Research Article

ฤทธิ์ต้านอนุมูลอิสระ และฤทธิ์ปกป้องเซลล์ของสารสกัดจากผลพืชสกุลมะเขือ 4 ชนิด

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บทคัดย่อ

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รับบทความ : 30 กรกฎาคม 2562 **แก้ไขบทความ:** 7 ตุลาคม 2562 **ตอบรับ:** 29 ตุลาคม 2562

ผลพืชสกุลมะเขือหลายชนิดได้แก่ *Solanum sanitwongsei* W. G. Craib (มะแว้ง), *Solanum stramonifolium* Jacq. (มะอึก), *Solanum torvum* Sw. (มะเขือพวง) และ *Solanum xanthocarpum* Schrad. & H. Wendl. (มะเขือขื่น) ถูกนำมาใช้เป็นสมุนไพรพื้นบ้านและเป็นผักรับประทาน การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาฤทธิ์ต้านอนุมูลอิสระในหลอดทดลอง วิเคราะห์หาปริมาณสารประกอบฟีนอลิกและฟลาโวนอยด์ และศักยภาพในการปกป้องเซลล์ตับ (HepG2) จากการเหนี่ยวนำเซลล์ให้เกิดภาวะ oxidative stress โดยใช้ *tert*-butyl hydroperoxide (*t*-BuOOH) ของผลพืชสกุลมะเขือ 4 ชนิด (*S. sanitwongsei*, *S. stramonifolium*, *S. torvum* and *S. xanthocarpum*) **วิธีการศึกษา:** สกัดสารจากผลพืชสกุลมะเขือทั้ง 4 ชนิดโดยใช้เมทานอล ศึกษาฤทธิ์ต้านอนุมูลอิสระในหลอดทดลองโดยการประเมินความสามารถในการกำจัดอนุมูลอิสระด้วยวิธี DPPH และ ABTS รวมทั้งประเมิน reducing power วิเคราะห์หาปริมาณสารประกอบฟีนอลิกและฟลาโวนอยด์โดยใช้วิธี Folin-Ciocalteu และ aluminium chloride ตามลำดับ สำหรับการศึกษาฤทธิ์ปกป้องเซลล์ทำโดยการประเมินอัตราการมีชีวิตรอดของเซลล์ด้วยวิธี MTT assay **ผลการศึกษา:** สารสกัดของผลพืชสกุลมะเขือทั้ง 4 ชนิดมีฤทธิ์ต้านอนุมูลอิสระ พบปริมาณสารประกอบฟีนอลิกและฟลาโวนอยด์มีค่าเท่ากับ 1031.23±11.87 ถึง 4474.36±23.74 mg GAE/100 g สารสกัด และ 1283.04±12.61 to 2984.98±10.21 mg CE/100 g สารสกัดตามลำดับ ทั้งนี้ฤทธิ์ต้านอนุมูลอิสระและปริมาณฟลาโวนอยด์ของสารสกัดที่ตรวจพบมีลำดับดังนี้ *S. xanthocarpum* > *S. stramonifolium* > *S. torvum* > *S. sanitwongsei* **สรุปผลการศึกษา:** สารสกัดของผลพืชสกุลมะเขือ *S. stramonifolium, S. torvum* และ *S. sanitwongsei* มีศักยภาพในการต้านอนุมูลอิสระและมีฤทธิ์ปกป้องเซลล์ตับจากการเหนี่ยวนำให้เกิดภาวะ oxidative stress ผลมะเขือทั้ง 3 ชนิดนี้จัดเป็นแหล่งกำเนิดของสารต้านอนุมูลอิสระที่มาจากธรรมชาติและอาจนำไปประยุกต์ใช้ในทางเภสัชกรรมเพื่อรักษาโรคต่าง ๆ ที่เกี่ยวข้องกับ oxidative stress

คำสำคัญ: มะเขือ, ฤทธิ์ต้านอนุมูลอิสระ, ฤทธิ์ปกป้องเซลล์

In Vitro Antioxidant and Cytoprotective Activities of Extracts from Four *Solanum* Fruits

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Abstract

In Vitro Antioxidant and Cytoprotective Activities of Extracts from Four _Solanum_ Fruits

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Many *Solanum* fruits such as *Solanum sanitwongsei* W. G. Craib, *Solanum stramonifolium* Jacq., *Solanum torvum* Sw. and *Solanum xanthocarpum* Schrad. & H. Wendl. are consumed as traditional herbal medicine and vegetables. The present study aimed to evaluate *in vitro* antioxidant activities, total phenolic and flavonoid content and cytoprotective potential against *tert*-butyl hydroperoxide (*t*-BuOOH)-induced oxidative stress in a human liver cell, HepG2 of four edible fruits of *Solanum* (*S. sanitwongsei*, *S. stramonifolium*, *S. torvum* and *S. xanthocarpum*). **Methods:** Methanol was used to extract of the four *Solanum* fruits. *In vitro* antioxidant activities were evaluated for radical scavenging abilities of DPPH and ABTS and reducing power. Total phenolic and flavonoid contents were determined using the colorimetric methods of Folin-Ciocalteu and aluminium chloride, respectively. For evaluation of cytoprotective effect, cell viability was evaluated by MTT assay. **Results:** All four *Solanum* fruit extracts displayed antioxidant activities. Total phenolic and flavonoid contents were in the range of 1031.23±11.87 to 4474.36±23.74 mg GAE/100 g extract and 1283.04±12.61 to 2984.98±10.21 mg CE/100 g extract, respectively. The order of antioxidant properties and total flavonoid content was *S. xanthocarpum* > *S. stramonifolium* > *S. torvum* > *S. sanitwongsei*. **Conclusion:** The extracts of *S. stramonifolium, S. torvum*, *S. sanitwongsei* fruits had antioxidant potentials and cytoprotective effects against *t*-BuOOH in the human liver cell. They could be considered as potential sources of natural antioxidants and might be used for pharmaceutical application to treat diseases associated with oxidative stress.

Keywords: *Solanum*; antioxidant; cytoprotective

Introduction

Oxidative stress, an imbalance between ROS production and antioxidant defenses, is an important factor in the etiology and pathogenesis of many acute and chronic diseases such as cardiovascular, acute and chronic kidney, neurodegenerative, and liver diseases (Liguori *et al.*, 2018; Nagata *et al.*, 2007). Antioxidants from plant source have attracted special interest because they can protect human body from radical related diseases with little or no side effects. Among the natural phytochemicals identified from plants, phenolic compounds such as phenolic acids, flavonoids and tannins represents important and interesting classes of biologically active compounds. These compounds may exist in all parts of plants including leaves, roots, barks and fruits. Their effectiveness was reported in the protective studies of various cell/tissue types from oxidative injury (Kumar *et al.*, 2016; Lima *et al.*, 2006; Omobowale *et al.*, 2018; Zou *et al.*, 2010).

Solanum sanitwongsei W. G. Craib, *Solanum stramonifolium* Jacq., *Solanum torvum* Sw. and *Solanum xanthocarpum* Schrad. & H. Wendl. fruits are edible and used as traditional herbal remedies. In South-East Asia especially in Indonesia, the fruit of *S. sanitwongsei* is traditionally used to treat diabetes (Simmonds and Howes, 2006). In Thailand, this fruit is popularly used in cough herbal remedies. Ethanol extract of the *S. sanitwongsei* fruit has diuretic and antihypertensive effects (Aminunsyah *et al.*, 2014). In traditional Indian folk medicine, the fruit of *S. stramonifolium* is used to treat nailside infection (Kshirsagar and Singh, 2001), and the fruit of *S. torvum* is used in the treatment of cough, liver and spleen enlargement (Siemonsma and Piluek, 1994). The *S. torvum* fruit possesses antimicrobial (Chah *et al.*, 2000), antioxidant (Ramamurthy *et al.*, 2012), antiviral (Arthan *et al.*, 2002), cardiovascular and anti-platelet aggregation (Nguelefack *et al.*, 2008) activities. The fruit of *S. xanthocarpum* is known for several traditional Indian medicine uses like anthelmintic, antipyretic, laxative, anti-inflammatory, urinary bladder, enlargement of the liver, antiasthmatic and aphrodisiac activities (Gupta *et al.*, 2011). The *S. xanthocarpum* fruit has shown antifungal (Dabur *et al.*, 2004), antioxidant (Demla and Verma, 2012) and hypoglycaemic activities (Kar *et al.*, 2006).

As stated, there were reports of the antioxidant activities of the fruits of *S. torvum* and *S. xanthocarpum* however, little is known about the antioxidant activities of the fruits of *S. sanitwongsei* and *S. stramonifolium*. In addition, the protection against oxidative stress in human liver cells of these *Solanum* fruits has not been reported. Therefore, the objective of this study was to investigate the *in vitro* antioxidant activities and the total phenolic and flavonoid contents as well as the potential cytoprotective effects against *tert*-butylhydroperoxide (*t*-BuOOH)-induced oxidative stress in the human liver cell of four edible fruits of *S. sanitwongsei*, *S. stramonifolium*, *S. torvum* and *S. xanthocarpum*.

Materials and methods

Chemicals and reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid diammonium salt (ABTS), catechin, 3-(4-5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and *t*-BuOOH were obtained from Sigma/Aldrich (St. Louis, USA). Ferric chloride ($FeCl_3.6H_2O$), Folin-Ciocalteu reagent, glacial acetic acid, hydrochloric acid, sodium carbonate, sodium hydroxide, and sodium nitrite were purchased from Carlo Erba Reagenti (Milano, Italy). 2,4,6-tripyridyl-*s*-triazine (TPTZ) were obtained from Acros Organics (Morris Plains, USA). Gallic acid was supplied by Fluka Chemie AG (Buchs, Switzerland). Aluminium chloride was obtained from BDH (Poole, UK). The human hepatoma cell line, HepG2 was purchased from Cell Lines Service (Eppelheim, Germany). Minimum Essential Medium Eagle (MEM), fetal bovine serum (FBS), phosphate buffer saline, and trypsin were obtained from GIBCO (Grand Island, USA). All other chemicals and reagents were of analytical grade.

Preparation of plant extracts

S. sanitwongsei, *S. stramonifolium*, *S. torvum* and *S. xanthocarpum* were collected from local gardens, Srisaked province, Thailand. Voucher specimens of the collected plants were deposited at Faculty of Pharmaceutical Sciences, Ubon Ratchathani University for future reference. The fresh fruits were cleaned, cut in to small pieces, sun-dried, and ground. A powdered sample (15 g) was extracted twice with methanol (150 mL) for 3 days each at room temperature. The combined extracts were evaporated to dryness under reduced pressure. The yields of dried extracts were 5-11% (w/w) of the dried plant materials (Table 1). All dry extracts were kept at 4 ºC until used.

Evaluation of antioxidant activity

The stock *Solanum* extracts were prepared in DMSO at concentrations of 5-30 mg/mL, and were diluted in DMSO to various concentrations (125–30000 µg/mL). At least five different concentrations of the test extract were used to evaluate antioxidant activity.

DPPH radical scavenging activity

Radical scavenging activity of *Solanum* species was estimated using a stable DPPH radical ($DPPH^{\bullet}$) assay (Brand-Williams *et al.*, 1995). Briefly, in a 96-well microplate, 20 µL of various concentrations (500-30000 µg/mL) of the test extract and 180 µL of $DPPH^{\bullet}$ solution (130 µM) were added. The reaction mixture was shaken well and incubated in the dark for 30 min. The mixture absorbance was measured at 530 nm. Percent radical scavenging activity was calculated according to the following equation:

$$\% \text{ radical scavenging activity} = (1-\text{Absorbance}_{sample}/\text{Absorbance}_{control}) \times 100$$

The concentration of extract required to scavenge free radical by 50% (EC_{50}) was estimated from the graph plotting percent radical scavenging activity against extract concentration (µg/mL).

ABTS radical cation scavenging activity

Radical scavenging activity of *Solanum* species was measured using an improved ABTS assay (Re *et al.*, 1999). The ABTS radical cation ($ABTS^{\bullet+}$) stock solution was prepared by the reaction of 7 mM ABTS and 2.45 mM potassium persulfate and incubated for 16 h in the dark at room temperature. The $ABTS^{\bullet+}$ solution was then diluted to obtain an absorbance of 0.712 ± 0.018 at 734 nm. One thousand microliters of the $ABTS^{\bullet+}$ solution was reacted to 150 µL of various concentrations (125-12500 µg/mL) of the test extract for 6 min and the absorbance was immediately measured at 734 nm. Results were expressed as EC_{50}.

Ferric reducing antioxidant power (FRAP)

The FRAP assay was performed as previously described by Benzie and Strain (1996) with some modifications. In this study, the method was modified for the 96-well microplate reader. Briefly, the FRAP reagent was prepared by mixing acetate buffer (300 mM, pH 3.6), a solution of 10 mM TPTZ in 40 mM hydrochloric acid, and 20 mM $FeCl_3.6H_2O$ at 10:1:1 (v/v/v). The reagent (180 µL) and sample solutions (20 µL, 500-10000 µg/mL) were added to each well and mixed thoroughly. After 4 min, the absorbance was immediately taken at 570 nm. A calibration curve of 100-1200 µM ferrous sulfate ($FeSO_4$) was used and results were expressed in units of mmol $FeSO_4$ per 100 g extract.

Determination of total phenolic and flavonoid contents

Total phenolic content

Total phenolic content was determined using *the Folin*-Ciocalteu colorimetric method reported previously (Akanitapichat *et al.*, 2010) with some modifications. Appropriately diluted test extracts (80 µL) were mixed with 40 µL of *Folin*-Ciocalteu reagent followed by the addition of 120 µL of 7% aqueous sodium carbonate. Distilled water was then added to adjust the final volume to 800 µL. After standing in the dark at room temperature for 3 h, the absorbance of the mixture was measured at 765 nm against reagent blank. The total phenolic content was expressed as gallic acid equivalents (GAE) in mg per 100 g extract.

Total flavonoid content

Total flavonoid content was measured using the aluminium chloride colorimetric method as described by Akanitapichat *et al* (Akanitapichat *et al.*, 2010) with some modifications involving the use of 96-well plates. Briefly, the reaction mixture contained 20 µl of extract, 50 µl of 0.5% sodium nitrite, and 50 µl of 2% aluminium chloride. The solution absorbance was measured 6 min after adding 80 µl of 0.425 M sodium hydroxide at 490 nm. The total flavonoid content was expressed as catechin equivalents (CE) in mg per 100 g extract.

Evaluation of cytotoxicity/cytoprotective effect

HepG2 cells were grown in a humidified incubator containing 5% CO_2 and 95% air at 37°C. They were cultured in MEM medium supplemented with 5% (v/v) FBS, 0.1 mM non-essential amino acid, and 2 mM glutamine. Only cells in exponential growth were used for the experiments.

Cells were seeded in 96-well plates at a density of 5000 cells/well and incubated at 37°C for 24 h. The cultured medium was replaced with serum-free medium and supplemented with test extracts for 24 h.

For cytotoxicity evaluation, cell viability was determined using the MTT assay (Mosmann, 1983) and results were expressed as % cell viability.

$$\% \text{ cell viability} = \frac{\text{(Absorbance of cells treated with } \textit{Solanum} \text{ extracts-Absorbance of blank)}}{\text{(Absorbance of cells treated without } \textit{Solanum} \text{ extracts-Absorbance of blank)}}$$

For evaluation of cytotorotective effect, the cells were placed in a medium containing 300 μM *t*-BuOOH and incubated for 4 h. Cell viability was determined using the MTT assay (Mosmann, 1983) and results were expressed as % cytoprotection.

% cytoprotection = % viability of cells pretreated with *Solanum* extracts - % viability of cells treated with *t*-BuOOH alone

Statistical analysis

All results were expressed as means ± standard deviations of three determinations. Statistical analyses were performed using SPSS version 15.0 software for Windows. Results of cytoprotective effect were subjected to ANOVA and differences between means were located using Bonferroni multiple-comparison test.

Table 1 Antioxidant activities of the four *Solanum* fruits.

Sample	%Yield (w/w)	Antioxidant activities		
		DPPH (EC_{50}, mg/mL)	ABTS (EC_{50}, mg/mL)	FRAP (mmol $FeSO_4$/100 g extract)
S. sanitwongsei	8.75	1.671±0.011[a]	0.671±0.002[a]	8.15±0.16[a]
S. stramonifolium	10.25	0.279±0.004[b]	0.117±0.001[b]	20.15±0.18[b]
S. torvum	11.00	0.520±0.006[c]	0.166±0.000[c]	13.90±0.16[c]
S. xanthocarpum	5.12	0.174±0.003[d]	0.065±0.001[d]	26.90±0.40[d]
Gallic acid	-	0.0024±0.0002[e]	0.0005±0.0000[e]	-

Values with the different superscript letter are statistically different ($p < 0.05$).

Table 2 Total phenolic and flavonoid content of the four *Solanum* fruits.

Sample	Total phenolic content (mg GAE/100 g extract)	Total flavonoid content (mg CE/100 g extract)
S. sanitwongsei	1031.23±11.87[a]	n.d.
S. stramonifolium	2634.59±21.40[b]	1437.87±19.09[b]
S. torvum	4347.59±17.80[c]	1283.04±12.61[c]
S. xanthocarpum	4474.36±23.74[d]	2984.98±10.21[d]

GAE – gallic acid equivalents; CE – catechin equivalents; n.d. – not detected.

Values with the different superscript letter are statistically different ($p < 0.05$).

Results

Antioxidant activities

Free radical scavenging capacities of the four *Solanum* fruit extracts were measured by DPPH and ABTS assays and the results are presented in Table 1. *S. xanthocarpum* was the most effective radical scavenger with the lowest EC_{50} values of DPPH and ABTS followed by *S. stramonifolium*, *S. torvum* and *S. sanitwongsei*. Antioxidant activities have been proposed to be related to reducing power (Duh, 1998). The reducing power of the *Solanum* fruit extracts was determined using the FRAP assay. The values were in the range of 8.15±0.16 to 26.90±0.40 mmol $FeSO_4$/100 g extract (Table 1). These observations were in accordance with the results obtained by DPPH and ABTS assays.

Total phenolic and flavonoid contents

The total phenolic and flavonoid contents of the methanolic extracts of the *Solanum* fruits ranged from 1031.23±11.87 to 4474.36±23.74 mg GAE/100 g extract and 1283.04±12.61 to 2984.98±10.21 mg CE/100 g extract respectively (Table 2). The highest levels of both phenolics and flavonoids were found in *S. xanthocarpum*, the lowest content of phenolics in *S. sanitwongsei*, and the lowest level of flavonoids in *S. torvum*. At the concentrations used in this study, flavonoids were not detected in *S. sanitwongsei*.

Potential cytoprotective effect against t-BuOOH-induced oxidative stress in the human liver cell

t-BuOOH (an oxidative insult), the human hepatoma cell line HepG2 and gallic acid (a positive control) were used in the present study. *t*-BuOOH, a short chain analog of lipid hydroperoxide, can be metabolized to free radical intermediates that can subsequently initiate lipid peroxidation, affect cell integrity, and form covalent bonds with cellular molecules resulting in cell injury (Rush *et al.*, 1985). HepG2 was demonstrated to be a good model to study *in vitro* toxicity to the liver since it retains many characteristics of normal human hepatocytes (Knasmüller *et al.*, 1998). Reportedly, a consistent cellular stress in HepG2 was evoked by *t*-BuOOH (Alia *et al.*, 2005). Published studies revealed the ability of gallic acid, a phenolic compound, to protect liver tissues against alloxan-induced oxidative stress, doxorubicin-induced hepatotoxicity in rats (Omobowale *et al.*, 2018; Ramkumar *et al.*, 2014). In the present study, to select appropriate extract concentrations of *Solanum* fruits for further cytoprotective study in HepG2 cells, non-cytotoxic concentrations were first determined. In cell treatment with extracts at 10, 50 and 100 µg/mL for 24 hour, the cell viability was ≥80% at 10-50 µg/mL of *S. xanthocarpum* and 10-100 µg/mL of *S. sanitwongsei*, *S. stramonifolium* and *S. torvum* (data not shown), indicating that all extracts of *Solanum* (except *S. xanthocarpum*) showed no cytotoxicity at ≤ 100 µg/mL. Therefore, only one concentration (50 µg/mL) of the *S. xanthocarpum* extract and two concentrations (50 and 100 µg/mL) of the other three extracts were used to evaluate potential cytoprotection in HepG2 cells. Subsequently, the possible protection of *Solanum* fruit extracts against *t*-BuOOH-induced loss of cell viability was performed by preincubating cells with and without *Solanum* extracts for 24 h, followed by treatment with the toxicant for 4 h. Treatment with 300 µM *t*-BuOOH alone resulted in 52.27±2.05% cell viability. As shown in Figure 1, all *Solanum* extracts except *S. xanthocarpum* extract significantly increased cell viability ($p < 0.05$) of *t*-BuOOH-exposed HepG2 cells by 6.95±2.25 to 53.09±2.60%. The cytoprotective activity of *S. sanitwongsei* was observed only in concentration of 100 µg/mL while those of *S. stramonifolium* and *S. torvum* were found in both concentrations of 50 and 100 µg/mL. In addition, the cytoprotective effect of *S. stramonifolium* was insignificantly different to that of gallic acid at equal concentration ($p > 0.05$).

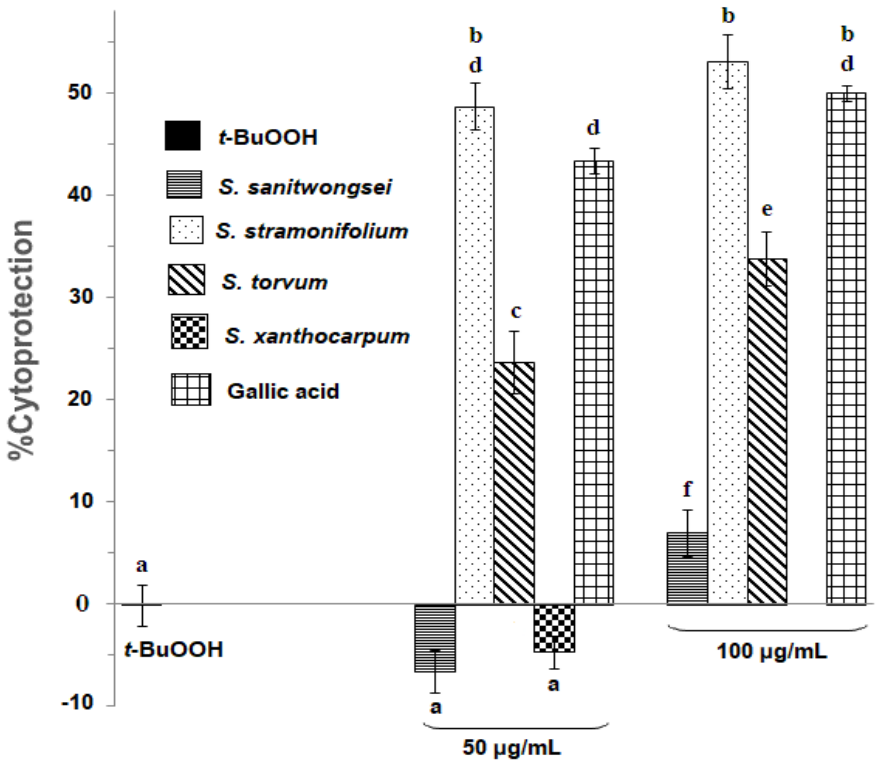

Figure 1 Protective effects of the four *Solanum* fruits against *t*-BuOOH-induced toxicity in HepG2 cells. Bars with no letters in common are significantly different ($p < 0.05$).

Discussion

The antioxidant activities of polyphenols are mainly due to their ability to act as radical scavengers and reducing agents (Mai *et al.*, 2009). *In vitro* antioxidant activities of the *Solanum* extracts in the present study were assessed by employing DPPH, ABTS and FRAP assays. The DPPH and ABTS are decolorization assays which measure the relative antioxidant abilities of the extracts to scavenge free radicals ($DPPH^{\bullet}$ and $ABTS^{\bullet+}$, respectively) generated in the assay system (Apak *et al.*, 2007) whereas the FRAP assay measures the reducing capability by increased sample absorbance based on the formation of ferrous ions (Prior *et al.*, 2005). In the present study, the results of the three antioxidant assays showed that the antioxidant activities of these four *Solanum* fruit extracts decreasing in the order were found to be *S. xanthocarpum* > *S. stramonifolium* > *S. torvum* > *S. sanitwongsei*. Previously, antioxidant activities of methanol extracts of *S. xanthocarpum* and *S. torvum* determined by DPPH assay were reported with EC_{50} values of 0.250 and 0.092 mg/mL, respectively whereas EC_{50} values of the two extracts in the present study were 0.174 and 0.520 mg/mL, respectively. Differences in the EC_{50} values might be because the reaction time used to incubate the test extract and $DPPH^{\bullet}$ solution was 20 or 30 min. In addition, fruits grown in different location and extraction in different condition might affect the amount of active constituents.

Phenolic compounds such as phenolic acids and flavonoids were commonly found in plants and have been reported to act as natural antioxidants (Rice-Evans *et al.*, 1997). Phytochemical investigation has shown that *S.torvum* fruit contains phenolic constituents including caffeic acid, catechin, ferulic acid, gallic acid, pyrogallol, quercetin and rutin (Kusirisin *et al.*, 2009; Ramamurthy *et al.*, 2012). *S. xanthocarpum* fruit comprises phenolic compounds like caffeic acid, methyl caffeate, and glycosides of quercitrin and apigenin (Gunaselvi *et al.*, 2010; Siddiqui, 1983). To the

best of our knowledge, data on the chemical composition of *S. sanitwongsei* and *S. stramonifolium* were not found. In the present study, the estimation of the total phenolic content found in the methanolic extracts of the four *Solanum* fruits decreased in the order: *S. xanthocarpum* > *S. torvum* >*S. stramonifolium* > *S. sanitwongsei*. Flavonoids in the *S. sanitwongsei* fruit extract indicate that the flavonoid level was too low to be measured by the method used in this study. Therefore, the total flavonoid content of these four *Solanum* extracts would be: *S. xanthocarpum* > *S. stramonifolium* > *S. torvum* > *S. sanitwongsei*. This decreasing order of the total flavonoid content present in the four *Solanum* extracts was similar to that of their antioxidant properties. It likely suggests that the antioxidant properties of these *Solanum* fruits can be predicted on the basis of their total flavonoid content

The present study demonstrated that at a maximal noncytotoxic concentration (100 µg/mL), *S. stramonifolium*, *S. sanitwongsei* and *S. torvum* were able to protect HepG2 cells against *t*- BuOOH- induced toxicity. The order of cytoprotection of these three *Solanum* extracts was *S. stramonifolium* > *S. torvum* > *S. sanitwongsei*, similar to that of antioxidant activities and total flavonoids. At a lower concentration (50 µg/ mL), the cytoprotective effects were observed in only *S. stramonifolium* and *S. torvum*, and the higher effect was in *S. stramonifolium*. These results likely suggest that flavonoids are active constituents with cytoprotection of these *Solanum* fruit extracts, and might be partly responsible for antioxidant activity to scavenge and reduce free radicals generated by *t*-BuOOH in HepG2 cells. The cytoprotective activity of *S. sanitwongsei* extract observed at only 100 µg/ mL but not at 50 µg/ mL could be due to the low amount of cytoprotective flavonoids found in this extract. The cytoprotective observation of the *S. torvum* fruit extract in the present study was in agreement with the previous report of its *in vivo* hepatoprotection against carbon tetrachloride- induced liver injury (Kayalvizhi *et al.*, 2012). Surprisingly, in the present study, the *S. xanthocarpum* fruit extract, the strongest antioxidant activities and the highest amount of total flavonoids, had no cytoprotective effect at the maximal noncytotoxic concentration (50 µg/ mL) . Reportedly, the *S. xanthocarpum* fruit extract defatted with petroleum ether and exhaustively macerated with 50% ethanol was shown to have the antioxidant dependent protective effect against liver injury in rats (Gupta *et al.*, 2011; Hussain *et al.*, 2012). The unobserved cytoprotection in the present study may be due to extraction with different method/ condition that affects the active flavonoids with cytoprotective effect, resulting in the low amount in the *S. xanthocarpum* fruit extract.

Conclusion

The present study demonstrated that edible fruits of *S. sanitwongsei*, *S. stramonifolium*, *S. torvum* and *S. xanthocarpum* possessed antioxidant activities. Of all four fruits, *S. xanthocarpum* displayed the strongest antioxidant properties and highest amount of total phenolics and flavonoids. Except *S. xanthocarpum* fruit, the other three *Solanum*s had the ability to protect cell against *t*-BuOOH in the human liver cell. Further studies would be worthwhile to isolate and identify active constituents with antioxidant and cytoprotective activities of these *Solanum* fruits as well as to investigate their possible mechanism of cytoprotection.

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