, and 7 days. After incubation, the cells were washed twice with phosphate-buffered saline and 300 µL of free-serum culture medium containing 1 mg/mL of MTT was added into each well and incubated further for 1h. The medium containing MTT was then replaced by 200 µL of DMSO. The blue crystals of the oxidized MTT (formazan) were quantified by spectrophotometry at 570 nm using ELISA microplate reader (Biotex-synergy-HT). Percentage of proliferation was plotted compared with the control (untreated) group. All assays were done in triplicate.

#### RNA extraction and reverse transcriptase PCR

Cells were treated with berberine and its derivatives, B2-B4, at 0.1 and 1.0 µM for 7 days. Then, total RNA from treated and non-treated fibrosarcoma cells were isolated using Trizol reagent (Invitrogen, USA). RNA purification and concentration were checked by measuring the absorbance at 260 and 280 nm. Same amount of RNA from each sample has taken for reverse transcription into cDNA using SuperScript RT kit (Invitrogen, USA) according to the manufacturer's instructions.

The primers (Table 1) were designed based on the sequences in The GenBank and used to amplify the target genes, SOD, CAT, and GAPDH using i-Taq kit (iNtRON Biotechnology). The PCR products from Bio-Rad C1000

were electrophoretically analyzed on a 2% agarose gel, stained by 2% ethidium bromide, and photographed (Klongpityapong et al., 2013; Supabphol et al., 2013). Each sample was assayed in triplicate.

#### Statistical analysis

SPSS (IBM Singapore Pte Ltd; Registration No.1975-01566-C) was used for statistical analyses. Data are expressed in form of mean value from at least three individual experiments±standard deviation of control. Statistical significance was determined when p<0.05. The Student's t-test was used for statistical comparisons between groups.

## Results

#### Isolated berberine and synthesized derivatives B2-B4

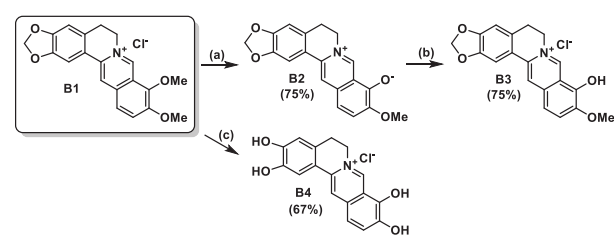
Berberine (B1) was isolated from stems of *C. fenestratum* in 4.8% yield and used as a lead structure to synthesize three phenolic derivatives (Figure 1). Compound B1 was demethylated at 190-200 °C under low pressure to give berberrubine (B2) in 75% yield with a single phenolic group at C-9. This compound was further acidified by 1 N HCl to obtain a chloride counter ion derivative, berberrubine chloride (B3), in 75% yield. 2,3,9,10-tetra-hydroxyberberine chloride (B4) was synthesized using anhydrous AlCl<sub>3</sub> at 150 °C in xylene to remove methyl and methylene groups in 67% yield with four phenolic groups.

#### The antioxidant activity of compounds B1-B4

An antioxidant capacity of compounds B1-B4 were determined using DPPH assay and IC<sub>50</sub> value was used to compare this activity (Figure 2). IC<sub>50</sub> values of B4, B3, and B2 were 10.72±1.76, 55.24±2.24, and 87.35±6.65 µM, respectively. Compound B1 showed the lowest antioxidant activity with an IC<sub>50</sub> value of more than 500 µM. Compounds B4 and B3 demonstrated a better antioxidant activity than BHT standard antioxidant (IC<sub>50</sub>=72.74±7.22 µM).

#### The cytotoxicity of compounds B1-B4 on HT1080

Human fibrosarcoma cells (HT1080) were treated with compounds B1-B4 for 1, 4, and 7 days. MTT assay was used to determine viable and dead cells. The result of cytotoxicity (Figure 3) showed that compounds B1, B2, and B4 significantly decreased the viability of HT1080 at all concentrations with both dose and time-dependent fashion (p<0.05). Compounds B1, B2, and B4 showed

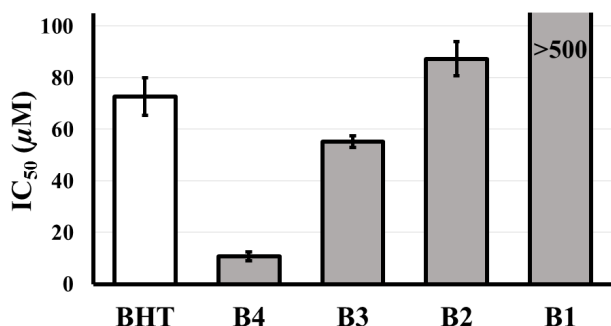


**Figure 1. The Scheme for Synthesis of Derivative B2-B4 from Berberine (B1).** (a) 190-200 °C, high vacuum, 1h; (b) 1 N HCl; (c) AlCl<sub>3</sub>, xylene, 150 °C, 1h

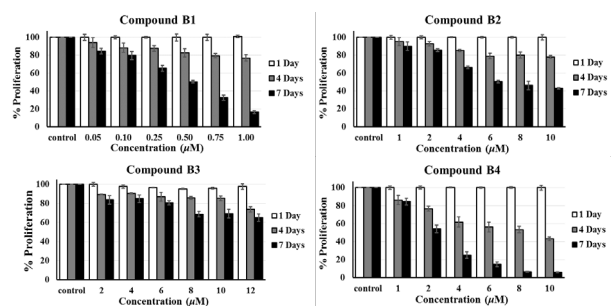
**Table 1. List of Primers were used for PCR Amplification**

Gene	Nucleotide sequence (5'-3')	Size (bp)
SOD	GAGACTTGGGCAATGTGACTG	201
	TTACACCACAAGCCAAACGA	
CAT	CCTGGAGCACAGCATCCAAT	85
	GAATGCCCGCACCTGAGTAA	
GAPDH	AGTCCACTGGCGTCTTCACC	119
	GTTACACCCATGACGAACATG	

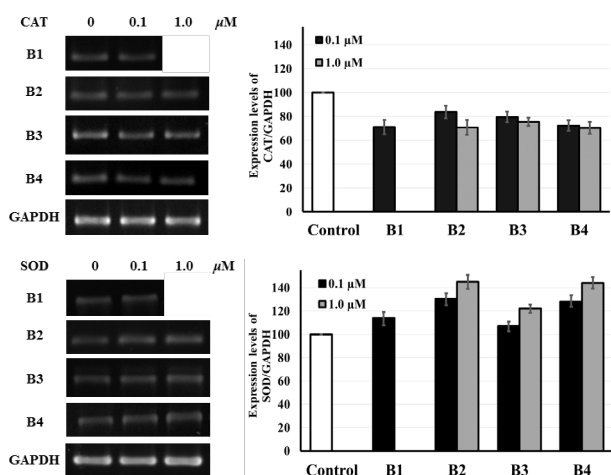
the IC<sub>50</sub> values at 0.44±0.03, 6.05±0.64, and 2.88±0.23 μM, respectively, for 7-day exposure. The IC<sub>50</sub> value of compound B3 was not able to determine due to a higher value than 12 μM.



**Figure 2. The IC<sub>50</sub> of Compounds B1-B4 and BHT.** Each data point represents mean±standard deviation from three independent experiments. All IC<sub>50</sub> were significantly different when compared with that of BHT (p<0.05)



**Figure 3. Effect of Compounds B1-B4 on % Proliferation of HT1080 for 1, 4 and 7 Days.** The % proliferation was calculated compared with control (untreated cells). Each data point represents mean±standard deviation from three independent experiments. Compound B1, B2, and B4 significantly reduced the viability of HT1080 at all concentrations with both dose- and time-dependent fashion for 4- and 7-day exposure only, p<0.05



**Figure 4. The Effect of Compounds B1-B4 on the Expression Levels of CAT and SOD.** HT1080 were treated with compounds B1-B4 for 7 days. Three independent experiments were performed. Compound B1-B4 significantly reduced the CAT expression and increased SOD expression of HT1080 at all concentrations for 7-day exposure, p<0.05

*The Antioxidant gene expression of compounds B1-B4 on HT1080*

Human fibrosarcoma cells (HT1080) were treated with compounds B1-B4 at 0.1 and 1.0 μM for 7 days while gene expression of SOD and CAT was examined via RT-PCR (Figure 4). Primers of SOD, CAT, and GAPDH are shown in Table 1. HT1080 could not survive for 7 days in berberine at 1.0 μM.

Compounds B1-B4 showed significantly down-regulation of CAT expression compared with untreated cells (p<0.05). In contrast, B2-B4 did not show the significant difference in CAT expression if compared with B1. Interestingly, compounds B1-B4 exhibited a significant up-regulation of SOD expression in a dose dependent fashion (p<0.05) compared with the untreated cells. B2 and B4 also showed a better SOD up-regulation than B1.

**Discussion**

Most of the active antioxidants contain more than one active functional group such as NH<sub>2</sub> or OH in *ortho* position. That is the reason why catechol, containing two hydroxyl groups in *ortho* position, is classified as the most active antioxidant compounds due to the ability to trap two peroxy radicals (Valgimigli et al., 2008). More phenolic groups were believed to produce more antioxidant activity as found in the previous report (Bors et al., 1996).

Our work used berberine (B1) isolated from *C. fenestratum* as a lead compound. The phenolic derivatives were synthesized to obtain three compounds, compound B2 with a single phenolic group, compound B3 with a single phenolic group and a chloride counter ion, and compound B4 with four phenolic groups. All compounds were characterized by <sup>1</sup>H and <sup>13</sup>C NMR and found that they were well matched with the reference structure in the previous work (Nair et al., 1992; Kim et al., 2001; Kim et al., 2002).

From DPPH assay, compounds B2-B4 exhibited a good antioxidant activity with IC<sub>50</sub> values lower than 100 μM, whereas that of berberine (B1) was higher than 500 μM. Moreover, compounds B3 and B4 exhibited a better DPPH scavenging activity than the standard antioxidant, BHT. Compound B4 exhibited the best antioxidant activity probably due to the presence of 1,2 hydroxyl groups in its molecule as mentioned in the previous report (Bendary et al., 2013). The better free radical scavenging activity of two hydroxyl groups in *ortho* position should be firstly functioned through the abstraction of first hydrogen atom to generate the stable phenoxy radical via an intramolecular hydrogen bonding and followed by second abstraction (Bendary et al., 2013). For compounds B2 and B3 containing single a phenolic group, chloride counter ion addition into the molecule can significantly increase the scavenging activity.

Compounds B2-B4 with a good scavenging activity were further investigated with human fibrosarcoma cells due to the relationship between antioxidant activity and chemoprevention previously reported (Tangjitjaroenkun et al., 2012; Klongpityapong et al., 2013). For cytotoxicity assay, IC<sub>50</sub> values at 1- and 4-day treatments cannot be

calculated because the survival cells were higher than 50%. That values at 7-day treatment of compound B3 cannot be estimated either. The sequence of IC<sub>50</sub> value at 7-day treatment stated B1 < B4 < B2 (0.44±0.03, 2.88±0.23, and 6.05±0.64 µM, respectively). The compound with a chloride counter ion (B3) might not improve cytotoxicity. In contrast, the compound without (B1) and with four phenolic groups (B4) gave a better cytotoxic effect than those with one phenolic group (B2 and B3).

The further studies were performed to investigate the indirect effect of these compounds to antioxidant enzymes, SOD and CAT in human fibrosarcoma cells (Figure 4). Berberine and its derivatives (B2-B4) showed the down-regulation on CAT expression and up-regulation on SOD expression compared with untreated cells. If the control (untreated cells) was excluded, CAT expression among B1-B4 were approximately the same level. SOD expression was found to be up-regulated in a dose-dependent fashion compared with untreated cells especially for compounds containing a single (B2) and four (B4) phenolic groups. If the control was excluded, SOD expression of B2 and B4 were still higher than B1.

The relationship between SOD-CAT function and mechanistic pathway can be used to suggest that the increasing of SOD expression should elevate the H<sub>2</sub>O<sub>2</sub> concentration from •O<sub>2</sub><sup>-</sup> (Khan et al., 2013). In addition, the decreasing of CAT expression made the redox imbalance because of the less decomposition of H<sub>2</sub>O<sub>2</sub> (Khan et al., 2010). Low CAT expression can also be found in the redox imbalance of endothelial cells exposed to nicotine (Supabphol and Supabphol, 2013). Consequently, the increasing concentration of H<sub>2</sub>O<sub>2</sub> which accumulates intracellularly might damage target biomolecules including protein, lipid, DNA, and RNA (Park et al., 2003). These damages might cause cell death *via* apoptotic or non-apoptotic mechanism (Giorgio et al., 2005).

In conclusion, our results showed that berberine derivatives containing phenolic groups can improve direct-antioxidant activity in cell-free system especially compound B4, because of *ortho* position of hydroxyl groups and the intramolecular hydrogen bonding. However, berberine possessed a better cytotoxic activity against HT1080 than its derivatives. Berberine derivatives containing a single (B2) and four phenolic groups (B4) exhibited a better up-regulation of SOD gene expression. Berberine derivatives with a phenolic group showed the antioxidant potential both extracellular fluid in a cell-free system and intracellular fluid in a cell-based system through the up-regulation of SOD gene expression. The cytotoxic action might not be the main target of berberine derivatives. Moreover, a chloride counter ion might not be important for an indirect antioxidant action. More pharmacological effects of these derivatives, such as antimicrobial, anti-inflammatory activities, should be further investigated for precise conclusion.

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