The Thai Journal of Pharmaceutical Sciences

Volume 35
Issue 4 *2011*

Article 4

1-1-2011

Antioxidant properties of squeezed mangosteen juice

Monrudee Sukma

Tanasait Ngawhirunpat

Praneet Opanasopit

Pongpun Siripong

Follow this and additional works at: https://digital.car.chula.ac.th/tjps

Part of the Pharmacology Commons

Recommended Citation

Sukma, Monrudee; Ngawhirunpat, Tanasait; Opanasopit, Praneet; and Siripong, Pongpun (2011) "Antioxidant properties of squeezed mangosteen juice," *The Thai Journal of Pharmaceutical Sciences*: Vol. 35: Iss. 4, Article 4.
Available at: https://digital.car.chula.ac.th/tjps/vol35/iss4/4

This Article is brought to you for free and open access by the Chulalongkorn Journal Online (CUJO) at Chula Digital Collections. It has been accepted for inclusion in The Thai Journal of Pharmaceutical Sciences by an authorized editor of Chula Digital Collections. For more information, please contact ChulaDC@car.chula.ac.th.

Original article

Antioxidant properties of squeezed mangosteen juice

Monrudee Sukma[1*], Tanasait Ngawhirunpat[2], Praneet Opanasopit[2] and Pongpun Siripong[3]

[1]*Department of Pharmacology and Toxicology, Faculty of Pharmacy, Silpakorn University, Muang, Nakhon Pathom, 73000 Thailand*

[2]*Department of Pharmaceutical Technology, Faculty of Pharmacy, Silpakorn University, Muang, Nakhon Pathom, 73000 Thailand*

[3]*Natural Products Research Section, Research Division, National Cancer Institute, Bangkok, Thailand*

**Corresponding author. Tel: +66-34-255800, Fax: +66-34-255801*

E-mail address: monrudee@.su.ac.th

Abstract:

The aim of this study was to investigate antioxidant effects of squeezed mangosteen *(Garcinia mangostana)* juice as preferable diet for human consumption. Mangosteen juice was prepared from the aril part. Antioxidant properties were evaluated by total antioxidant capacity (TAC), ion chelating and biological relevant radical scavenging assays. TAC was performed by 2, 2-azinobis 3-ethylbenzothiazoline 6-sulfonate (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), galvinoxyl radical scavenging and ferric reducing antioxidant power (FRAP) assay. Radical scavenging properties were evaluated by hydroxyl (OH·), superoxide ($O^{\cdot}_2$), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), hypochlorous acid (HOCl) and peroxynitrite ($ONOO^-$) scavenging assays. This investigation reveals free radical scavenging (FRS) activity of mangosteen juice. We found that the IC_{50} values for ABTS and DPPH were 0.23 ± 0.46 and 1.91 ± 0.65 mg/ml respectively. The EC_1 of FRAP was 301.10 ± 8.37 mg/ml. The IC_{50} values for the OH·, $O^{\cdot}_2$ and $ONOO^-$ scavenging were 1.99 ± 0.10, 2.41 ± 0.85 and 0.21 ± 0.03 mg/ml respectively. These findings suggest that mangosteen juice could be another potential source of natural dietary antioxidants. Unfortunately, we found that single application of mangosteen juice (1-300 μg/ml) did not provide the protective effects on H_2O_2- and glutamate-induced neuroblastoma (NG 108-15) cell death and 6-hydroxydopamine-induced human neuroblastoma (SH-SY5Y) cell death. Future neuroprotective studies should be performed by using repeated and long-term applications or a combination of mangosteen juice with other antioxidants.

Keywords: Antioxidant; Free radical scavenging; *Garcinia mangostana;* Mangosteen; Juice

Introduction

Mangosteen (*Garcinia mangostana* Linn.) is a fruit widely grown in Thailand. Thailand is the world's leading supplier of mangosteen. The value of fruits exported from Thailand has increased considerably from USD 28.5 million (74,751 tons) in 1992 to USD 150 million (341,321 tons) in 2002 [1]. In addition, mangosteen remains a large extent for domestic consumption. Mangosteen arils have been consumed in Thailand as fresh fruit and juice, and also desserts. Nowadays, there are numerous commercialized mangosteen products, for example, canned, frozen, freeze-dried and dehydrated mangosteen arils, jam, wine and pasteurized juice. The most famous commercialized product is the juice blends which consist of whole fruit of mangosteen pureed and other combination ingredients. It is claimed for antioxidant, anti-inflammatory and protective effect on neurodegenerative diseases [2]. However, current reports reveal limit clinical efficacy [3] and possible toxicity i.e. severe lactic acidosis [4]. Consequently, interest is shift from the blended mangosteen juice to mangosteen juice from the aril. Unfortunately, little is known about health benefit of mangosteen juice from the aril part. In order to provide basic health-related information for consumer and manufacturer, we aimed to study antioxidant and cytoprotective activities of squeeze mangosteen juice. Although there is an important body of works in the literature on the antioxidant capacities of mangosteen aril, studies on mangosteen juice from the aril are non-existent. In addition, unlike previous publications, in this research, experimental sample was prepared from lyophilized mangosteen juice squeezed from the aril part which is closer to natural intake.

Material and Methods

Chemicals

2,2-diphenyl-1-picrylhydrazyl (DPPH), 2, 2-azinobis 3-ethylbenzothiazoline 6-sulfonate (ABTS), Trolox®, ferrous sulfate, NADH, polyornithine hydrobromide, bovine serum albumin, and trypan blue were purchased from Sigma (St. Louis, MO, USA). Potassium persulfate, 2,4,6,-tripyridyl-s-triazine complex (TPTZ), N-methylphenazonium methyl sulfate (PMS), nitrotetrazolium blue chloride (NBT), Folin-Ciocalteu, gallic acid, and tannic acid were purchased from Fluka (Switzerland). Sodium carbonate, dimethyl sulfoxide (DMSO), and H_2O_2 were purchased from Merck (Darmstadt, Germany).

Samples and preparation

Fresh mangosteen fruits *(G. mangostana)* from Nakorn Pathom province, Thailand were selected in August 2009. They were at same stage of ripeness. The edible portions were squeezed and then filtered. The filtrate was concentrated using a freeze drier. The samples were stored at -20°C until the bioactive properties were analyzed.

Cell line and culture

The neuroblastoma NG 108-15 cells and human neuroblastoma SH-SY5Y cells were donated by Associate Professor Tohda Michihisa, Institute of Natural Medicine, University of Toyama. NG 108-15 cells were grown in condition as previously report [5,6]. SH-SY5Y cells were grown in DMEM containing 10% fetal bovine serum. Cell cultures were maintained in a humidified incubator with 5% CO_2-95% air at 37°C.

Standardization of extract

Determination of the total phenolic content

The amounts of phenolic compounds in the extracts were determined using the Folin-Ciocalteu method as described by Kawpoomhae *et al.* [7], and gallic acid was used as a standard phenolic compound. Fifty microliter aliquots of the extracts (1 mg/ml) were added to a mixture of 2.5 ml of 10% Folin-Ciocalteu reagent and 2 ml of 7.5% Na_2CO_3. After incubation at 45°C for 30 min, the absorbance was measured at 765 nm. A linear dose-response regression curve was generated using absorbance reading of gallic acid (0.1-1.0 mg/ml). The content of total polyphenols in the extract (c) is calculated from C = A/B and expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g) of extracts; A is the equivalent concentration of gallic acid established from the calibration curve (mg); and B is the dry weight of the extract (g).

Determination of tannic acid content

The tannic acid content in the extracts was determined using the method described by Kawpoomhae *et al.* [7]. First, 2 mg of bovine serum albumin was mixed with 1 ml of the sample extract (1 mg/ml) and then kept at room temperature for 20 min. Then, the mixtures were centrifuged and the sediment was dissolved with 0.1% sodium dodecyl sulfate (2 ml), triethanolamine (2 ml) and 10 mM $FeCl_3$ (1 ml). The suspensions were measured spectrophotometrically at 510 nm. The calibration curve was established using standard tannic acid (1.6-2.4 mg/ml).

Determination of ascorbic acid content

The vitamin C samples were pre-derivatised as adapted from method of Raghu *et al.* [8]. The ascorbic acid was oxidised to dehydroascorbic acid (DHAA). The oxidised form was then reacted with o-phenylene diamine (OPDA) to form a flourophore. The DHAA-OPDA derivative was analysed using the excitation wavelength 350 nm and emission wavelength 430 nm. Quantitation of the DHAA-OPDA derivative was made, which was based on a calibration curve generated from standard vitamin C derivative (1-300 μg).

Antioxidant assays

Total antioxidant capacity assays

Scavenging activity for DPPH radicals

Free radical scavenging (FRS) potentials were tested in a methanolic solution of DPPH. The degree of decoloration of the solution indicates the scavenging efficiency of the added substance. DPPH radical form has an absorption band at 515 nm which disappears upon reduction by antiradical compounds. A 200 μM DPPH solution (100 μl) was added to 100 μl of sample. The mixture was left standing at room temperature for 30 min; the absorbance was then measured spectrophotometrically at a wavelength of 515 nm (Fusion Universal Microplate Analyser Model). The results of the assay were expressed as IC_{50}, which represents the concentration of the juice (mg/ml) required to inhibit 50% of FRS activity. The FRS activity was assessed using the equation:

$$\% \text{ inhibition} = (1-(A_{sample}/A_{control})) \times 100 \qquad (1)$$

where A_{sample} and $A_{control}$ are the absorbance in the presence of sample and control respectively. The IC_{50} values were calculated by linear regression of plots where the x-axis represented the various concentrations (mg/ml) of test plant extracts while the y-axis represented the % inhibition.

Scavenging activity for ABTS radicals

ABTS method for screening of antioxidant activity is reported as a decolorization assay applicable to both lipophillic and hydrophilic antioxidants, including flavonoids, hydroxycinnamates, carotenoids, and plasma antioxidants. The pre-formed radical monocation ABTS is generated by oxidation of ABTS with potassium persulfate and is reduced in the presences of both the inhibition of the radical cation absorption when determining the antioxidant activity. $ABTS^+$ radical cation was generated by the interaction of 7 mM ABTS and 4.95 mM potassium persulfate. The mixture was kept in the dark at room temperature for 12-16 h before used. Prior to the assay, the solution was diluted with phosphate buffer pH 7 to give an absorbance of 0.7 ± 0.02 at 734 nm in a 1-cm cuvette. Then, 3.9 ml of the working solution was mixed with 0.1 ml of sample at various concentrations or negative control (phosphate buffer) or positive control (Trolox®). After keeping for 10 min at room temperature, the samples were measured at the wavelength of 734 nm. The inhibition percentage and IC_{50} values were calculated as described in the DPPH assay.

FRAP assay

FRAP assay measures ability of the antioxidants in the investigated samples to reduce ferric-tripyridyltriazine (Fe^{3+}-TPTZ) to a ferrous form (Fe^{2+}), which absorbs light at 593 nm. FRAP reagent was freshly prepared and consisted of acetate buffer (pH 3.6), ferric chloride solution (20 mM) and TPTZ solution (10 mM). The 200 μl of the diluted sample was added to FRAP reagent. Then sodium acetate buffer was added to a final volume of 4 ml. The tubes were vortexed and left for exactly 30 min, and the absorbance was measured at the wavelength of 593 nm. The measurement was compared to a standard curve of $FeSO_4 \cdot 7H_2O$ solutions and expressed as an EC_1 value, which means the

concentration of antioxidant in the reactive system having a ferric-TPTZ reducing ability equivalent to that of 1 mM $FeSO_4 \cdot 7H_2O$.

Scavenging activity onto galvinoxyl radicals

Galvinoxyl, another stable phenoxyl radical can be reduced by hydrogen-donating free radical scavengers. This activity was determined as described in Kawpoomhae *et al.* [7]. A total volume of 900 µl of 1 mM galvinoxyl methanol solution was added to 90 µl of test samples to make a final volume of 990 µl and the mixture was allowed to react at 37°C. After 20 min, the absorbance value was measured at the wavelength of 420 nm. L-ascorbic acid served as a positive control.

Scavenging capacity on biologically relevant oxidants

$O^{\cdot}_2$, OH· and H_2O_2 scavenging

$O^{\cdot}_2$, OH· and H_2O_2 scavenging activities of mangosteen juice was evaluated as reported by Kawpoomhae *et al.* [7].

$^1O_2$ sscavenging

The production of $^1O_2$ was determined by monitoring N, N-dimethyl-4-nitrosoaniline (RNO) bleaching. $^1O_2$ was generated by a reaction between NaOCl and H_2O_2. and the bleaching of RNO was monitored at 440 nm. The scavenging assay was performed in accordance with method described by Hazra *et al.* [9]. The reaction mixture contained 45 mM phosphate buffer (pH 7.1), 100 mM NaOCl, 100 mM H_2O_2, 100 mM histidine, 500 µM RNO and various concentrations of mangosteen juice (0.1-10 mg/ml) in a final volume of 1 ml. It was incubated at 30°C for 40 min and the decrease in RNO absorbance was measured at 440 nm. IC_{50} values of the sample and positive controls, L-ascorbic acid, were calculated using method as described in DPPH assay.

HOCl scavenging capacity

HOCl scavenging capacity was determined according to Padraza-Chaverri *et al.* [10]. HOCl was prepared immediately before use by adjusting the pH of a 1% (v/v) solution of NaOCl to pH 6.2 with 0.6 M sulphuric acid. The concentration of HOCl was further determined spectrophotometrically at 235 nm using the molar extinction coefficient of 100 M^{-1} cm^{-1}. TNB or 5-thio-2-nitrobenzoic acid was prepared. The 20 mM sodium borohydride was added to a 1 mM solution of TNB in 50 mM potassium phosphate buffer, pH 6.6, containing 5 mM EDTA. The solution was incubated at 37°C for 30 min. The concentration of TNB was determined by measuring the absorbance at 412 nm using the molar extinction coefficient of 13,600 M^{-1} cm^{-1}. The assay for scavenging HOCl was performed. The reactions were performed by using 0.1 ml of 60 µM TNB, 0.5 ml of distilled water or test samples and 0.1 ml of 70 µM HOCl. The absorbance was measured at 412 nm before and 5 min after the addition of HOCl. A HOCl scavenger inhibits the oxidation of TNB resulting in high % TNB remaining. Scavenging of HOCl was ascertained by using L-ascorbic acid as a reference scavenger, which inhibited TNB oxidation in a concentration dependent manner.

$ONOO^-$ scavenging activity

$ONOO^-$ was synthesized as previously described [9] with slight modification. Five ml of an acidic solution (1.2 M HCl) of H_2O_2 (7 M) was mixed with 5 ml of 2 M KNO_2 on an ice bath for 1s and the reaction was quenched with 5 ml of ice-cold 4.2 M NaOH. Residual H_2O_2 was removed using granular MnO_2 prewashed with 4.2 M NaOH and the reaction mixture was then left overnight at -20°C. The resulting yellow liquid layer on the top of the frozen mixture was collected for the experiment. Concentrations of $ONOO^-$ were determined before each experiment at 302 nm using a molar extinction coefficient of 1,670 M^{-1} cm^{-1}.

$ONOO^-$ scavenging activity was measured by Evans Blue bleaching assay. The reaction mixture contained 50 mM phosphate buffer (pH 7.4), 0.1 mM diethylene-triamine-pentacetic acid (DTPA), 9 mM NaCl, 0.5 mM KCl, 12.5 µM Evans Blue, various doses of mangosteen juice (0-200 mg/ml) and 1 mM $ONOO^-$ in a final volume of 1 ml. After incubation at 25°C for 30 min the absorbance was measured at 611 nm. The percentage scavenging of $ONOO^-$ was calculated by comparing the results of the test and blank samples. Gallic acid was used as a reference compound.

Metal ion chelating

The ferrous ion chelating activity was measured

by a decrease in absorbance at 562 nm of the iron (II)-ferrozine complex. Metal ion chelating was evaluated using method of Kawpoomhae *et al.* [7]. The different concentrations of mangosteen juice (0.1 ml) or EDTA (0.1 ml), a standard compound, were added to a solution of 50 mM of $FeSO_4$ (0.1 ml) and 0.7 ml of 0.15 M NaCl solution. The reaction was initiated by the addition of ferrozine at 300 mM (0.1 ml) and the mixture was shaken vigorously and left standing at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm in a spectrophotometer.

Neuroprotective studies

Cytotoxicity assay

The MTT assay was performed to assess the toxicity on NG 108-15 cells. For, H_2O_2-induced cytotoxicity study, NG 108-15 cells were plated out at 2×10^3 cells/well in a polyornithine-coated 96-well plate. After 48 h, the sample was added. The plates were then incubated for 4 h. After incubation, the cells were treated with MTT solution (final concentration, 1 mg/ml) for 3 h. The dark blue formazan crystals formed in intact cells were solubilized by DMSO, and then the absorbance at 550 nm was measured using a microplate reader. Percent cell viability was calculated, assuming that the absorbance of the control cells was 100%. For glutamate-induced cytotoxicity study, NG 108-15 cells (2×10^3 cells/well) were seeded into 96-well plates and incubated at 37°C for 36 h. After plating, they were treated with various concentrations of mangosteen juice (1-300 μg/ml) for 22 h 30 min. After that, neuronal survival was quantified using MTT assay.

SH-SY5Y cells (1×10^4 cells/well) were seeded into 96-well plates and incubated at 37°C for 24 h. After plating, the cells were treated with various concentrations of mangosteen juice for 50 h. Cell survival was quantified using MTT assay.

Protective effect of mangosteen juice in H_2O_2-induced cytotoxicity

NG 108-15 cells (2×10^3 cells/well) were seeded into 96-well plates and incubated at 37°C for 48 h. After plating, they were treated with various concentrations of mangosteen juice (1-3000 μg/ml) for 2 h, and then H_2O_2 solution was added to yield a final concentration of 150 μM. Cell survival was quantified using MTT assay.

Protective effect of mangosteen juice in 6-OHDA-induced cytotoxicity

The protective effect was carried out based on method described by Levites *et al.* [11] and Nobre Junior *et al.* [12]. SH-SY5Y cells (1×10^4 cells/well) were seeded into 96-well plates and incubated at 37°C for 24 h. After plating, they were treated with various concentrations of mangosteen juice (1-300 μg/ml) 2 h, and 6-hydroxydopamine (6-OHDA) solution was added to yield a final concentration of 100 μM and then incubated at 37°C for 48 h. Cell survival was quantified using MTT assay.

Protective effect of mangosteen juice in glutamate-induced cytotoxicity

The protective effect on glutamate-induced cytotoxicity study was determined by the method of Okamoto *et al.* [13] with some modifications. NG 108-15 cells (2×10^3 cells/well) were seeded into 96-well plates and incubated at 37°C for 36 h. After plating, they were treated with various concentrations of mangosteen juice (1-300 μg/ml) for 30 min, for the excitotoxity insult, the cells were exposed to 100 mM L-glutamic acid for 22 h. After that, neuronal survival was quantified using MTT assay.

Statistical analysis

Each of the measurements described above was carried out in triplicate and the results were reported in terms of means and standard deviations. In addition, one way analysis of variance (ANOVA) was used to analyze the results with post-hoc analysis, and where appropriate, Turkey's test was employed, using a statistical software package (Open Stat). The *p* values of less than 0.05 were considered significant.

Results and Discussion

Analysis of total phenolic content, ascorbic acid and tannic acid

Total phenolic, ascorbic acid and tannic acid contents of mangosteen juice are presented in Table 1.

Total phenolic and ascorbic acid contents were slightly different from previous reports [14-17]. When compared with study carried out by Lim *et al.* [17], mangosteen juice in our study showed lower total phenolic content (18.54 ± 2.44 vs. 54 ± 7 mg GAE/100 g) but higher ascorbic acid content (7.08 ± 0.64 vs. 5.8 ± 0.8 mg/100 g). Factors such as regional differences, harvest time, storage conditions, sample preparation (lyophilized squeezed mangosteen juice vs. 50% ethanol extract of the aril part) and analytical procedures might contribute to these differences.

Total antioxidant capacity, radical scavenging and ion chelating assays

On the basis of the chemical reactions involved, major antioxidant capacity assays can be roughly divided into two categories: 1) hydrogen atom transfer (HAT) reaction based assay and 2) single electron transfer (SET) reaction based assay. SET reaction based assay measures an antioxidant's reducing capacity. HAT-based assays quantify hydrogen atom donating capacity. HAT reaction is more relevant to the radical chain-breaking antioxidant capacity which is related to prevent lipid peroxidation in cell membrane [18]. The assay involving HAT reaction in this study is galvinoxyl scavenging assay. The SET reaction based reaction is FRAP, DPPH and ABTS radical scavenging assays. We found that mangosteen juice showed reducing power on ABTS, FRAP and DPPH assays but it was not able to scavenge galvinoxyl radical (Table 2). These results suggested that mangosteen juice has antioxidant capacity which mainly occurs via SET based reaction. Hence, in further experiments, biological relevant radical scavenging activities were determined.

Table 1 Total phenolic, ascorbic acid and tannic acid contents of mangosteen juice

Total phenolic compounds (mg GAE/100 g)	Ascorbic acid (mg/100 g)	Tannic acid (mg/100 g)
18.54 ± 2.44	7.08 ± 0.64	84.77 ± 8.46

GAE = Gallic acid equivalent

Table 2 Antioxidant activities of mangosteen juice (n=3)

	Test/Sample	MJ (mg/ml)	References	
Total antioxidant capacity	ABTS (IC_{50})	0.23 ± 0.46	1.27 ± 0.46 μg/ml	Trolox®
	FRAP (EC_1)	301.10 ± 8.37	115.61 ± 12.51 μg/ml	Trolox®
	DPPH (IC_{50})	1.91 ± 0.65	1.27 ± 0.10 μg/ml	L-ascorbic acid
	Galvinoxyl radical (IC_{50})	-	1.34 ± 0.02	L-ascorbic acid
Ion chelating activity	(IC_{50})	-	3.89 ± 1.60 μM	EDTA
Radical scavenging	Hydroxyl (IC_{50})	1.99 ± 0.10	6.71 ± 0.20 mM	Mannitol
	Superoxide (IC_{50})	2.41 ± 0.85	1.13 ± 0.20 μg/ml	L-ascorbic acid
	Peroxynitrite (IC_{50})	0.21 ± 0.03	0.21 ± 0.03 mM	Gallic acid
	Singlet oxygen (IC_{50})	-	0.42 ± 0.03 mg/ml	L-ascorbic acid
	Hydrogen peroxide(IC_{50})	-	272.91 ± 1.61 μg/ml	BHT

MJ = mangosteen juice; BHT = butylated hydroxytoluene

Experimental evidence suggested that there are major reactive oxygen species (ROS) causing damage in the human body such as O_2^{-}, H_2O_2, OH·, $^1O_2$, HOCl and $ONOO^{-}$ [19]. We found that mangosteen juice could scavenge OH·, O_2^{-}, $ONOO^{-}$ (Table 2) and HOCl (Fig. 1). However, we could not find the scavenging effects on $^1O_2$ and H_2O_2 (Table 2) while L-ascorbic acid and butylated hydroxytoluene (BHT), reference substances, showed scavenging effects on $^1O_2$ and H_2O_2 with IC_{50} values of 0.42 ± 0.03 mg/ml and 272.91 ± 1.61 μg/ml respectively (Table 2).

To further investigate antioxidant property of mangosteen juice, iron ion chelating activity was evaluated. Ferrous iron (Fe^{2+}) is very important in the Fenton reaction to give the OH· which is extremely harmful to human body [19]. We found that mangosteen juice (1-10 mg/ml) did not show ion chelating activity (Table 2) while EDTA, reference substance, showed positive results with the IC_{50} value as 3.89 ± 1.60 μM. These findings were consistent with the result of Lim *et al.* [17] which reported the low chelating power of 50% ethanol extract of the edible portion of mangosteen.

From aforementioned experiments it can be suggested that mangosteen juice has antioxidant activities which mainly occur via SET based reactions. Form the results, mangosteen juice also had scavenging effect on OH·, O_2^{-}, $ONOO^{-}$ and HOCl. There are numerous reports support neurotoxicity of OH·, O_2^{-}, $ONOO^{-}$ and HOCl [19]. Therefore, neuroprotective effect of mangosteen juice was evaluated.

Neuroprotective effects

It is importance to study whether the sample containing antioxidant has cytoprotective effect against oxidant-induced cell death. The findings are beneficial to the implication for disease treatment/prevention.

Neurodegenerative diseases are characterized by the loss of neuronal cells in the brain. It is well known

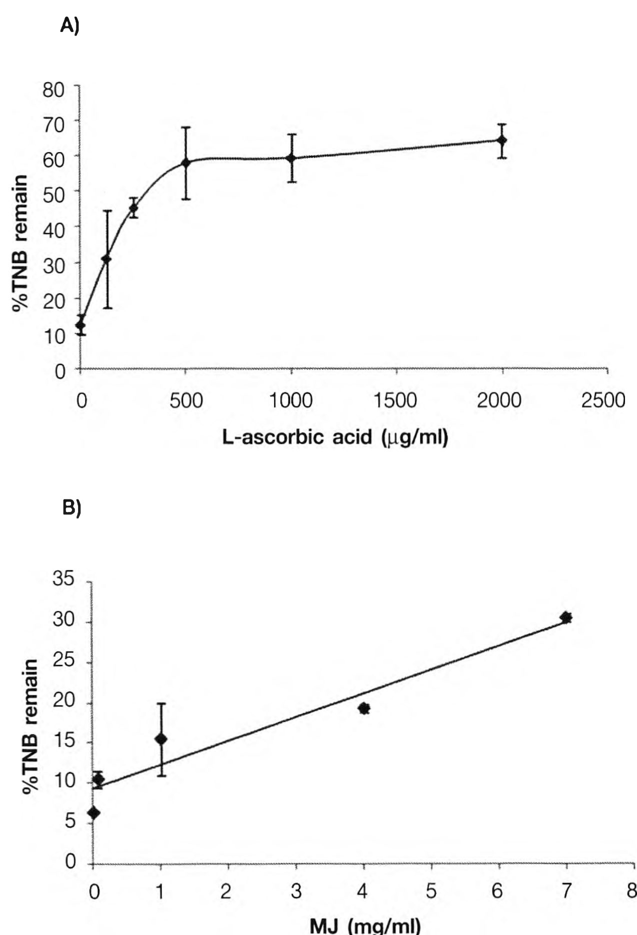

Figure 1 HOCl scavenging capacity of L-ascorbic acid, a reference, (A) and mangosteen juice (MJ) (B). Data are expressed as means of % TNB remain ± S.D. (n=5) of three independent replicates

that ROS may involve in the etiologies of these diseases. Antioxidants in plants or herbs may be useful in delaying or preventing oxidation damage [20]. In this study, H_2O_2-induced neuroblastoma NG 108-15 cell death model was used because this model is accepted as a good model for study of oxidant implicated neuronal diseases. The data from this model is correlated to *in vivo* study and clinical results [21-24]. Prior to the neuroprotection studies, the cytotoxic effect of mangosteen juice was evaluated. We found that mangosteen juice at a concentration up to 3 mg/ml was not toxic to the NG108-15 cells (Fig. 2A). For the cytoprotective experiment, we found that Trolox®, reference antioxidant, could protect the cells from H_2O_2-induced cell death (Fig. 2D). However, we could not find the protective effect of mangosteen juice on H_2O_2-induced cell death even though the highest concentration of 3000 μg/ml was applied (Fig. 2E). The results suggested limitation of efficacy of mangosteen

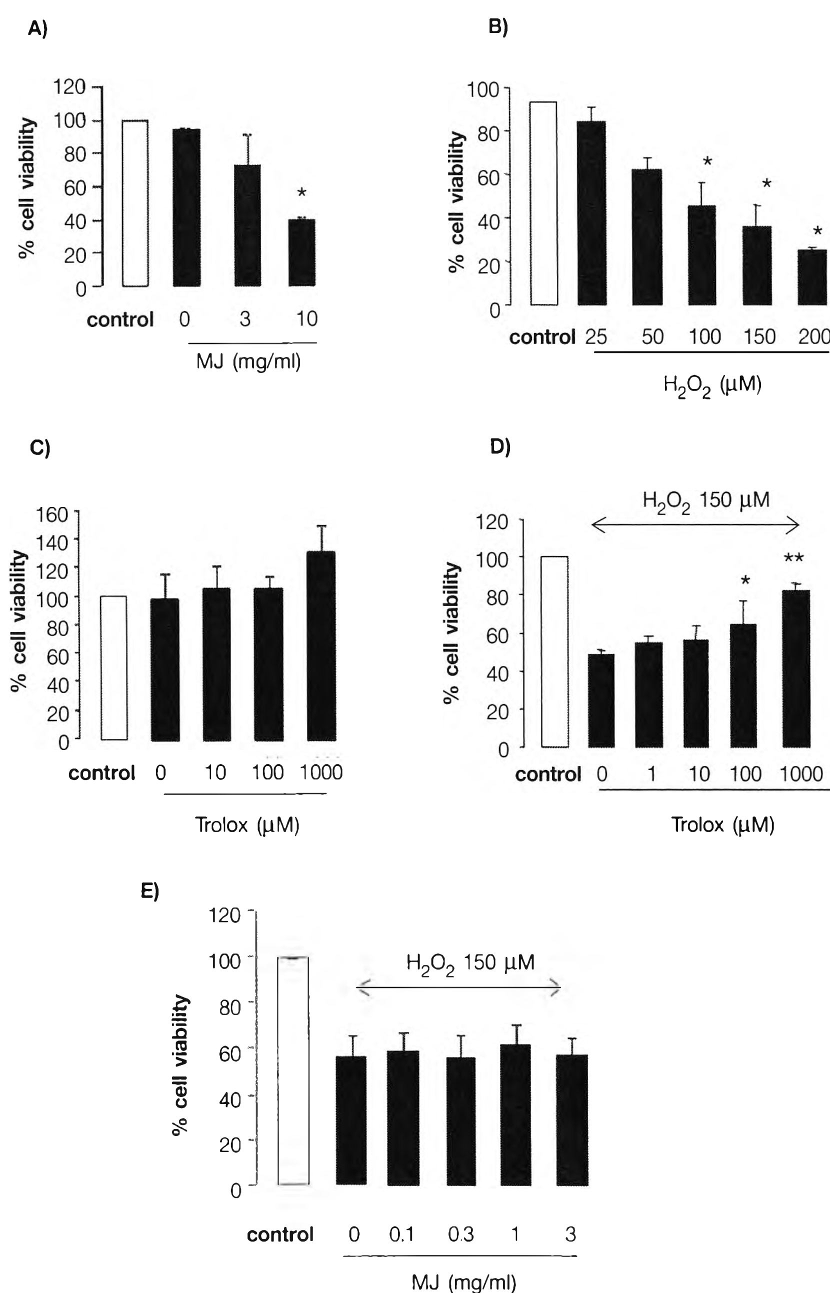

Figure 2 Effects of test compounds on H_2O_2-induced cell damage in NG 108-15 cells. Cytotoxic effects of mangosteen juice (MJ) (A), H_2O_2 (B) and Trolox®(C). For protective effects, NG 108-15 cells were pretreated with various concentrations of Trolox®(D) and mangosteen juice (MJ) (E) for 2 h. Then a stock solution of H_2O_2 solution was added to yield a final concentration of 150 μM. Neuronal survival was quantified using MTT assay. Data are expressed as means of cell viability ± S.D. (n=5) of three independent replicates. *$p \leq 0.05$ compared to the control group, **$p \leq 0.01$ compared to the control group

juice on protection/treatment of oxidants-induced neuronal disease. The negative findings on the cytoprotective study of mangosteen juice were different from results of the peel extracts' studies which demonstrated that the methanol and water extracts of mangosteen peel showed neuroprotective activity on H_2O_2-induced NG108-15 cell death [5,25]. These might be due to a difference of bioactive components containing in the samples. The methanol and water extracts might contain nonpolar constituents as compared to polar constituents in mangosteen juice, which is hardly entered to the cell. In addition, the different bioactive component might lead to unique antioxidant potency and capacity of the samples. There were some recent reports revealed differences of polyphenols of the aril and the peel. Chaovanalikit *et al.* reported that mangosteen peel have higher total phenolic content compared to the aril part [26]. In another paper, Zadernowski *et al.* showed differences of phenolic content and profiles of the peel and the aril [27]. They found that protocatechuic acid was the major phenolic acid in the peel, while p-hydroxybenzoic acid was the predominant phenolic acid in the aril and caffeic acid was detected only in the peel [24]. These differences might lead to the different antioxidant activity. For example, in DPPH assay, IC_{50} values of mangosteen juice and methanol extract of mangosteen peel were 1.91 ± 0.65 mg/ml and 14.7 ± 1.3 µg/ml, respectively [28]. For OH· scavenging activity, IC_{50} values of mangosteen juice and the methanol extract of mangosteen peel were 1.99 ± 0.10 mg/ml and 1.38 ± 0.15 mg/ml, respectively [25]. For $O^{\cdot}_2$ radical scavenging activity, IC_{50} values of mangosteen juice and the methanol extract of mangosteen peel were 2.41 ± 0.85 mg/ml and 172.1 ± 7.2 µg/ml, respectively [28].

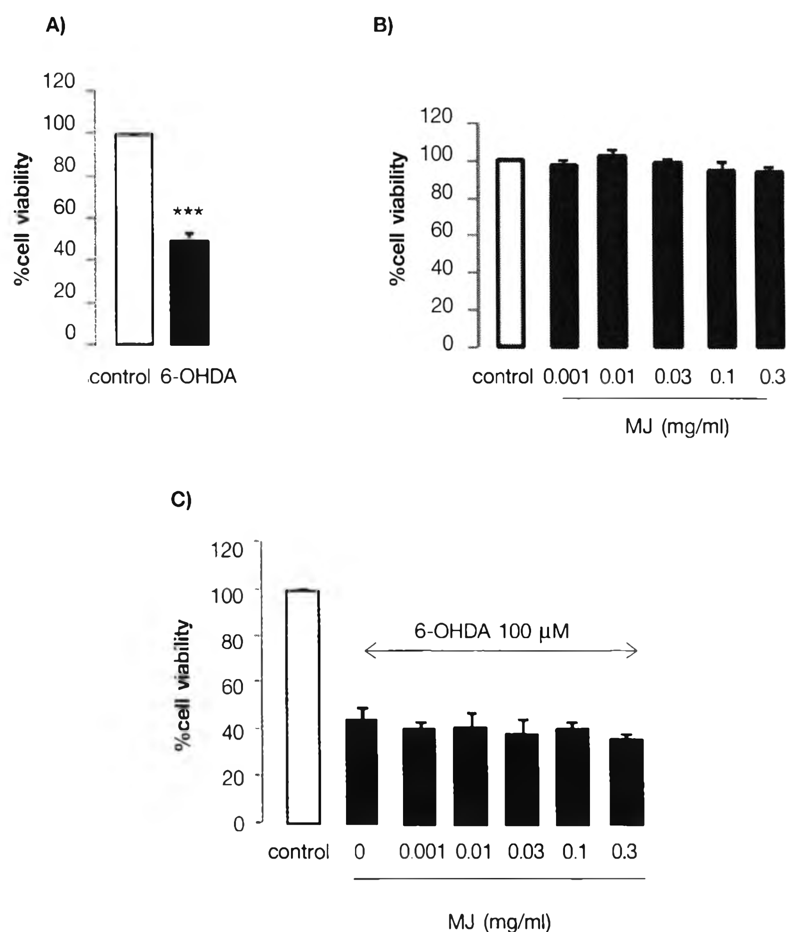

Figure 3 Effects of mangosteen juice on 6-OHDA-induced cell damage in SH-SY5Y cells. Cytotoxic effects of 100 µM of 6-OHDA (A) and mangosteen juice (MJ) (B). For protective effect, SH-SY5Y cells were pretreated with mangosteen juice (MJ) (C) for 2 h. Then a stock solution of 6-OHDA solution was added to yield final concentration of 100 µM. The cells were incubated at 37°C for 48 h. Neuronal survival was quantified using MTT assay. Data are expressed as means of cell viability ± S.D. (n=5) of three independent replicates. ***$p \leq 0.001$ compared to the control group.

Based on biological relevant radicals, OH·, O_2^{-}, and $ONOO^{-}$, scavenging effects of mangosteen juice, other two models related to oxidative stress implicated neurotoxicity were performed in an attempt to demonstrate other applications of mangosteen juice. There were 6-OHDA and glutamate induced neuronal cell death. 6-OHDA-induced neuronal cell death is widely used to investigate possible active substance for treatment and/or prevention of Parkinson's disease. Parkinson's disease is neurodegenerative disease cause by degeneration of dopaminergic neuron in substantial nigra pars compacta. 6-OHDA is a hydroxylated analogue of the natural neurotransmitter dopamine. One of proposed toxicity mechanism to dopaminergic neurons of 6-OHDA is thought to involve the generation of free radicals especially the OH· [29]. Mangosteen juice could scavenge OH·, therefore the protective effect using this model was performed. In this study, human neuroblastoma SH-SY5Y cells were selected because they are dopamine-neuron-derived cell lines which are suitable for examining mechanisms of dopaminergic neuronal degeneration and for screening new pharmacological agents [30]. Fig. 3B illustrates the cytotoxic effect of mangosteen juice. Mangosteen juice at the concentration up to 300 μg/ml was not toxic to the SH-SY5Y cells. Unfortunately, although mangosteen juice was able to scavenge OH·, we could not find protective effect of mangosteen juice on 6-OHDA-induced cell death as can be seen in Fig. 3C.

For the second model, glutamate-induced neuronal

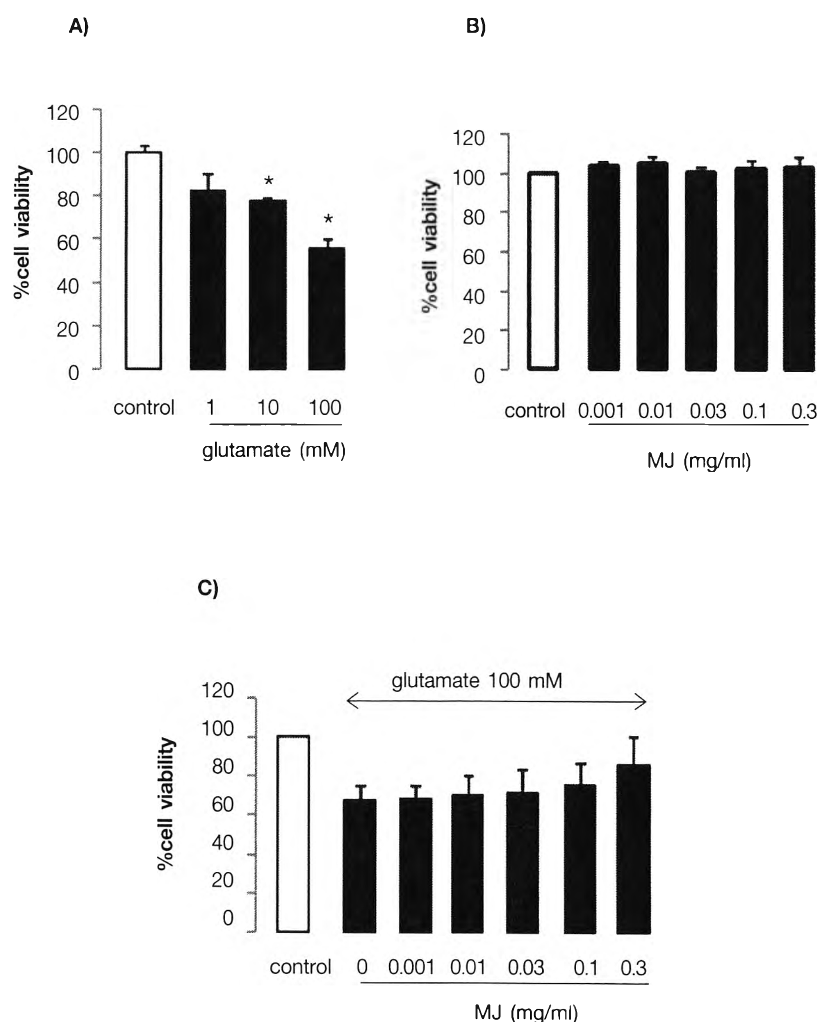

Figure 4 Effects of mangosteen juice on glutamate-induced cell damage in NG 108-15 cells. Cytotoxic effects of glutamate (A) and mangosteen juice (MJ) (B). For protective effect, NG 108-15 cells were treated with various concentrations of mangosteen juice (MJ) (C) for 30 min; neurons were exposed to 100 mM L-glutamic acid for 22 h. Then neuronal survival was quantified using MTT assay. Data are expressed as means of cell viability ± S.D. (n=5) of three independent replicates. *$p \leq 0.05$ compared to the control group

cell death model was chosen. This model is usually used for investigate probable drug for treatment of acute neurodegenerative conditions such as stroke transient ischemic condition or seizure. In this condition, there are an abundant increase in ROS and stimulation of glutamate release from presynaptic nerves terminals and astrocyte to extracellular space. Glutamate activates nerve cell and nervous system via glutamate receptors such as N-methyl-D-aspartate (NMDA) receptors and non-NMDA receptors. Over-activation of NMDA and non-NMDA glutamate receptors cause abrupt rises in the concentration of cytoplasmic Ca^{2+} in neurons. This Ca^{2+} overload leads to enhanced ROS production and depolarization of the mitochondrial membrane. One of the mechanisms to enhance ROS production is stimulation of nitric oxide syntase to produce nitric oxide. Nitric oxide reacts with O_2^- to form $ONOO^-$, then it reacts with tyrosine amino acid to release OH• [31]. Because mangosteen juice showed scavenging effects on OH•, O_2^- and $ONOO^-$, we hope that it can prevent glutamate damage. Prior to the neuroprotection studies, the cytotoxic effect of mangosteen juice was evaluated. We found that mangosteen juice at the concentration up to 300 μg/ml did not toxic to the NG108-15 cells (Fig. 4B). Unfortunately, single application of mangosteen juice (1-300 μg/ml) did not show protective effect on glutamate-induced neuroblastoma NG108-15 cells death (Fig. 4C). The negative findings in both 6-OHDA- as well as glutamate-induced cell death experiments may be a result from weak antioxidant capacity of mangosteen juice. In the future, studies using repeated and long-term application of mangosteen juice and combination of mangosteen juice with other antioxidants are needed.

Conclusion

We firstly found the antioxidant activity of mangosteen juice. Its antioxidant activity results from free radical scavenging activity. The FRS occurs via electron transfer reaction. For biological relevant radicals, mangosteen could scavenge O_2^-, OH•, HClO and $ONOO^-$. These findings suggest that it could be another potential source of natural dietary antioxidants. Unfortunately, single application of mangosteen juice was not able to protect neuronal cells from H_2O_2, glutamate and 6 OHDA-induced neuronal cell death. In the future, studies using repeated and long-term application of mangosteen juice as well as combination of mangosteen juice with other antioxidants should be investigated.

Acknowledgements

This work was supported by the Thailand Research Fund and the Commission of Higher Education, Ministry of Education, Thailand (MRG 4880110) and the Thailand Research Fund (Thai-Fruits-Functional Fruits Project. The authors are also thankful to Miss Areerat Sripattanaporn for her experimental assistance.

References

[1] M. B. Osman, and A. R. Milan. Mangosteen-*Garcinia mangostana*. Southampton Centre for Underutilized Crops, University of Southampton, Southampton, 2006, pp. 32.

[2] Mangosteen supplement benefit, side effects, extract, fruit and juice information, xanthones research and the role of these substances against cancer, do these pills help prevent or treat tumors and cancers? [Internet]. Mangosteen pill Organic, direct from Thailand, Cited 8 Oct 2011, Available from: http://www.mango-steen.org.

[3] J. K. Udani, B.B. Singh, M. L. Barrett, and V. J. Singh. Evaluation of mangosteen juice blend on biomarkers of inflammation in obese subjects: a pilot, dose finding study, *Nutr J.* 8: 48 (2009).

[4] L. P. Wong, and P. J. Klemmer. Severe lactic acidosis associated with juice of the mangosteen fruit *Garcinia mangostana*. *Am. J. Kidney Dis.* 51: 829-833 (2008).

[5] M. Sukma, T. Ngawhirunpat, P. Opanasopit, U. Sotanaphan, and P. Siripong, Antioxidative and neuroprotective activities of barakol, and extracts from the fruit hull of mangosteen (*Garcinia mangostana* Linn.), the 21st Congress of Federation of Asian Pharmaceutical Associations (FAPA), Yokohama, 2006.

[6] M. Sukma, M. Tohda, and H. Watanabe. Chronic treatment with imipramine inhibits cell growth and enhances serotonin 2C receptor mRNA expression in NG 108-15 cells, *J. Pharmacol. Sci.* 92: 433-436 (2003).

[7] K. Kawpoomhae, M. Sukma, T. Ngawhirunpat, P. Opanasopit, and A. Sripattanaporn. Antioxidant and neuroprotective effects of standardized extracts of *Mangifera indica* leaf,

Thai J. Pharm. Sci. 34: 32-43 (2010).

[8] V. Raghu, K. Platel, and S. K. Comparison of ascorbic acid content of *Emblica officinalis* fruits determined by different analytical methods, *J. Food Composit. Anal.* 20: 529-533 (2007).

[9] B. Hazra, S. Biswas, and N. Mandal. Antioxidant and free radical scavenging activity of *Spondias pinnata, BMC Complementary and Alternative Medicine* 8: 63 (2008).

[10] J. Pedraza-Chaverrí, G. Arriaga-Noblecía, and O. N. Medina-Campos. Hypochlorous acid scavenging capacity of garlic, *Phytotherapy Res.* 21: 884-888 (2007).

[11] Y. Levites, M. B. H. Youdim, G. Maor, and S. Mandel. Attenuation of 6-hydroxydopamine (6-OHDA)-induced nuclear factor-kappaB (NF-κB) activation and cell death by tea extracts in neuronal cultures, *Biochem. Pharmacol.* 63: 21-29 (2002).

[12] H. V. Nobre Junior, G. M. A. Cunha, F. D. Maia, R. A. Oliveira, M. O. Moraes, and V.S.N. Rao. Catechin attenuates 6-hydroxydopamine (6-OHDA)-induced cell death in primary cultures of mesencephalic cells, *Comparative Biochemistry and Physiology Part C: Toxicology & amp; Pharmacol.* 136: 175-180 (2003).

[13] M. Okamoto, S. Mori, and H. Endo. A protective action of chondroitin sulfate proteoglycans against neuronal cell death induced by glutamate, *Brain Res.* 637: 57-67 (1994).

[14] S. Grinstein, S. Poovarodom, H. Leontowicz, M. Leontowicz, J. Namiesnik, S. Vearasilp, R. Haruenkit, P. Ruamsuke, E. Katrich, and Z. Tashma. Antioxidant properties and bioactive constituents of some rare exotic Thai fruits and comparison with conventional fruits: *In vitro* and *in vivo* studies, *Food Res. Int.* 44: 2222-2232 (2011).

[15] R. Haruenkit, S. Poovarodom, H. Leontowicz, M. Leontowicz, M. Sajewicz, T. Kowalska, E. Delgado-Licon, N. E. Rocha-Guzman, J. A. Gallegos-Infante, S. Trakhtenberg, and S. Gorinstein. Comparative study of health properties and nutritional value of durian, mangosteen, and snake fruit: experiments *in vitro* and *in vivo, J. Agric. Food Chem.* 55: 5842-5849 (2007).

[16] M. Isabelle, B. L. Lee, M. T. Lim, W. P. Koh, D. Huang, and C. N. Ong. Antioxidant activity and profiles of common fruits in Singapore, *Food Chem.* 123: 77-84 (2010).

[17] Y. Y. Lim, T. T. Lim, and J. J. Tee. Antioxidant properties of several tropical fruits: a comparative study, *Food Chem.* 103: 1003-1008 (2007).

[18] S. M. Phipps, M. H. M. Sharaf, and V. Butterweck. Assessing Antioxidant Activity in Botanicals and Other Dietary Supplements, *Pharmacopeial Forum* 33: 810-814 (2007).

[19] K. A. Jellinger. Advances in our understanding of neurodegeneration. In: G.A. Qureshi, and S. H. Parvez (eds.), *Oxidative Stress and Neurodegenerative Disorders*, Elsevier, Oxford, 2007, pp.1-58.

[20] G. Oboh, A. J. Akinyemi, and A. O. Ademiluyi. Antioxidant and inhibitory effect of red ginger (*Zingiber officinale* var. Rubra) and white ginger (*Zingiber officinale* Roscoe) on Fe^{2+} induced lipid peroxidation in rat brain *in vitro*, *Experimental and Toxicologic Pathology* (2011) In Press.

[21] P. Mahakunakorn, M. Tohda, Y. Murakami, K. Matsumoto, and H. Watanabe. Antioxidant and free radical-scavenging activity of Choto-san and its related constituents, *Biol. Pharm. Bull.* 27: 38-46 (2004).

[22] P. Mahakunakorn, M. Tohda, Y. Murakami, K. Matsumoto, H. Watanabe, and O. Vajaragupta. Cytoprotective and cytotoxic effects of curcumin: dual action on H_2O_2-induced oxidative cell damage in NG108-15 cells, *Biol. Pharm. Bull.* 26: 725-728 (2003).

[23] P. Mahakunakorn, M. Tohda, Y. Murakami, H. Watanabe, and K. Matsumoto. Effects of Choto-san and its related constituents on endogenous antioxidant systems, *Biol. Pharm. Bull.* 28: 53-57 (2005).

[24] H. Watanabe, Q. Zhao, K. Matsumoto, M. Tohda, Y. Murakami, S. H. Zhang, T. H. Kang, P. Mahakunakorn, Y. Maruyama, I. Sakakibara, N. Aimi, and H. Takayama. Pharmacological evidence for antidementia effect of Choto-san (Gouteng-san), a traditional Kampo medicine. *Pharmacol. Biochem.* Behav. 75: 635-643 (2003).

[25] W. Weecharangsan, P. Opanasopit, M. Sukma, T. Ngawhirunpat, U. Sotanaphun, and P. Siripong. Antioxidative and neuroprotective activities of extracts from the fruit hull of mangosteen (*Garcinia mangostana* Linn.), *Med. Princ. Pract.* 15: 281-287 (2006).

[26] A. Chaovanalikit, and A. mingmuang. Anthocyanin and total phenolic content of mangosteen and its juices, *Srinakharinwirot science journal.* 23: 68-78 (2007).

[27] R. Zadernowski, S. Czaplicki, and M. Naczk. Phenolic acid profiles of mangosteen fruits *(Garcinia mangostana). Food Chemistry.* 112: 685-689 (2009).

[28] T. Ngawhirunpat, P. Opanasopi, M. Sukma, C. Sittisombut, A. Kat, and I. Adachi. Antioxidant, free radical-scavenging activity and cytotoxicity of different solvent extracts and their phenolic constituents from the fruit hull of mangosteen *(Garcinia mangostana). Pharm Biol.* 48: 55-62 (2010).

[29] D. Blum, S. Torch, N. Lambeng, M.-F. Nissou, A.-L. Benabid, R. Sadoul, and J.-M. Verna. Molecular pathways involved in the neurotoxicity of 6-OHDA, dopamine and MPTP: contribution to the apoptotic theory in Parkinson's disease, *Progress in Neurobiology.* 65: 135-172 (2001).

[30] Y. Glinka, M. Gassen, and M.B. Youdim. Mechanism of 6-hydroxydopamine neurotoxicity, *J Neural Transm Suppl.* 50: 55-66 (1997).

[31] R. Sattler, and M. Tymianski. Molecular mechanisms of glutamate receptor-mediated excitotoxic neuronal cell death, *Mol Neurobiol.* 24: 107-129 (2001).