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Comparison of the protective effects of seven selected herbs against oxidative stress

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ABSTRACT

Objective: To compare the protective effects of the water extracts of seven herbs, including *Solanum indicum* L., *Mallotus repandus* (Wild) Muell-Arg. (MRM), *Bombax malabarica* DC (BMDC), *Tadehagi triquetrum* (L.) Ohashi (TTLO), *Clinacanthus nutans* (Burm f.) Lindau, *Salvia plebeia* R. Br (SPRB), *Ixeris chinensis* Mak (ICM), against *tert*-butylhydroperoxide (*t*-BHP)-induced oxidative stress in Clone 9 cells.

Methods: To evaluate the antioxidant properties of water extracts from seven herbs, reducing ability, metal-chelating activity and radical-scavenging activity such as 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) radical cation and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were determined. In cellular systems, *t*-BHP was used as a model oxidant to induce oxidative stress. 2′,7′-Dichlorofluorescin diacetate and chloromethylfluorescein-diacetate were used as fluorescence probe to determine reactive oxygen species generation and glutathione level in *t*-BHP-induced Clone 9 cells, respectively. In addition, total tannins, total anthocyanins, total polyphenolics and flavonoids were determined.

Results: According to the data obtained from the trolox equivalent antioxidant capacity method, DPPH radical scavenging assay and reducing ability determination, MRM, SPRB, and BMDC showed relatively high antioxidant properties while TTLO and ICM were in the middle and *Solanum indicum* and *Clinacanthus nutans* had relatively low activity. In cellular model systems, SPRB, BMDC, and TTLO showed higher protective effects against *t*-BHP-induced oxidative stress. BMDC, ICM, and TTLO displayed higher inhibitory effects on reactive oxygen species generation in *t*-BHP-induced Clone 9 cells. In addition, SPRB, MRM, and BMDC showed significantly positive modulated glutathione levels. Tannins, anthocyanins, flavonoids and polyphenolics were present in the herbs, which may in part contribute to regulating the oxidative stress.

Conclusions: These results indicated that the seven selected herbs may play a crucial role in regulating oxidative stress, especially BMDC.

1. Introduction

Free radicals, in the form of reactive oxygen and nitrogen species, are generated in the human body. However, an over-production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) can occur due to oxidative stress brought about by the imbalance of the body's antioxidant defense system and free radical formation[1]. These excessive amounts of ROS and RNS may react with biomolecules, leading to cell injury and death[2]. However, the inhibition of ROS formation by exogenous and endogenous bioactive substances may be a useful and effective therapy to prevent oxidative damage and the progression of diseases[3]. Therefore, investigations of natural antioxidants and bioactive compounds for scavenging oxidative stress and certain degenerative disorders have received a great deal of attention.

Chinese herbs are widely accepted and well known medicines in Asia. The biological activities of medicinal herbs are attributed

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mainly to the bioactive compounds which are produced in relatively small amounts in herbs[4]. These medicinal herbs play a crucial role in maintaining human health and improving the quality of human life. It is estimated that over 60% of Americans use complementary medicines, among which the use of herbals, botanicals, vitamins and minerals is significantly popular[5]. Many medical herbs contain phytochemicals such as polyphenol, which plays an important role in inhibiting ROS and RNS formation. These phytochemicals with biological characteristics are associated with lower mortality rates of diseases[6].

Solanum indicum L. (SIL), *Mallotus repandus* (Wild) Muell-Arg. (MRM), *Bombax malabarica* DC (BMDC), *Tadehagi triquetrum* (L.) Ohashi (TTLO), *Clinacanthus nutans* (Burm f.) Lindau (CNL), *Salvia plebeia* R. Br (SPRB) and *Ixeris chinensis* Mak (ICM) have been used as folk medicines in Asia for ages. The choice of the herbs investigated in this study, including SIL, MRM, BMDC, TTLO, CNL, SPRB and ICM as substrates-tested, was based on the fact that these herbs are found in many Asia countries and make significant contributions against a variety of diseases such as hepatoprotection, detoxification, antipyretic, nephralgic remission, antiuratic, liver protective action, and anti-inflammatory for SIL, MRM, BMDC, TTLO, CNL, SPRB and ICM, respectively. However, the literature regarding scientific experiments is limited. Although these seven herbs are commonly used as folk medicines, it remains unclear whether they have any biological effects on modulating oxidative stress and protecting biomolecules. Thus, the aim of the present study was to compare the antioxidant properties of the seven selected herbs and modulation of oxidative stress in a cellular model system.

2. Materials and methods

2.1. Sample preparation

Seven herbs, including SIL, MRM, BMDC, TTLO, CNL, SPRB and ICM were purchased from local market. Each sample (300 g) was extracted twice with boiling water (3 000 mL) for 5 min. Then, the filtrate was freeze-dried and weighed.

2.2. The trolox equivalent antioxidant capacity (TEAC) of herbs

This method is based on the capacity of herbs to scavenge the 2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) radical cation ($ABTS^+$) compared with trolox in a concentration-dependent response. The $ABTS^+$ radical-scavenging activity was measured as previously described[7]. The radical-scavenging capacity was plotted as a function of concentration and the TEAC was calculated against a trolox calibration curve.

2.3. Determination of reducing activity and chelating activity of herbs

The reducing ability and chelating activity of herbs on Fe^{2+} were measured according to the method described by Jain *et al.*[8] and Boonpeng[7], respectively.

2.4. Determination of condensed tannin and total anthocyanins

The content of proanthocyanidins (condensed tannin) and total anthocyanins of herbs were determined according to the method of Liu *et al.*[9] and Elfalleh *et al.*[10] respectively. The total anthocyanins absorbance was calculated using the following formula: $A = [(A_{510} - A_{700})_{pH\ 1.0} - (A_{510} - A_{700})_{pH\ 4.5}]$. Delphindin was used as the standard for a calibration curve.

2.5. Determination of total polyphenolics and flavonoids

The levels of total polyphenolics and flavonoids were measured according to the methods of Dorman *et al.*[11], respectively. The levels of total polyphenolics and flavonoids were determined as gallic acid and rutin equivalents, respectively.

2.6. Measurement of Clone 9 cells viability

Clone 9 cells (ATCC number: CRL-1439) were purchased from Bioresources Collection and Research Center (Shin-chu, Taiwan) and cultured in 100 mL F-12 medium containing 10 mL fetal bovine serum, 2 mmol/L glutamine, and maintained in humidified 95 liters air containing 5 L CO_2 at 37 ℃. After cells were cultured with samples, in the presence of 2 mmol/L *tert*-butylhydroperoxide (*t*-BHP) or not, cell viability was determined by colorimetric measurement of the reduction product of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). After the original medium was removed, MTT (final 0.5 g/L) were added to each well. After 1 h of incubation, the reaction was terminated and the plates were incubated for 30 min to solubilize the formazan dye by addition of dimethyl sulfoxide. The optical density of each well was measured at 570 nm.

2.7. Measurement of intracellular ROS

Intracellular ROS were determined by using the 2′,7′-dichlorofluorescin diacetate (DCFH-DA) fluorescence probe. DCFH-DA was nonfluorescent until removal of the acetate groups by intracellular esterases and reacts with ROS to produce the fluorescent product, dichlorofluorescein (DCF) within the cells. Prior to *t*-BHP stimulation, cells were cultured with DCFH-DA (10 μmol/L). After 20 min of incubation, various concentrations of samples were added to cells for 30 min, and then incubated with *t*-BHP (0.2 mmol/L) for 30 min. The control wells, containing dye, samples and *t*-BHP, did not produce any fluorescent signal. After incubation, ROS produced from cells was determined using a Bio-Tek FLx800 microplate fluorescence reader (Winooski, VT, USA) with excitation and emission wavelengths of 485 and 535 nm, respectively.

2.8. Evaluation of glutathione (GSH) in Clone 9 cells

Intracellular GSH levels were determined after staining cell

with 5-chloromethylfluorescein diacetate (CMF-DA)[12]. CMF-DA passed freely through cell membranes, but once inside the cell, were transformed into cell-impermeant products and react with the thiol group of GSH. After Clone 9 cells were pretreated with samples for 30 min, 0.2 mmol/L *t*-BHP were added to the medium and incubated at 37 ℃ for 4 h. After incubation, cells were washed with phosphate buffer solution and treated with CMF-DA (5 μmol/L) for 30 min. Then, cells were washed with phosphate buffer solution and intracellular GSH was detected by using a Bio-Tek FLx800 microplate fluorescence reader (Winooski, VT, USA) with excitation and emission wavelengths of 485 and 528 nm, respectively.

2.9. Statistical analysis

Statistical analysis involved use of the Statistical Analysis System software package. Analysis of variance was performed by ANOVA procedures. Significant differences between means were determined by Duncan's multiple range tests at a level of $P < 0.05$.

3. Results

Table1 summarizes the antioxidative properties of seven herbal extracts. The scavenging activity of seven herbal extracts at 400 μg/mL on the $ABTS^+$ radical was remarkable (92.74%-93.95%). In addition, in the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging model system, MRM at 400 μg/mL showed significantly higher activity of 92.59% followed by ICM (88.24%), SPRB (87.68%), TTLO (85.73%), BMDC (78.23%), SIL (25.28%) and CNL (13.26%). Clearly, MRM, ICM, SPRB, TTLO and BMDC demonstrated significant hydrogen donating abilities, however, SIL and CNL showed weak scavenging activity.

Table 1

Antioxidant capacities of water extracts of seven herbs.

Sample	ABTS inhibition (%)	DPPH inhibition (%)	Reducing ability (AAE g/kg)	Chelating capacity (%)
SIL	93.76 ± 0.70^{b}	25.28 ± 1.18^{c}	6.41 ± 0.27^{c}	16.04 ± 4.49^{a}
MRM	93.95 ± 0.16^{b}	92.59 ± 0.19^{f}	29.14 ± 3.00^{a}	23.57 ± 2.89^{a}
BMDC	92.83 ± 0.16^{a}	78.23 ± 0.59^{d}	33.24 ± 0.58^{f}	43.97 ± 0.54^{b}
TTLO	92.74 ± 0.74^{a}	85.73 ± 0.13^{e}	23.99 ± 1.35^{e}	26.49 ± 8.79^{a}
CNL	93.11 ± 0.43^{ab}	13.26 ± 1.19^{b}	3.30 ± 0.33^{b}	28.66 ±12.07^{a}
SPRB	92.74 ± 0.56^{a}	87.68 ± 0.21^{a}	29.55 ± 2.07^{a}	23.02 ±11.37^{a}
ICM	93.20 ± 0.32^{ab}	88.24 ± 1.01^{a}	20.91 ± 0.53^{d}	43.17 ± 2.53^{b}

The concentration of sample was 400 μg/mL. Results are mean ± SD (n = 3). Values with different superscripts in a column are significantly different ($P < 0.05$). Reducing ability plotted as a function of concentration and ascorbic acid equivalent (AAE) of sample was calculated against the ascorbic acid calibration curve.

The reducing ability expressed as equivalent to ascorbic acid, was also shown in Table 1. In general, the reducing ability of the seven herbal extracts at 400 μg/mL extracts followed the descending sequence: BMDC > MRM = SPRB > TTLO > ICM > SIL > CNL.

The chelating actions of the samples at 400 μg/mL for BMDC, ICM, CNL, TTLO, MRM, SPRB and SIL were 43.97%, 43.17%, 28.66%, 26.49%, 23.57%, 23.02% and 16.04%, respectively (Table 1). Clearly, the seven herbal extracts showed a weak chelating effect on ferrous ions, compared to the chelating effect of ethylene diamine tetraacetic acid.

In the present study, we further examined the cytotoxic effects of *t*-BHP on Clone 9 cells in the presence and absence of the seven herbal extracts for 4 h. Then, cell viability was determined by the MTT method. It was found that the cell viability in the presence of the seven herbal extracts in the range of 100-400 μg/mL was > 95%, indicating the seven herbal extracts had no cytotoxicity. With the addition of 0.2 mmol/L *t*-BHP to the Clone 9 cells, the cell viability was 59.0% compared to the control group, indicating that 0.2 mmol/L *t*-BHP showed significant cytotoxicity on Clone 9 cells. However, pretreatment of Clone 9 cells with herbal extracts at 400 μg/mL for 0.5 h prior to the addition of 0.2 mmol/L *t*-BHP significantly increased cell viability ($P < 0.05$), with the exception of SIL, compared to the cells treated with *t*-BHP alone (Figure 1).

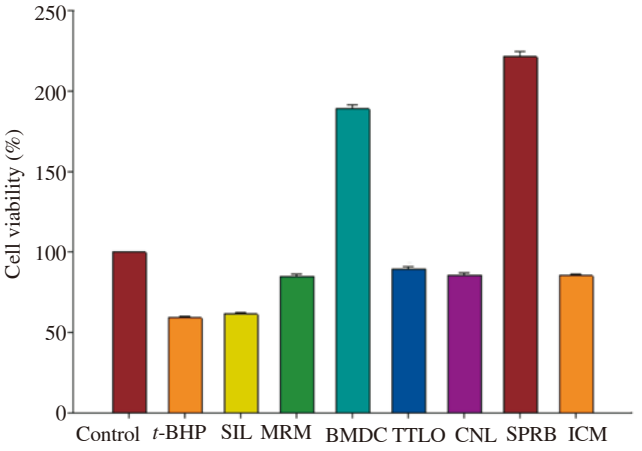

Figure 1. Effect of water extracts of seven herbs on Clone 9 cells viability induced by 0.2 mmol/L *t*-BHP.

To explain the observed cytoprotective effects on Clone 9 cells induced by 0.2 mmol/L *t*-BHP, two markers of cellular oxidative stress, including ROS formation and GSH levels, were examined. As shown in Figure 2, the fluorescent intensity was dramatically increased to 303% of ROS when Clone 9 cells were exposed to 0.2 mmol/L *t*-BHP, indicating that a marked increase in ROS generation was found in Clone 9 cells. However, pretreatment of Clone 9 cells with 400 μg/mL of herbal extracts showed ROS levels of 75.82%, 81.43%, 90.94%, 110.65%, 116.12%, 179.57% and 207.34%, induced by 0.2 mmol/L *t*-BHP for BMDC, ICM, TTLO, MRM, SPRB, SIL and CNL, respectively, compared to the control group (100%). No differences were found among BMDC, ICM, and TTLO in the inhibition of ROS generation ($P > 0.05$).

Figure 3 shows the effects of the seven herbal extracts on the content of GSH in 0.2 mmol/L *t*-BHP-induced Clone 9 cells. The GSH content was dramatically decreased when Clone 9 cells were exposed to 0.2 mmol/L *t*-BHP. However, pretreatment with SPRB, MRM, and BMDC not only prevented against *t*-BHP-induced GSH depletion, but also significantly and positively modulated GSH levels, compared to the cells treated with 0.2 mmol/L *t*-BHP. The positive modulation in this cellular model system was in the order of SPRB > MRM > BMDC. However, no significant differences ($P>0.05$) were found between the other herbal extracts and the cell group treated with 0.2 mmol/L *t*-BHP alone.

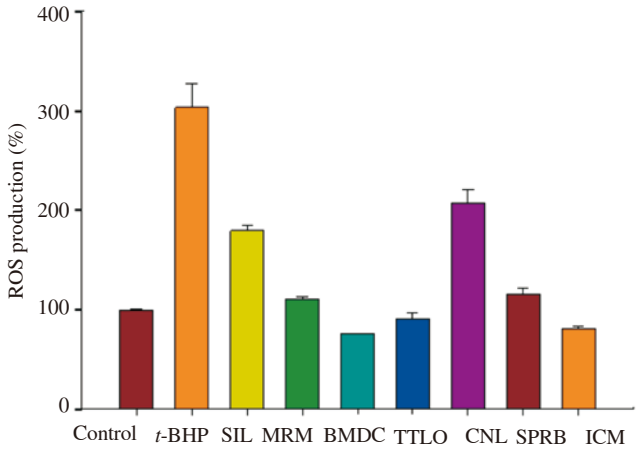

Figure 2. Effect of water extracts of seven herbs on intracellular ROS production in Clone 9 cells induced by 0.2 mmol/L *t*-BHP.

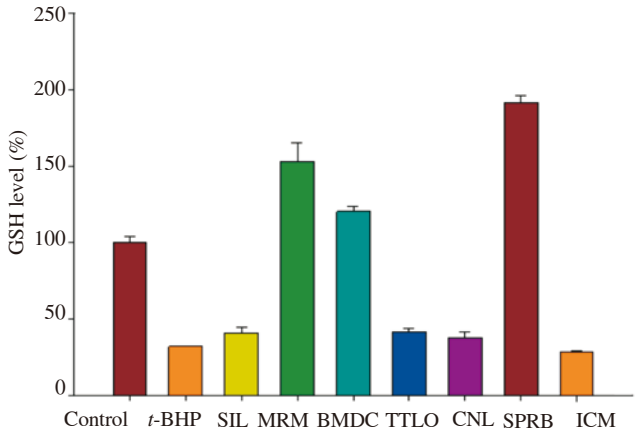

Figure 3. Effect of water extracts of seven herbs on GSH levels in Clone 9 cells induced by 0.2 mmol/L *t*-BHP.

Table 2 summarizes the contents of bioactive compounds, including total phenolics, flavonoids, condensed tannins, total anthocyanins and ascorbic acid, in the seven herbal extracts. Of the seven herbal extracts, MRM, BMDC and TTLO contained condensed tannins, and BMDC contained 0.01 g/kg total anthocyanins of extract. Moreover, among the seven herbal extracts, BMDC contained the highest contents of polyphenolics followed by SPRB, TTLO, MRM, ICM, SIL and CNL. With regard to the contents of total flavonoids, ICM appeared to contain the highest amount of total flavonoids followed by SPRB, BMDC, TTLO, SIL, MRM, and CNL.

Table 2

Contents of phytochemicals in water extracts of seven herbs (g/kg).

Sample	Condensed tannins (catechin)	Total anthocyanins	Total polyphenols (gallic acid)	Flavonoids (rutin)
SIL	ND	ND	50.170 ± 0.022^{f}	15.220 ± 0.003^{e}
MRM	1.24 ± 0.01^{b}	ND	102.220 ± 0.032^{d}	3.440 ± 0.001^{f}
BMDC	1.05 ± 0.05^{a}	0.010 ± 0.001	170.440 ± 0.101^{a}	23.810 ± 0.004^{c}
TTLO	1.94 ± 0.01^{c}	ND	123.660 ± 0.055^{c}	19.050 ± 0.001^{d}
CNL	ND	ND	35.590 ± 0.013^{g}	3.130 ± 0.002^{g}
SPRB	ND	ND	123.880 ± 0.016^{b}	92.970 ± 0.011^{b}
ICM	ND	ND	76.950 ± 0.055^{e}	118.420 ± 0.011^{a}

Results are mean ± SD (n = 3). Values with different superscripts in a column are significantly different ($P < 0.05$). ND: not detected.

4. Discussion

Due to the complexity of oxidation-antioxidation reaction, no simple method is capable of providing a comprehensive survey of the antioxidant profile of a tested sample[13]. Therefore, antioxidant activities of tested samples are often determined by using a range of testing model systems *in vitro*. Thus, several assays were used to evaluate the antioxidant properties of seven herbal extracts.

Free radicals consist of ROS and RNS, which are highly reactive species and involved in many pathological conditions[14]. The seven herbal extracts not only significantly scavenged the $ABTS^{+}$ free radical, a stable hydrophilic free radical, but also, with the exception of SIL and CNL, showed scavenging activity on the DPPH radical, indicating that herbal extracts were able to pair with the odd electrons of $ABTS^{+}$ and DPPH radicals.

The reducing ability was expressed as equivalent to ascorbic acid, because ascorbic acid is a remarkable antioxidant and acts as a reductant. Therefore, the seven herbal extracts exhibited reducing ability, consequently contributing to antioxidant activity. We speculate that the reductants in extracts may be derived from some naturally occurring materials present in extracts. According to the data obtained from the TEAC method, DPPH radical scavenging assay and reducing ability determination, the three model systems provided some interesting results. That is, MRM, SPRB, and BMDC showed relatively high antioxidant properties while TTLO and ICM were in the middle and SIL and CNL had relatively low activity.

The chelating action of the seven herbal extracts on metal ions differed in regard to free radical scavenging activity as well as reducing ability. Some studies have noted that the ability of phenolic compounds to chelate metal ions depends on the availability of a properly oriented functional group[1,15]. In addition, in a complex mixture, sugar, amino acids and organic acids can sequester metal ions[1]. In other words, the various chelating actions of the seven herbal extracts may be the result of different components present in the extracts. In this regard, our results coincide with those of an earlier report[1] which noted no clear relationship between free radical scavenging activity, reducing ability and metal ion chelation activity. Although the seven herbal extracts showed weak chelating effects on ferrous ions compared to ethylenediaminetetraacetic acid, they exhibited significant meaningfulness because the chelating activity of seven herbal extracts toward ferrous ions reduces the concentration of the transition metal in lipid peroxidation[16].

To further understand the antioxidant potential of the seven selected herbal extracts against the oxidative damage in a cellular system, *t*-BHP is used as a model oxidant to induce oxidative stress[17]. Based on the data obtained, SPRB and BMDC are in the high protective group with survival of over 100%. TTLO, CNL, ICM and MRM are in the middle protective group with protective action leading to survival ranging between 85.0%-89.7% as compared to the control group. Of the seven herbal extracts, SIL showed no protection, compared to the cells treated with 0.2 mmol/L *t*-BHP.

The levels of intracellular ROS in Clone 9 cells induced by

0.2 mmol/L *t*-BHP were determined by a fluorescence meter using a peroxide-sensitive fluorescent probe, DCFH-DA. SPRB was ranked first in the protection against Clone 9 cell death induced by 0.2 mmol/L *t*-BHP, but it was fifth in scavenging ROS generation. BMDC was ranked second in the protection against Clone 9 cells death induced by 0.2 mmol/L *t*-BHP, but it ranked first in scavenging ROS generation. The inhibitory effect of the seven herbal extracts on ROS generation differed in relation to the protection against Clone 9 cell death. Among the seven herbal extracts, BMDC consistently showed the highest scavenging ROS generation and the second best protection against Clone 9 cell death induced by 0.2 mmol/L *t*-BHP. SIL showed the lowest protection against Clone 9 cell death and the second lowest scavenging ROS generation among the samples. Yang *et al.* noted that *t*-BHP is a well-known oxidant that induces acute oxidative stress *in vitro* and *in vivo*[18]. It is metabolized to free radical intermediates, such as *t*-butoxyl and methyl radicals, by cytochrome P450, which can cause lipid peroxidation, GSH depletion and DNA damage. The result showed that in the cytotoxicity assay, 0.2 mmol/L *t*-BHP induced Clone 9 cell growth inhibition, and pretreatment with herbal extracts decreased the ROS generation and increased Clone 9 cell survival. In addition, the formation of peroxy radicals generated from 0.2 mmol/L *t*-BHP in the cytosol may interact with ferrous ions, which is similar to Fenton reaction that causes a further increase in ROS production[19], and accelerates lipid peroxidation chain reaction[20]. The results showed that all the samples possessed scavenging of $ABTS^{+}$ and DPPH radical activity, reducing ability as well as chelating ferrous ions. Furthermore, the seven herbal extracts may exert their antioxidative properties, thereby decreasing the level of intracellular ROS. Thus, we suggest that the protective effects of the seven herbal extracts on *t*-BHP-induced Clone 9 cell death are at least in part attributed to the ROS scavenging activity derived from their antioxidant properties.

Concerning the preventive mechanisms of the seven selected herbal extracts, we therefore determined the level of GSH in the Clone 9 cells induced by 0.2 mmol/L *t*-BHP. Owing to the cysteine moiety of GSH, the reduced form of GSH functions both as a reductant and as a nucleophile in order to convert a variety of electrophilic substances under physiological conditions[21]. In addition, GSH, the most abundant non-protein thiol source in the cells, is an important intracellular antioxidant that protects against oxidative stress[22]. Therefore, severe GSH depletion causes cells to be more vulnerable to oxidative damage. Thus, the potential of SPRB, MRM, and BMDC to modulate the maintenance of GSH at remarkably high levels could be expected to protect the cell against *t*-BHP-induced toxicity[23]. In addition, the treatment of SPRB, MRM, and BMDC decreased the *t*-BHP-induced increase in ROS formation, and prevented the decrease in GSH levels induced by *t*-BHP. SPRB was ranked first in modulating GSH content in 0.2 mmol/L *t*-BHP-induced Clone 9 cells. This appeared in parallel with the protection against Clone 9 cell death induced by 0.2 mmol/L *t*-BHP. BMDC was ranked second against protection of Clone 9 cell death and first in scavenging ROS generation induced by 0.2 mmol/L *t*-BHP, but it ranked third in modulating GSH content in 0.2 mmol/L *t*-BHP-induced Clone 9 cells. In addition, SIL, TTLO, CNL and ICM showed no protective effects on the loss of GSH in 0.2 mmol/L *t*-BHP-induced Clone 9 cells, however, they showed antioxidant properties in total antioxidant activity, scavenging of DPPH radicals, reducing ability, chelating iron and reduced ROS generation. These discrepancies could be due to the different intracellular model systems and different experimental protocols.

To evaluate the effects of the phytochemicals of the extracts on oxidative reactions, the contents of bioactive compounds in the seven herbal extracts were determined, including total phenolics, flavonoids, condensed tannins, total anthocyanins and ascorbic acid. The trend of the levels of total polyphenolics in each of the herbal extracts differed in the same order as different total flavonoids. Apparently, the levels of total flavonoids varied in each herbal extract and were not associated with the total polyphenolics in the herbal extracts. Djeridane *et al.* noted that the antioxidant activity of polyphenolics is mainly attributed to their redox properties which make them act as reducing agents, hydrogen donors, ROS scavengers and metallic chelators as well[24]. In addition, many studies have shown that antioxidant properties may be concomitant with the development of reducing ability[8,24]. In the present study, the reducing ability correlated ($r = 0.92$) well with the polyphenolic contents of a given sample, indicating that polyphenolics play an important role in antioxidant capacity of water extracts of seven herbs. Tannins with high molecular weight (Mw > 500) and many phenolic groups are more effective at quenching peroxyl radicals than simple phenolics or trolox[25]. Anthocyanins are a group of abundant and widely consumed flavonoid constituents that occur ubiquitously in the plant kingdom, providing potential effects in reducing the risk of cardiovascular disease, the most common cause of mortality among men and women[26]. BMDC contained condensed tannins and total anthocyanins which were not found in other samples. In addition, BMDC exhibited the highest amounts of total polyphenolic compounds among the seven herbal extracts. This finding may be associated with scavenging of ROS generation and prevention of the depletion of GSH levels in 0.2 mmol/L *t*-BHP-induced Clone 9 cells, resulting in a significantly protective effect against Clone 9 cell death. Nevertheless, unknown compounds other than phenolic compounds also seem to contribute to the protective effect on the oxidative stress of Clone 9 cells. These compounds may be due to the synergism of the components with one another, therefore, accounting for the antioxidant effects of the extracts.

In conclusion, the results obtained in this study clearly indicate that the seven selected herbs showed antioxidant activity in different model systems. Among the seven herbal extracts, BMDC showed the highest inhibitory effect on ROS generation and marked prevention of depletion of GSH levels in *t*-BHP-induced Clone 9 cells. Most likely, BMDC exerted their protective action on cytotoxicity and oxidative damage as a result of bioactive compounds present in BMDC. In addition, these results may

provide an antioxidant background of the efficacies of the seven selected herbs. However, further research is required through *in vivo* testing to validate the relevance of these results.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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