Research Article

ฤทธิ์ต้านอนุมูลอิสระ ความเป็นพิษต่อเซลล์ และฤทธิ์ปกป้องเซลล์ ของสารสกัดน้ำจากกิ่งของต้นพญารากดำ

นพรัตน์ นันทรัตนพงศ์[1]*, วาสนา เรียมอมตกุล[2], ภัทรดา สถาปัตยานนท์[3], รสิตา เพชรไทย[3], รินทร์นภา อธิกิจ[3]

[1] ภาควิชาเภสัชวิทยาและพิษวิทยา คณะเภสัชศาสตร์ มหาวิทยาลัยศิลปากร นครปฐม 73000 ประเทศไทย
[2] นักศึกษาหลักสูตรปรัชญาดุษฎีบัณฑิต สาขาวิทยาการทางเภสัชศาสตร์ คณะเภสัชศาสตร์ มหาวิทยาลัยศิลปากร นครปฐม 73000 ประเทศไทย
[3] นักศึกษาหลักสูตรปริญญาบัณฑิต ภาควิชาเภสัชวิทยาและพิษวิทยา คณะเภสัชศาสตร์ มหาวิทยาลัยศิลปากร นครปฐม 73000 ประเทศไทย
* **ติดต่อผู้นิพนธ์:** นพรัตน์ นันทรัตนพงศ์, ภาควิชาเภสัชวิทยาและพิษวิทยา คณะเภสัชศาสตร์ มหาวิทยาลัยศิลปากร นครปฐม 73000
โทรศัพท์ +66(0)34255800; โทรสาร +66(0)34250941; อีเมล์: nuntharatanapon_n@su.ac.th

บทคัดย่อ

ฤทธิ์ต้านอนุมูลอิสระ ความเป็นพิษต่อเซลล์ และฤทธิ์ปกป้องเซลล์ของสารสกัดน้ำจากกิ่งของต้นพญารากดำ

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พญารากดำเป็นพืชสมุนไพรที่มีการนำมาใช้เป็นยาพื้นบ้านเพื่อใช้รักษาโรคต่างๆโดยใช้ส่วนของเนื้อไม้และรากเป็นยาลดไข้และลดการอักเสบ หลายการศึกษาพบว่าเปลือกไม้ของต้นพญารากดำมีฤทธิ์ต้านเชื้อมาลาเรียและฤทธิ์ต้านอักเสบ การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาฤทธิ์ต้านอนุมูลอิสระ ความเป็นพิษต่อเซลล์และฤทธิ์ปกป้องเซลล์ของสารสกัดจากส่วนของใบและกิ่งของต้นพญารากดำ **วิธีการศึกษา:** สกัดสารจากใบและกิ่งพญารากดำโดยใช้เอทานอลและน้ำ ทำการศึกษาฤทธิ์ต้านอนุมูลอิสระในหลอดทดลองด้วยวิธี DPPH radical scavenging assay พร้อมทั้งหาปริมาณสารประกอบฟีนอลิกและฟลาโวนอยด์ในสารสกัด ทำการทดสอบความเป็นพิษต่อเซลล์และฤทธิ์ปกป้องการตายของเซลล์จากการเหนี่ยวนำด้วยสารไฮโดรเจนเปอร์ออกไซด์ในเซลล์ HepG2 ด้วยวิธี MTT assay **ผลการศึกษา:** สารสกัดด้วยเอทานอลและน้ำจากกิ่งของต้นพญารากดำมีฤทธิ์ต้านอนุมูลอิสระสูงโดยสารสกัดด้วย 80%เอทานอลมีฤทธิ์สูงที่สุด ในขณะที่สารสกัดจากใบมีฤทธิ์ต้านอนุมูลอิสระต่ำ ฤทธิ์ต้านอนุมูลอิสระมีความสัมพันธ์กับปริมาณสารประกอบฟีนอลิกและฟลาโวนอยด์ที่พบในพืช จากผลการศึกษาความเป็นพิษต่อเซลล์พบว่าสารสกัดด้วยน้ำจากกิ่งพญารากดำไม่แสดงความเป็นพิษต่อเซลล์ HepG2 แม้จะได้รับในความเข้มข้นสูง ในขณะที่อัตราการมีชีวิตรอดของเซลล์ HepG2 ลดลงกว่าร้อยละ 50 เมื่อได้รับสารสกัดด้วยเอทานอลความเข้มข้น 1000 ไมโครกรัมต่อมิลลิลิตร นอกจากนี้ยังพบว่าสารสกัดด้วยน้ำจากกิ่งพญารากดำในขนาด 10-100 ไมโครกรัมต่อมิลลิลิตรมีฤทธิ์ปกป้องการตายของเซลล์จากสารไฮโดรเจนเปอร์ออกไซด์ได้อย่างมีนัยสำคัญและไม่แตกต่างจาก silymarin ที่ใช้เป็นสารมาตรฐาน **สรุปผลการศึกษา:** สารสกัดด้วยน้ำจากกิ่งพญารากดำน่าจะเป็นแหล่งของสารต้านอนุมูลอิสระที่มีในธรรมชาติและมีฤทธิ์ปกป้องการตายของเซลล์จากการถูกเหนี่ยวนำให้เกิดสภาวะเครียด

คำสำคัญ: พญารากดำ, ฤทธิ์ต้านอนุมูลอิสระ, ความเป็นพิษต่อเซลล์, ฤทธิ์ปกป้องเซลล์, สารสกัดน้ำ

Antioxidant, Cytotoxicity and Cytoprotective Activity of *Polyalthia cerasoides* Branch Aqueous Extract

Nopparat Nuntharatanapong[1]*, Wassana Riam-amatakun[2], Patrada Satapattayanont[3], Rasita Phetthai[3], Rinnapa Atikij[3]

[1] Department of Pharmacology and Toxicology, Faculty of Pharmacy, Silpakorn University, Nakhon Pathom 73000, Thailand
[2] Ph.D Candidate in Pharmaceutical Sciences, Faculty of Pharmacy, Silpakorn University, Nakhon Pathom 73000, Thailand
[3] Undergraduate Student, Department of Pharmacology and Toxicology, Faculty of Pharmacy, Silpakorn University, Nakhon Pathom 73000, Thailand
*** Corresponding author**: Nopparat Nuntharatanapong, Department of Pharmacology and Toxicology, Faculty of Pharmacy, Silpakorn University, Nakhon Pathom 73000 Tel. +66(0)34255800; Fax. +66(0)34250941; E-mail: nuntharatanapon_n@su.ac.th

Abstract

Antioxidant, Cytotoxicity and Cytoprotective Activity of *Polyalthia cerasoides* Branch Aqueous Extract

Nopparat Nuntharatanapong[1]*, Wassana Riam-amatakun[2], Patrada Satapattayanont[3], Rasita Phetthai[3], Rinnapa Atikij[3]

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Polyalthia cerasoides wood and root have been traditionally used for antipyretic and anti-inflammatory activities. Several studies showed that the extracts from stem bark of *P. cerasoides* have biological effects including antimalarial and anti-inflammatory activities. The present study was aimed to investigate antioxidant property, cytotoxicity and cytoprotective activity of ethanolic and aqueous extracts of *P. cerasoides* branches and leaves. **Methods:** Ethanol and water extracts of *P. cerasoides* branches and leaves were examined for antioxidative activity *in vitro* by a DPPH radical scavenging assay. Total phenolic and flavonoid contents of the extracts were determined. The cytotoxicity test and cytoprotective activity against hydrogen peroxide-induced cell death were performed in HepG2 cells and evaluated by MTT assay. **Results:** In DPPH assay, the ethanolic and aqueous extracts from *P. cerasoides* branch showed superior scavenging effect, with the highest activity of 80% ethanolic extract, whereas the leaf extracts showed weaker activity. The antioxidative activity was also positively associated with the total phenolic and flavonoid contents of the extracts. The cytotoxicity results revealed that an aqueous extract of *P. cerasoides* branch was not toxic to HepG2 cells even at a high concentration. However, when the cells were treated with 1000 µg/mL of ethanolic branch extract, the percentage cell viability of HepG2 cells decreased to less than 50%. Furthermore, *P. cerasoides* aqueous branch extract as well as silymarin, a standard hepatoprotective agent, exhibited protective effect against oxidative damage induced by hydrogen peroxide in HepG2 cells. **Conclusion:** These results suggested that the aqueous branch extracts of *P. cerasoides* might be useful as a natural source of potential antioxidant and could provide the novel source for the treatment of diseases associated with oxidative stress.

Keywords: *Polyalthia cerasoides*, Antioxidant, Cytotoxicity, Cytoprotective activity, Aqueous extract

Introduction

Herbal plants have been known as the important sources of medicinal compounds. Some conventional medications originated from botanical sources, for instance, aspirin was derived from willow bark (Norn *et al.*, 2009) and quinine was derived from cinchona bark (Achan *et al.*, 2011). Nowadays, several plant extracts are widely investigated on their biological effects. Antioxidative activity of medicinal plants is one of the most pharmacological interest (Xu *et al.*, 2017; Mahadlek *et al*., 2017). Many chronic diseases, including neurodegenerative diseases (Kim *et al.*, 2015), cancer (Prasad *et al.*, 2016) and cardiovascular diseases (Siti *et al.*, 2015), resulted from the imbalance between excessive reactive oxygen species (ROS) production and decreased endogenous antioxidative defenses. Oxidative stress-related disorders are one of the major health problems at the present day. Therefore, the searches for naturally occurring antioxidants are needed to help protect cells and DNA from oxidative damage.

Polyalthia cerasoides (Roxb.) Benth. ex Bedd., or Phaya-rakdum in Thai, is a medium-sized tree in the family of Annonaceae that is widely distributed throughout Thailand, India and Southeast Asian region. Several parts of *P. cerasoides*, especially wood and root, have been traditionally used as tonic, analgesics, antipyretics and anti-inflammatory agent. Phytochemicals that have been found in this plant are phenolics (Ravikumar *et al.*, 2008), flavonoids (Kanchanapoom *et al.*, 2002), alkaloids (Kanokmedhakul *et al.*, 2007) and terpenoids (Goudarshivananavar *et al*., 2015). It has been reported in previous studies that extracts from stem bark and leaf of *P. cerasoides* have antimicrobial (Treeratanapiboon *et al.*, 2011; Surekha *et al.*, 2011) and antifungal properties (Surekha *et al*., 2011). The stem bark extracts also showed anti-inflammatory potential in carrageenan rat paw edema model (Goudarshivananavar *et al*., 2015). The root extracts of *P. cerasoides* exhibited antimalarial and anti-tuberculosis activities *in vitro* (Kanokmedhakul *et al*., 2007). Animal studies with *P. cerasoides* have revealed the hepatoprotective effect against carbon tetrachloride-induced liver injury (Goudarshivananavar *et al*., 2015; Padma *et al.*, 1999). Three studies have shown *in vitro* antioxidative effect of *P. cerasoides* extracts from stem bark (Goudarshivananavar *et al*., 2015; Ravikumar *et al*., 2008; Treeratanapiboon *et al*., 2011). Even though antioxidative activity is the most studied pharmacological action of *P. cerasoides*, there is still a limited information available on this action. It is noteworthy that the part of *P. cerasoides* conducted in these studies is restricted only to stem bark. To date, there is no existing evidence on antioxidative activity from the branch and leaf extracts of *P. cerasoides*.

In general, Thai folk medicines of *P. cerasoides* were prepared by boiling the wood and root with water. However, the extraction of *P. cerasoides* was usually done by organic solvent-based procedures. The solvents used in these studies included hexane, chloroform, dichloromethane, ethyl acetate, methanol and ethanol. (Goudarshivananavar *et al*., 2015; Kanokmedhakul *et al.*, 2007; Ravikumar *et al.*, 2008; Treeratanapiboon *et al*., 2011) According to Prat *et al*. (2014), some of these solvents are considered as problematic or hazardous, classified by its environmental, health and safety impacts. For examples, hexane causes neurotoxicity in rats (Takeuchi *et al.*, 1980) and human (Chang, 1987). Substitution of harmful solvents with less toxic solvents will provide a safer extraction method.

This study aims to evaluate the antioxidative activity of *P. cerasoides* extracts from branch and leaf, which are accessible parts of the plant, and to screen for the cytotoxicity of aqueous and ethanolic extracts, and investigate the cytoprotective ability against oxidative stress**.**

Materials and Methods

Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH), Trolox®, silymarin, hydrogen peroxide and MTT reagent were purchased from Sigma (St.Louis, MO,USA). Folin-ciocalteu,

gallic acid and quercetin were purchased from Fluka (Switzerland). Ethanol and methanol were obtained from RCI Labscan (Thailand). All the chemicals, solvents and reagents used were of analytical grade. HepG2 cell line was kindly gift from Professor Dr.Praneet Opranasopit. DMEM medium, fetal bovine serum and 1% penicillin/streptomycin were obtained from Gibco (Thailand).

Preparation of P. cerasoides branch and leaf extracts:

The fresh leaves and branches of *P. cerasoides* were collected from botanical garden, Ratchaburi province, Thailand. A voucher specimen of the collected plant is preserved in the Department of Pharmacology and Toxicology, Silpakorn University for future reference. The plants were washed with tap water and allowed to air dry in room temperature then chopped into small pieces. Both parts of plant were placed in hot air oven at 50^{o}C until completely dry. The dried leaves and branches were crushed separately into fine powder and stored at 4^{o}C before the extraction process started. One gram of plant powder was soaked with 100 mL of indicated solvent for 2 hr. The solvents used in this study were 80%ethanol, 50%ethanol and purified water. Plant mixtures were heated on water bath for 15 min at 60^{o}C and 80^{o}C for ethanol and water extraction, respectively. Plant extracts were filtered and the solvents were removed to obtain concentrated extracts. Solvent removal was performed by using water bath for 80%ethanol extract and freeze dryer (Labconco 7670530, USA) for 50%ethanol and water extracts. The percentage yield of dried extracts was calculated with reference to the dried plant material initially taken. All extracts were stored at -20^{o}C for further investigation.

Assessment of in vitro antioxidative activity:

The antioxidative activity of plant extracts was conducted by using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay (Moore and Yu, 2007). In brief, 100 µL of DPPH solution in ethanol (0.1 mM) was added to 100 µL of plant extracts solution in ethanol at different concentrations (5-3000 µg/mL). The plate was gently shaken before the absorbance (A) of the mixture was measured by a microplate reader (Fusion A153601) at 550 nm. Trolox was used as a reference and ethanol was used as a negative control. A reduction in the absorbance of the reaction mixture specified free radical scavenging capacity of the plant extracts. The antioxidative activity was calculated as percentage of inhibition using the following formula,

$$\% \text{ inhibition} = [(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100.$$

Where A is the measured absorbance. The absorbance of sample was corrected by subtracting background readings (plant extract solution without DPPH) from sample readings. The data were expressed as half maximum inhibitory concentration (IC50, µg/mL).

***Determination of phenolic content in P. cerasoides extracts*:**

Total phenolic content of *P. cerasoides* extracts was determined by Folin-Ciocalteu reagent assay (Makkar *et al.*, 1993). Briefly, 300 µL of the Folin-Ciocalteu reagent was mixed with 300 µL of plant extract in methanol (1 mg/mL). 2,400 µL of sodium carbonate (5 mg/mL) was then added to the mixture after 2 min of incubation. The mixture was incubated 30 min at room temperature, protected from light, before the absorbance was measured at 765 nm. The total phenolic content in plant extracts was reported as mg gallic acid equivalent per gram of dried extract, using a standard curve of gallic acid (mg GAE/g extract).

***Determination of total flavonoid content in P. cerasoides extracts*:**

Total flavonoid content of the extracts was performed by the aluminium chloride colorimetric method (Woisky and Salatino, 1998). Briefly, 0.5 mL of plant extract was mixed with 0.5 mL of 2% aluminium chloride solution. The absorbance was measured at 420 nm after standing at room temperature for 1 hr. Quercetin was used as a standard. The flavonoid concentration in the extracts was calculated from a standard curve, and the data were expressed as mg quercetin equivalent per gram of dried extract (mg QE/g extract).

Assessment of cytotoxicity:

The cytotoxic effect of *P. cerasoides* branch extracts was determined by MTT assay (Mosmann, 1983). Briefly, HepG2 cells were seeded at 1 x 10^4 cells/well in a 96-well plate in 100 µL of DMEM medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin, and then incubated for 24 hr at 37°C and 5%CO_2. The freshly prepared *P. cerasoides* extract solution (1-1000 µg/mL) was added to HepG2 cells. After 24 h of incubation, the medium was then removed and 25 µL of MTT solution (0.5 mg/mL) was added to each well and further incubated for 4 hr. MTT was then removed and 100 µL of DMSO was added to solubilize blue formazan crystal. The absorbance was quantified by a microplate reader at 550 nm. Cell viability was calculated as percentage relative to control.

***Assessment of cytoprotective activity against oxidative stress*:**

To determine the cytoprotective ability of *P. cerasoides* aqueous branch extracts against hydrogen peroxide (H_2O_2), cell viability was conducted by MTT assay. HepG2 cells were seeded overnight into 96-well plate at 1 x 10^4 cells/well. Cells were pretreated with aqueous branch extracts (1-1000 µg/mL) for 2 hr and then exposed to 50 µM H_2O_2. Untreated cells and cells treated with H_2O_2 alone were used as controls. The data were expressed as percentage cell viability relative to untreated control.

Statistical analysis:

All assays were done in triplicate. The data were expressed as mean ± SD values. The data were analyzed using one-way ANOVA followed by Duncan's multiple range test. *P* value < 0.05 was considered statistically significant. Correlation between antioxidative activity and total phenolic and flavonoid content was determined. The data were calculated by computer programs: Microsoft Excel and SPSS version 11.0

Results

In this experiment, branch and leaf powders of *P. cerasoides* were extracted by 80%, 50% ethanol and water. As shown in Table 1, the extracts from leaves of *P. cerasoides* showed higher percentage yield than branch extracts with 21-36% and 11-16% yield, respectively, whereas aqueous extracts from both branch and leaf gave higher percentage yield relative to ethanolic extracts.

Table 1 Yield percentage of *P. cerasoides* extracts from leaf and branch using different solvents

Extracts	% Yield	
	Leaf	Branch
80% ethanol	21.35 ± 1.38 [a, b]	10.67 ± 1.33 [a, b]
50% ethanol	30.92 ± 2.30 [a, b]	14.33 ± 2.90 [b]
Water	36.19 ± 2.43 [a, b]	16.33 ± 1.56 [b]

Value are expressed as mean ± SD (n=3)

[a]Significant difference (p-value<0.05) between the same column

[b]Significant difference (p-value<0.05) between the same row

Antioxidative activity

Antioxidant activities of *P. cerasoides* extracts were evaluated by DPPH method. The results were shown in Table 2. When compared between branch and leaf extracts for the same solvent, branch extracts showed lower IC_{50} than leaf extracts (*p*-value < 0.05). The IC_{50} of 50% ethanolic and aqueous branch extracts were not significantly different. 100 µg/mL of branch extracts, regardless of the type of solvents, exhibited similar antioxidant capacity (89.89-94.97% inhibition) to 10 µg/mL of antioxidant standard

Trolox (89.87% inhibition). On the other hand, the percent inhibition of DPPH of leaf extracts (14.46-46.81% inhibition) was lower than Trolox significantly. The data of percent inhibition of the extracts were shown in Figure 1.

Total phenolic and flavonoid contents of P. cerasoides extracts:

The total phenolic and flavonoid contents of *P. cerasoides* extracts were calculated from standard curve as mentioned above. Maximum phenolic and flavonoid contents were found in 80% ethanolic extracts from both branch and leaf. Moreover, the amount of phenolics and flavonoids found in branch extracts were higher when compared to leaf extracts by the same solvent. Figure 2 and 3 depicted the total phenolic and flavonoid content in *P. cerasoides* extracts in different solvents. The total phenolic and flavonoid contents were associated with antioxidative activity of *P. cerasoides* extracts with the correlation coefficient of +0.7007 and +0.6069, respectively.

As previously mentioned, branch extracts showed stronger antioxidative activity and correspondingly contained higher phenolic and flavonoid content than that of leaf extracts. For these reasons, *P. cerasoides* extracts from branch were selected for further examination.

Table 2 The IC_{50} of DPPH radical of *P. cerasoides* extracts in different solvents

Extracts	DPPH IC_{50} (µg/mL)	
	Leaf	Branch
80% ethanol	123.08 ± 29.60[a, b]	24.42 ± 4.64 [a, b]
50% ethanol	182.15 ± 17.20 [a, b]	32.64 ± 1.70 [b]
Water	> 500 [b]	42.72 ± 6.40 [b]

Value are expressed as mean ± SD (n=3)

[a]Significant difference (p-value<0.05) between the same column

[b]Significant difference (p-value<0.05) between the same row

IC_{50} value of Trolox = 4.69 ± 0.40 µg/mL

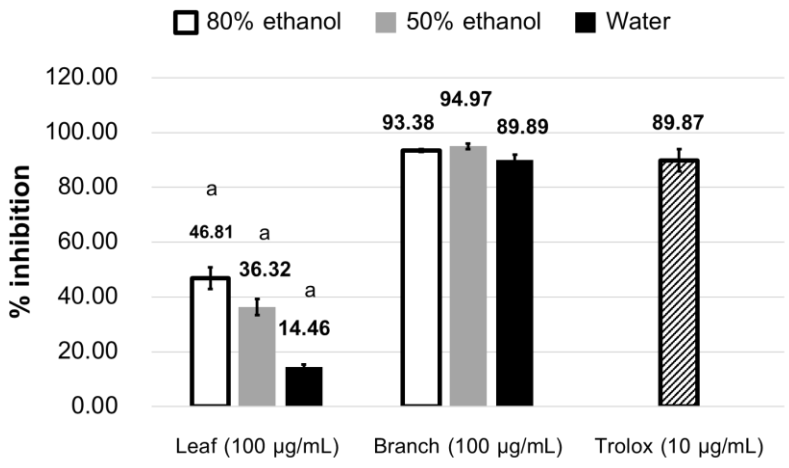

Figure 1 DPPH radical scavenging activity of *P. cerasoides* extracts. [a]Significant difference (p-value<0.05) compared with Trolox 10 µg/mL.

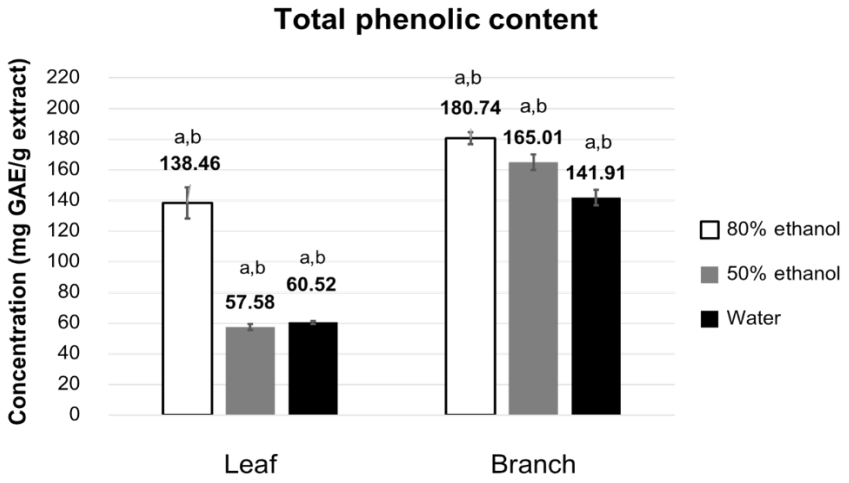

Figure 2 Total phenolic content of *P. cerasoides* extracts. Significant differences within and between groups are shown by letters [a] and [b], respectively (p-value<0.05).

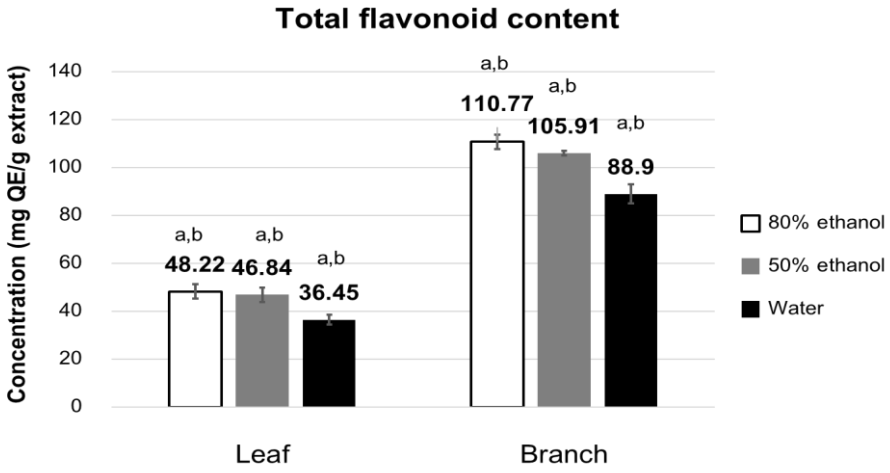

Figure 3 Total flavonoid content of *P. cerasoides* extracts. Significant differences within and between groups are shown by letters [a] and [b] respectively (p-value<0.05).

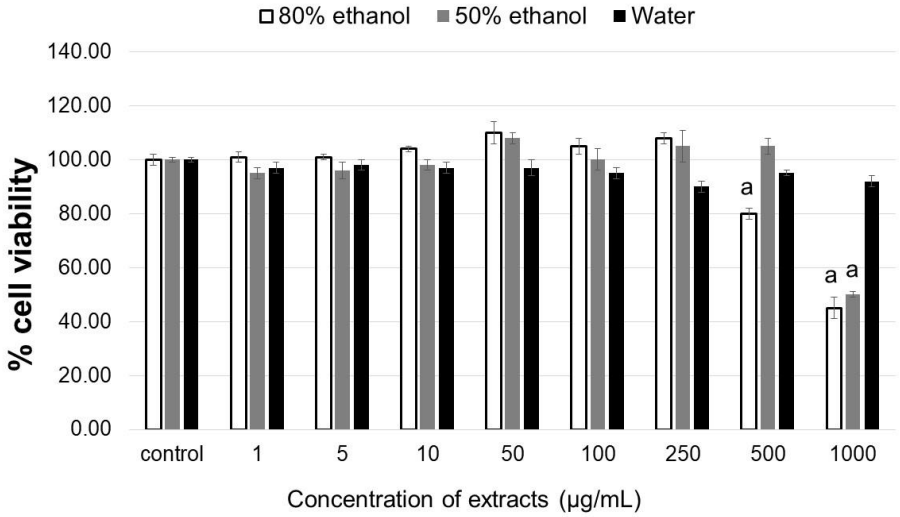

Figure 4 Cell viability of *P. cerasoides* branch extracts at different concentration.

[a]Significant differences compared to control, $p < 0.05$.

Cytotoxicity of P. cerasoides branch extracts:

Cytotoxic activity of the branch extracts was tested and the results were expressed as percentage of cell viability relative to control. At low concentration (up to 250 µg/mL), the plant extracts from all solvents did not affect the cell viability (Figure 4). At high concentration (1000 µg/mL), 50% and 80% ethanolic extracts decreased the cell viability to be 50.02% and 45.08% of the control, respectively. In contrast, the aqueous extract at 1000 µg/mL demonstrated more than 90% viability of HepG2 cells, indicating that the aqueous extract had lower toxicity to HepG2 cells compared to ethanolic extracts at higher concentration. With its effective antioxidative activity, the aqueous branch extract of *P. cerasoides* was chosen to perform additional experiment.

***Cytoprotective activity of P. cerasoides aqueous branch extract against oxidative stress*:**

In this experiment, H_2O_2 was used to induce the oxidative stress in HepG2 cells. After the 24 hr exposure of H_2O_2, cell viability decreased significantly compared to untreated control. The effects of *P. cerasoides* aqueous branch extract on the survival of HepG2 cells treated with H_2O_2 were presented in Figure 5. Pretreatment with the plant extracts (10-100 µg/mL) significantly increased the viability of H_2O_2-treated HepG2 cells with percentage cell viability of 78 ± 2.10%, 92 ± 4.62%, 99 ± 4.57% and 91 ± 4.91% at concentration of 5 µg/mL, 10 µg/mL, 25 µg/mL, 50 µg/mL, and 100 µg/mL, respectively (p-value < 0.01). Similar to *P. cerasoides* extracts, silymarin treatment (5-100 µg/mL) greatly improved the survival of H_2O_2-treated HepG2 cells. The viability of cells treated with *P. cerasoides* extracts and silymarin at each concentration was significantly different to untreated control cells, except for 50 µg/mL of *P. cerasoides* extract.

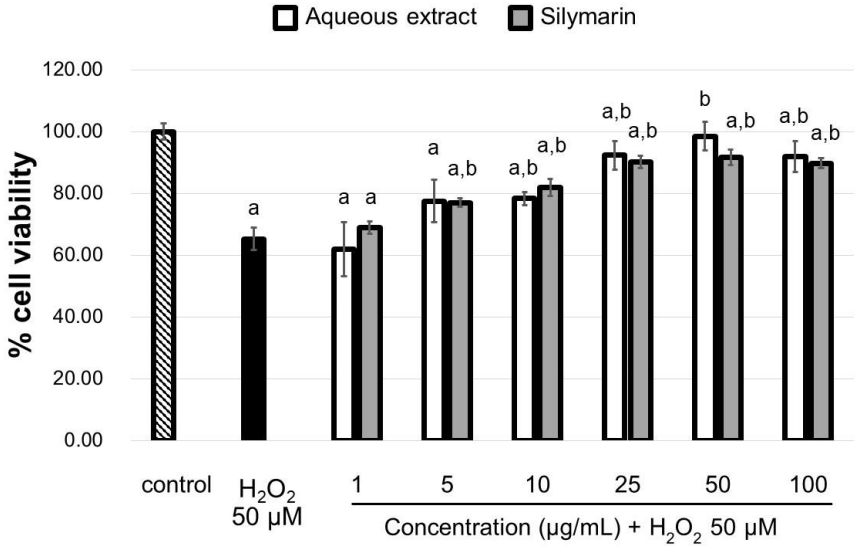

Figure 5 Cytoprotective effect of *P. cerasoides* aqueous branch extract and silymarin after H_2O_2-exposure in HepG2 cells. $^{a}p < 0.05$ compared with untreated control and $^{b}p < 0.01$ compared with H_2O_2-treated cells.

Discussion

The current study investigated the antioxidant potential of *P. cerasoides* branch and leaf extracts in various solvents. Stem bark was commonly used in the previous works to study the antioxidative effects of *P. cerasoides* (Goudarshivananavar *et al.*, 2015; Ravikumar *et al.*, 2008). In this study, branch and leaf were selected since they were easily obtained and a study on antioxidative effect of these parts did not exist.

The results showed that the branch extracts had significantly higher antioxidative activity than the leaf extracts, although the percentage yield of the leaf extracts was higher than the branch extracts. Their antioxidative activity was positively correlated with total phenolic and flavonoid contents. These results were similar to the prior study of *P. cerasoides* extracts from stem bark (Ravikumar *et al*., 2008) and other plant extracts (Babbar *et al.*, 2014; Erkan *et al.*, 2008; Saeed *et al.*, 2012). Phenolic and flavonoid compounds have been known as antioxidants due to its ability of scavenging free radicals (Balasundram *et al.*, 2006; Huang *et al.*, 2010). The results suggested that antioxidative activity could be based on phenolic and flavonoid components that were found in the extracts.

The selection of solvent is a key factor to the antioxidant capacity and the quantity of phenolic and flavonoid compounds of the extracts (Babbar *et al*., 2014). Polar solvents are suitable for gaining phenolic and flavonoid compounds from plants (Do *et al.*, 2014). Specifically, the mixture of ethanol and water was considered as a proper solvent because of its good extraction ability (Do *et al*., 2014). Regarding health and safety issues, ethanol and water were categorized as recommended solvents for extraction (Prat *et al.*, 2014).

Previously, dichloromethane stem bark extract of *P.cerasoides* was reported to show DPPH activity with IC_{50} of 100.76 µg/mL and no activity with hexane, ethyl acetate and methanol extracts (Treeratanapiboon *et al*., 2011). The antioxidative activity of dichloromethane extract, could possibly arise from triterpenes and stigmasterol (Ramadan *et al*., 2007; Prachayasittikul *et al*., 2010). According to the study by Ravikumar *et al*. (2008), alcoholic extract of *P.cerasoides* stem bark was reported to show high antioxidant capacity with moderate toxicity against L929 cell line.

Our results indicated that 80% ethanolic extracts showed the highest antioxidative activity, and greater amount of phenolic and flavonoid compounds than these of 50% ethanol and water. The cytotoxicity of the branch extracts in different solvents was further performed. At higher concentrations, the branch extracts with increasing ethanol: water ratio showed higher cytotoxicity. Although ethanol was more efficient solvent for extraction, the aqueous branch extract was less toxic to cells. Aqueous branch extract was consequently determined for cytoprotective ability against H_2O_2-induced toxicity. The results demonstrated that the aqueous branch extracts of *P. cerasoides* at concentration of 10-100 µg/mL can partially protected HepG2 cells against H_2O_2-induced toxicity. Interestingly, a 50 µg/mL concentration of *P. cerasoides* extract reversed the effects of H_2O_2 and the cell viability did not significantly differ from that of untreated control.

Conclusion

The present study demonstrated that antioxidative activity, total phenolic and flavonoid contents of *P. cerasoides* branch extract were higher than its leaf extract. Among three solvents used for extraction, 80% ethanol provided better results than those of other solvents. However, high concentration of ethanolic extraction (1000 µg/mL) showed cytotoxicity with less than 50% cell viability. Water, as a solvent for *P. cerasoides* extraction, offered advantages over ethanol since it is easy to use, inexpensive with low toxicity. The aqueous branch extract also showed protecting properties against H_2O_2-induced cytotoxicity. Thus, the aqueous branch extract of *P. cerasoides* may be beneficial and provide the novel source for the treatment of diseases associated with oxidative stress. The further studies are needed to isolate and identify active compounds in aqueous branch extract of *P. cerasoides* and to determine the possible protective mechanism of this plant.

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