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ORIGINAL ARTICLE

Assessments of Antioxidant, Antilipid Peroxidation, and *In-vitro* Safety of *Derris scandens* Vine Extracts from Southern Thailand

Rawiwan Nooin[1,2], Pornsiri Pitchakarn[3], Chaturong Kanchai[1], Churdsak Jaikang[1]

Departments of [1]Forensic Medicine and [3]Biochemistry, [2]Master's Degree Program in Toxicology Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand

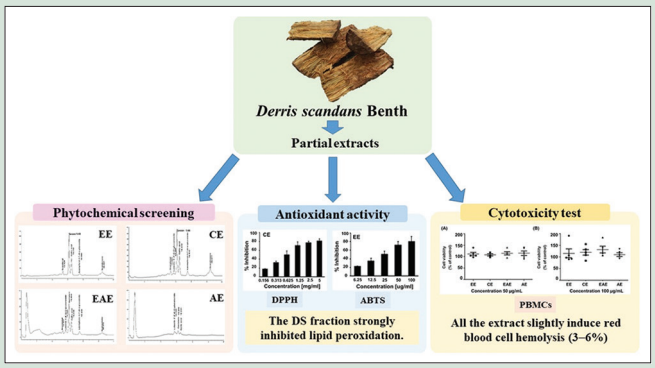
ABSTRACT

Background: *Derris scandens* (DS) has been used in Thai traditional medicine recipes for musculoskeletal pain relief in Southern Thailand. Changing of terrain and climate affected its phytochemical constituents and led to change antioxidant and toxic properties. **Objective:** The aim of this study was to study phytochemical contents, antioxidant properties, and toxicity of the DS extracts' *in vitro* models. **Materials and Methods:** The DS ethanolic extract (EE) was partially extracted with chloroform (CE), ethyl acetate (EAE), and water (aqueous extract [AE]). Phytochemical contents, antioxidant properties, and toxicity in Caco-2 cells, peripheral blood mononuclear cells (PBMCs), and red blood cells (RBCs) were explored. **Results:** Genistein and gallic acid were rich in the EE and CE. The CE demonstrated the highest 2,2 – diphenyl – 1 – picrylhydrazyl scavenging activity with half-maximal Radical cavenging concentration (SC_{50}) value – 0.81 ± 0.07 mg/mL. The EE showed the highest 2, 2'–azino – bis (3 – ethylbenzothiazoline – 6 – sulfonic acid) radical scavenging activity (SC_{50} = 22.05 ± 3.91 μg/mL) and all the extracts strongly inhibited lipid peroxidation. The EE and CE were more toxic than the EAE on Caco-2 cells with IC_{50} values – 26.45 ± 3.57 and 36.36 ± 6.87 μg/mL, respectively. At high dose, all fractions were not toxic to human's PBMCs, but they slightly induced RBC hemolysis by 3-6 percent. **Conclusions:** The DS grown in Southern Thailand had antioxidant properties, toxic to Caco-2 cells and not toxic to normal cells. High dose and continuous consumption of the DS must be concerned. Further safety assessment models including animals and humans should be performed to find safety dose of the DS.

Key words: Antioxidant activity, *Derris scandens*, genistein, phytochemical contents, toxicity

SUMMARY

- The *Derris scandens* (DS) grown in Southern Thailand contained phenolic and flavonoid compound especially genistein, luteolin 3',4'-di-O-glucuronide, and gallic acid
- The DS extracts had potential to scavenge 2,2 – diphenyl – 1 – picrylhydrazyl and 2, 2'–azino – bis (3 – ethylbenzothiazoline – 6 – sulfonic acid) radical and inhibited lipid peroxidation properties in dose-response manner
- The DS extracts at high dose decreased Caco-2 cells viability, slightly induced human red blood cell hemolysis, and were not toxic to human peripheral blood mononuclear cells.



Abbreviations Used: ABTS: 2, 2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), DMEM: Dulbecco's Modified Eagle Medium, DMSO: Dimethyl sulfoxide, DPPH: 2,2-diphenyl-1-picrylhydrazyl, HLM: Human liver microsome, HLM: Human liver microsome, HPLC: High-performance liquid chromatography, IC_{50}: Half inhibitory concentration, MDA: Malondialdehyde, OD: Optical density, PBMC: Peripheral blood mononuclear cell, RBC: Red blood cell, RPMI: Roswell Park Memorial Institute Medium, SC_{50}: Halfmaximal radical scavenging concentration, SRB: Sulforhodamine B assay.

Correspondence:
Dr. Churdsak Jaikang,
Department of Forensic Medicine,
Faculty of Medicine,
Chiang Mai University, Chiang Mai 50200,
Thailand.
E-mail: churdsak.j@cmu.ac.th
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INTRODUCTION

Derris scandens (DS) belongs to the family Leguminosae and in Thailand is known as "Thao – Wan – Priang." It has been recommended by Thailand National List of Essential Medicines for alleviate musculoskeletal pain. Dried powder (0.5–1 g) contained in capsules (dose 0.5–1 g immediately after meal 3 times a day), cocktail (dose 0.9–1.5 g immediately after meal 3 times a day), decoction and ethanolic extract (EE) (dose 400 mg immediately after meal 2 times a day) were major consumption recipes.[1] Flavonoids and phenolic compounds contained in the DS act as antioxidant,[2] anticancer activity, and cardiovascular preventive agents.[3-5] The phytochemicals provide health benefits; however, they may have side effects on consumers. Many reports have been indicated that the phytochemicals especially tannins and alkaloids might be related to the toxicity both in humans and animals.[6,7] Some flavonoids found in the DS could interact with biological membrane of peripheral blood mononuclear cells (PBMCs) and red blood cells (RBCs) leading to hemolysis.[8-10] Previous clinical study showed that a major adverse effect of the DS consumption was gastrointestinal symptoms.[11] The evidence of their toxicity and epidemiology studies are necessary to provide the truth of their claims. Variation of terrain and climate can change phytochemical constituents and toxicological properties in plants. Therefore, the phytochemical contents, antioxidant activity, and *in vitro* safety of the DS from Southern Thailand were investigated in

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this study. The results might be describe side effects of the DS and its products for surveillance and provide highly biological efficiency, safety, and friendliness to consumers.

MATERIALS AND METHODS

Plant material

Vines of DS were collected during March 2016 from Lang Suan District, Chumphon Province, Thailand. Voucher number of the DS (023204) was deposited at Department of Pharmaceutical Sciences, Faculty of Pharmacy, Chiang Mai University. Dried DS powder was soaked in 95% ethanol for 24 h in the dark. The solvent was evaporated under reduced pressure and low temperature. Distilled water was added in the crude EE and partially extracted with chloroform (CE) and ethyl acetate (EAE). Organic solvents were removed and dried under nitrogen gas. The aqueous extract (AE) was dried using lyophilizer, and all the extracts were kept at −20°C before use.

Determination of phytochemical contents in the *Derris scandens* extracts

Total phenolic content

Total phenolic content was determined by Folin–Ciocalteu reagent according to Abozed *et al.*'s protocol.[12] The DS extracts (1 mg/mL) in methanol were mixed with 10% Folin–Ciocalteu solution and 1 M Na_2CO_3. After incubation for 30 min in the dark, the reaction mixture was determined using spectrophotometer at 765 nm. The results were expressed as gallic acid equivalent per gram of dried plant (mg gallic acid equivalent/g dried plant).

Total flavonoid content

Total flavonoid content was determined by aluminum chloride method.[13] Briefly, the DS extracts (1 mg/mL) in methanol were added in the test tube containing 10% aluminum chloride and 1 M potassium acetate. Total flavonoid content was measured at 415 nm by spectrophotometer after incubation for 30 min in the dark. The results were presented as quercetin equivalent per gram of dried plant (mg quercetin equivalent/g dried plant).

Total tannin content

Total tannin content was determined by Folin–Dennis reagent.[13] Briefly, the DS extracts (1 mg/mL) in methanol was mixed with 10% Folin–Dennis and 1M Na_2CO_3 solution. After incubation at room temperature for 30 min, total tannin content was measured at 725 nm using spectrophotometer. The results were expressed as tannic acid equivalent per gram of dried plant (mg tannic acid equivalent/g dried plant).

Total alkaloid content

Total alkaloid content was evaluated by modifying method of John *et al.*[14] Briefly, 1 mg/mL of the DS extracts in methanol was mixed with 5 mL of phosphate buffer pH 4.7 and 5 mL of bromocresol green solution. 10 mL of CE was added in the mixture, and the organic layer was measured at 470 nm using spectrophotometer. The results were presented as caffeine equivalent per gram of dried plant (mg caffeine equivalent/g dried plant).

Bioactive compounds' screening in the *Derris scandens* extracts using high-performance liquid chromatography

Flavonoid and phenolic contents in the DS extracts were analyzed by high-performance liquid chromatography (HPLC)-diode array (Agilent 1260 infinity Binary LC). The DS extracts were dissolved in dimethyl sulfoxide (DMSO) and separated by C-18 column (Purospher® STAR RP-18e; 150 × 4.60, 5 μm). The mobile phase was composed of acetonitrile (a) and methanol (b) at a flow rate of 0.8 mL/min, and injection volume was 5 μL. Gradient elution program was used as followed: (time, min/%A): 0/20, 15/30, 20/40, 25/60, and 30/70, respectively. The spectrums were determined by diode array at 330 nm and were collected spectrums between 200 and 400 nm for bioactive compounds' identification.[15]

2, 2-Diphenyl-1-picryl-hydrazyl free radical scavenging assay

The 2,2 – diphenyl – 1 – picrylhydrazyl (DPPH) radical scavenging activity of the DS extracts was examined following the method of Khachitpongpanit *et al.*[13] Briefly, 20 μL of the DS extracts in DMSO (0.156–5 mg/mL) was mixed with 0.004% DPPH solution in methanol and then incubated in the dark for 30 min. The absorbance of the mixtures was measured at 515 nm by spectrophotometer and trolox was used as a positive control. The percentage of inhibition was calculated using the following formula:

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

2,2-Azino-bis3-ethylbenzothiazoline-6-sulfonic acid free radical scavenging assay

The 2, 2'–azino – bis (3 – ethylbenzothiazoline – 6 – sulfonic acid) (ABTS) radical scavenging assay was carried out according to the method of Olszowy *et al.*[16] The ABTS radical cation was generated from 7.0 mM ABTS solution and 2.45 mM potassium persulfate solution in the ratio of 1:1 (v/v) and stood in the dark at room temperature for 12 h before use. The $ABTS^{\bullet}$ solution was diluted with distilled water to give absorbance between 0.8 and 0.9 at 734 nm. 10 μL of the DS extracts was dissolved in DMSO and added in 190 μL of $ABTS^{\bullet}$ solution. The absorbance of the samples was measured by UV-visible spectrophotometer at 734 nm, and trolox was used as a positive control. Percent inhibition was calculated following the equation:

$$\% \text{ Inhibition} = (OD_{control} - OD_{sample})/OD_{control}) \times 100$$

Lipid peroxidation assay

Antilipid peroxidation assay of the DS extracts was assessed by modified method of Upadhyay *et al.*'s protocol.[17] Human liver microsome was used as lipid source and isolated from human liver tissues (Ethics Committee for Human Research, Chiang Mai University; FOR2559-04348). Briefly, 50 μL of the DS extracts in DMSO (1–12.5 μg/mL) was mixed with the microsomal solution. Then, 25 μL of 0.25 M $FeSO_4$ was added and incubated for 30 min. 750 μL of 20% acetic acid, 450 μL of 0.5% normal saline solution, 200 μL of thiobarbituric acid, and 1 mL 0.6 M trichloroacetic acid were added. The mixture was boiled at 95°C, for 30 min. After cooling, the mixture was centrifuged at 3000 rpm for 5 min. The absorbance of supernatant was measured at 532 nm using spectrophotometer. Percent inhibition was calculated following the equation:

$$\% \text{ Inhibition} = (OD_{control} - OD_{sample})/OD_{control}) \times 100$$

Cell culture and treatments

Human intestinal Caco-2 cell line (ATCC HTB-37) was seeded and the cells were routinely maintained at 37°C in 95% air, 5% CO_2 in complete Dulbecco's Modified Eagle's Medium.[18] For the treatment, the cells were plated at 1×10^4 cells/well in 96-well plates for 24 h and treated with the DS extracts in DMSO (0–400 μg/mL) for 48 h. The cell viability was assessed by means of the sulforhodamine B assay (SRB) colorimetric method.[19]

Cytotoxicity of the *Derris scandens* extracts on human peripheral blood mononuclear cells

PBMCs were isolated from 5 healthy volunteers according to the method of Umsumarng *et al.*[20] The PBMCs were maintained in Roswell Park Memorial Institute Medium media containing 10% fetal bovine serum, 1% penicillin and streptomycin. The PBMCs were plated (5×10^4 cells/well) in 96-well plates for 24 h and subsequently treated with the DS extracts in DMSO (0–100 μg/mL) for 48 h. The cell viability was measured by SRB assay according to Vichai and Kirtikara method.[19] The absorbance of cell viability was measured at 510 nm and compared with the control group.

In vitro hemolysis assay

RBCs were isolated from 5 healthy volunteers according to the method of Umsumarng *et al.*[20] Hemolysis was induced by the DS extracts and analyzed following Žabar Method.[21] Briefly, 5% RBCs solution was mixed with the DS extracts in DMSO (0–100 μg/mL) and then incubated at 37°C for 3 h. The treated RBCs were centrifuged at 500 g for 5 min at 25°C and supernatant was collected. The absorbance of the supernatant was measured at 540 nm. Percent hemolysis was calculated and compared with 0.05% Triton X-100 (positive control).

Statistical analysis

In this study, results were from triplicate independent experiments ($n = 3$) and expressed as mean ± standard deviation. Differences between groups were analyzed by one-way analysis of variance following by Tukey test. $P < 0.05$ was considered as statistically significant.

RESULTS

In this study, the DS was extracted with 95% ethanol and crude EE was partially separated with CE, EAE, and water. The CE gave the highest yield partial extract following the AE and EAE. Secondary metabolites contained in the extracts including phenolic compounds, flavonoids, tannins, and alkaloids were determined using colorimetric methods. All the phytochemicals were the richest in the EE ($P < 0.05$). For partial extraction, the CE had the highest total phenolic, flavonoid, tannin, and alkaloid contents ($P < 0.05$) compared with the EAE and AE. The results of percent yield extracts and phytochemical contents are shown in Table 1.

Phytochemical screening contained in the *Derris scandens* extracts using high-performance liquid chromatography

Scandenal, luteolin 3',4'–di – 0 – glucuronide, genistein, gallic acid, kaemferol-3, 7 – di – glucoside, and scandenone were good separated in the extracts and HPLC chromatograms are shown in Figure 1. The chromatogram showed flavonoid content in the EE more than the CE and EAE. Luteolin 3',4'–di – 0 – glucuronide, genistein, and gallic acid were found in very lower intensity of absorbance and not found kaemferol-3, 7 – di – glucoside and scandenone in the AE.

Free radical scavenging

The DS extracts showed low potential DPPH radical scavenging and half-maximal scavenging concentration (SC_{50}) values were in the range of 0.81–5.91 mg/mL [Figure 2]. The CE showed the most effective radical scavenger with SC_{50} value –0.81 mg/mL. Trolox was used as a positive control and had higher effect than the DS extracts. All the DS extracts scavenged ABTS radicals in a dose-dependent manner and the results are shown in Figure 3. The SC_{50} was calculated and presented in Table 2. The DS extracts were highly potential to inhibit lipid peroxidation induced by ferrous ion ranging 6.00–10.88 mg/mL in dose-dependent manner. The EE, CE, and EAE had potential to inhibit lipid peroxidation higher than the AE ($P < 0.05$). All the DS extracts showed ability, and rank order was established following order significantly ($P < 0.05$): EE~EAE~Trolox>CE~AE. The results of antilipid peroxidation are shown in Figure 4 and Table 2.

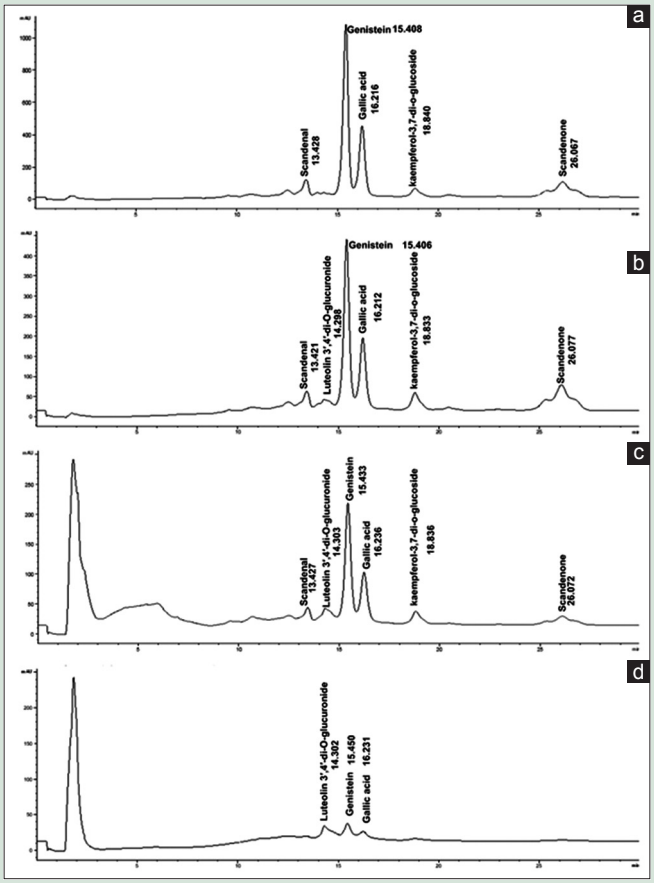

Figure 1: Chromatogram profiles of the DS extracts by high-performance liquid chromatography chromatogram at 330 nm. (a) EE, (b) CE, (c) EAE, and (d) AE. DS: *Derris scandens*; EE: Ethanolic extract; CE: Chloroform extract; EAE: Ethyl acetate extract; AE: Aqueous extract

Table 1: Percent yield extracts and phytochemical contents of the *Derris scandens* extracts

Extracts	Percentage yield	Total phenolic (mg GAE/g)	Total flavonoid (mg QE/g)	Total tannin (mg TE/g)	Total alkaloid (mg CE/g)
EE	2.83	4.08±0.11[a]	4.93±0.15[a]	0.84±0.14[g]	0.38±0.09[j]
CE	1.65	1.98±0.12[b]	1.99±0.06[b]	0.34±0.03[h]	0.16±0.04[k]
EAE	0.19	0.14±0.01[c]	0.07±0.01[e]	0.02±0.00[i]	0.02±0.00[l]
AE	0.96	0.50±0.01[d]	0.05±0.03[f]	0.02±0.01[f]	0.02±0.01[f,l]

Small capital letters are significant difference from the other group at $P<0.05$. GAE: Gallic acid equivalent; QE: Quercetin equivalent; TE: Tannic acid equivalent; EE: Ethanolic extract; CE: Chloroform; EAE: Ethyl acetate extract; AE: Aqueous extract

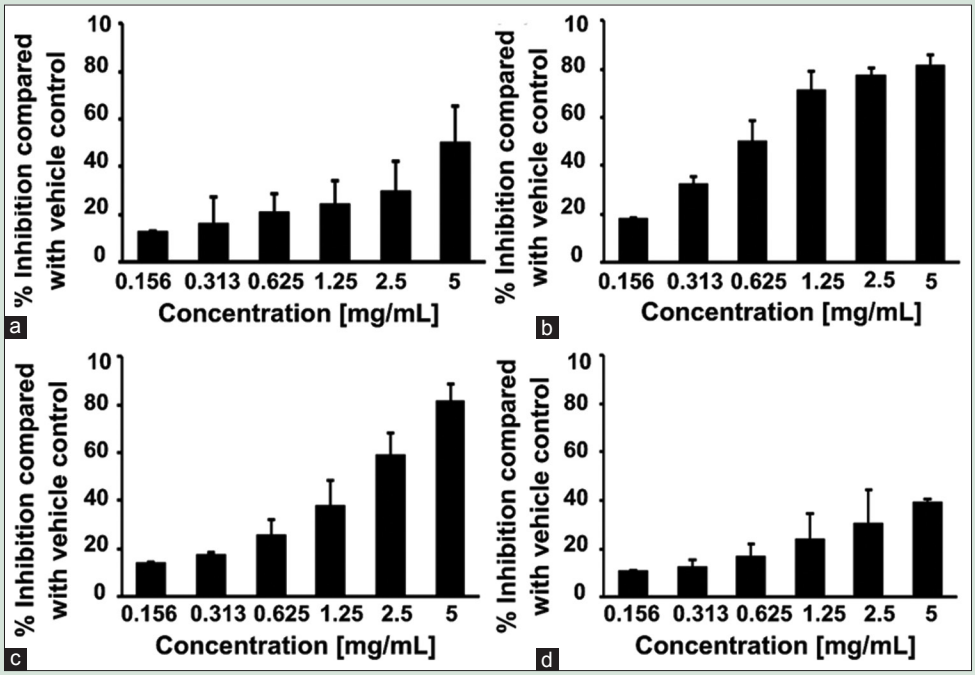

Figure 2: 2,2 – Diphenyl – 1 – picryl – hydrazyl radical scavenging activity of the DS extracts. (a) EE, (b) CE, (c) EAE, and (d) AE. DS: *Derris scandens*; EE: Ethanolic extract; CE: Chloroform extract; EAE: Ethyl acetate extract; AE: Aqueous extract

Table 2: Antioxidant activity of the *Derris scandens* extracts

Fraction	SC_{50}		IC_{50}
	DPPH· (mg/mL)	ABTS· (µg/mL)	MDA (µg/mL)
EE	4.60±0.73[a]	22.05±3.91[d]	6.15±0.63
CE	0.81±0.07[b]	53.63±4.03[e]	6.01±0.01
EAE	3.98±1.25[a]	27.55±2.50[d]	6.13±0.13
AE	5.91±0.16[a]	52.76±4.02[e]	10.88±1.63[f]
Trolox (µM)	0.04±0.01[c]	25.01±0.01[d]	-
GA	-	-	5.25±0.25

Small capital letters are significant difference from the other group at $P<0.05$. Values are express in mean±SEM (n=3). SEM: Standard error of mean; GA: Gallic acid; CE: Caffeine equivalent; EE: Ethanolic extract; CE: Chloroform extract; EAE: Ethyl acetate extract; AE: Aqueous extract; DPPH: 2,2–diphenyl–1 – picrylhydrazyl; ABTS: 2, 2′–azino–bis (3–ethylbenzothiazoline–6 – sulfonic acid); MDA: Malondialdehyde

Viability of Caco-2 cells treated with the *Derris scandens* extracts

The cells were treated with the DS extracts (0–400 µg/mL) for 4 and 48 h. The inhibition concentration at 20% value (IC_{20}) showed that the EE and CE were more toxic to Caco-2 cells than the EAE and AE. The cells incubated with the extracts at the longer time (48 h) were more toxic than shortened period incubation (4 h), and the results are shown in Table 3.

The effect of the *Derris scandens* extracts on human peripheral blood mononuclear cells

The human PBMCs were isolated and treated with various concentrations of EE, CE, EAE, and AE for 24 h, and cell viability was assessed by SRB method. The DS extracts at concentrations up to 100 µg/mL did not display toxic on the PBMCs when compared with the control group. The results of the cell viability after treating with the extracts at 50 and 100 µg/mL are shown in Figure 5.

Cytotoxicity of the *Derris scandens* extracts on human red blood cell

The percentage of human RBCs' hemolysis was assessed, and the results are illustrated in Table 4. After treating with the CE and EE at 100 µg/mL, the RBCs were hemolyzed about 5% and 6%, respectively. However, the EAE and AE less induced RBC hemolysis.

DISCUSSION

Extraction is an important step for bioactive compounds' identification from natural products. Partial extraction by increasing polarity of the solvents is often used for pharmacological studies.[22] Ethanol extraction was imitated the Thai traditional consumption by soaking with 40% ethanol. Ethanol, an amphiphilic, nontoxic, and biodegradable solvent, can elute both nonpolar and polar compounds resulting in the highest percent yield extract. CE is suitable organic solvent for hydrophobic compounds' extraction including flavonoid that gave higher percent yield extract than the other partial fractions. Whereas, EAE or hot water can extract hydrophilic compounds which are present in the DS extracts. The DS grown in Kanchanaburi Province, Thailand was extracted with 50% ethanol and provided 7.5% yield extract.[23] While the DS collected from Sing Buri Province, Thailand during June 2006 showed yield aqueous extract about 1.5%–2%.[24] The different type of organic solvents, source, and season may affect percent yield extract and amount of phytochemical contents.[25,26]

The EE and CE showed highly phenolic and flavonoid contents resulting from their solubility properties in organic solvents. The colorimetric methods showed that the total flavonoids in the EE were higher than total phenolic content due to chemicals' interference.[27] Thus, these results were confirmed with HPLC method, and the wavelength at 330 and 250 nm was selected for flavonoid and phenolic identification, respectively. The HPLC chromatograms showed that flavonoids were highly content than phenolics. Both genistein and gallic acid showed major compounds in all the DS extracts. Genistein was a major bioactive compound in the vine of DS,[1] and osajin, scandinone,

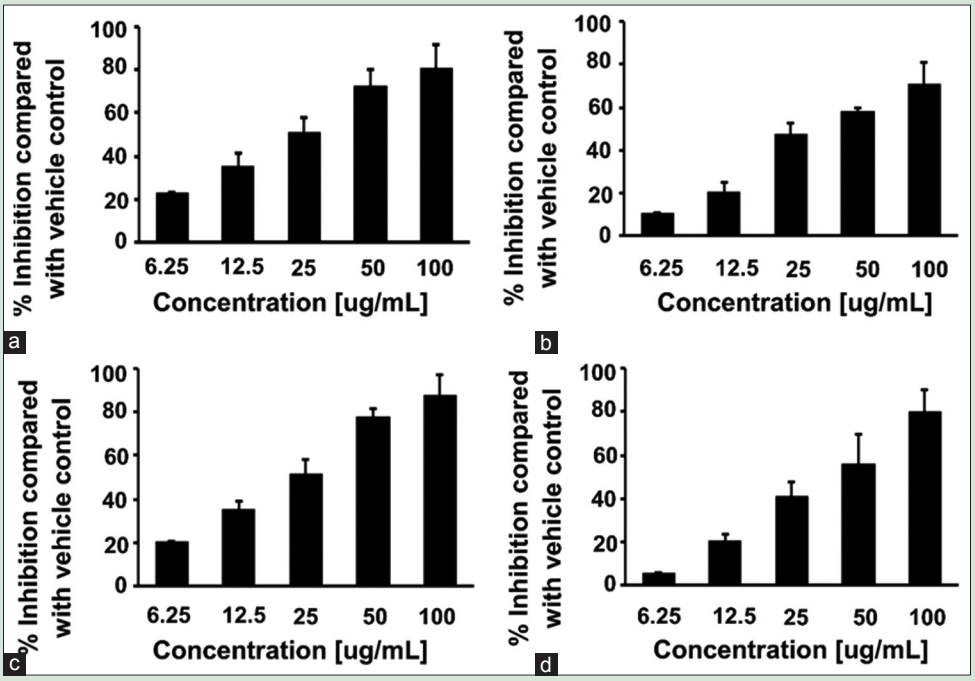

Figure 3: 2,2 – Azino – bis – 3 – ethylbenzothiazoline – 6 – sulfonic acid free radical scavenging activity of the DS extracts. (a) EE, (b) CE, (c) EAE, and (d) AE. DS: *Derris scandens*; EE: Ethanolic extract; CE: Chloroform extract; EAE: Ethyl acetate extract; AE: Aqueous extract

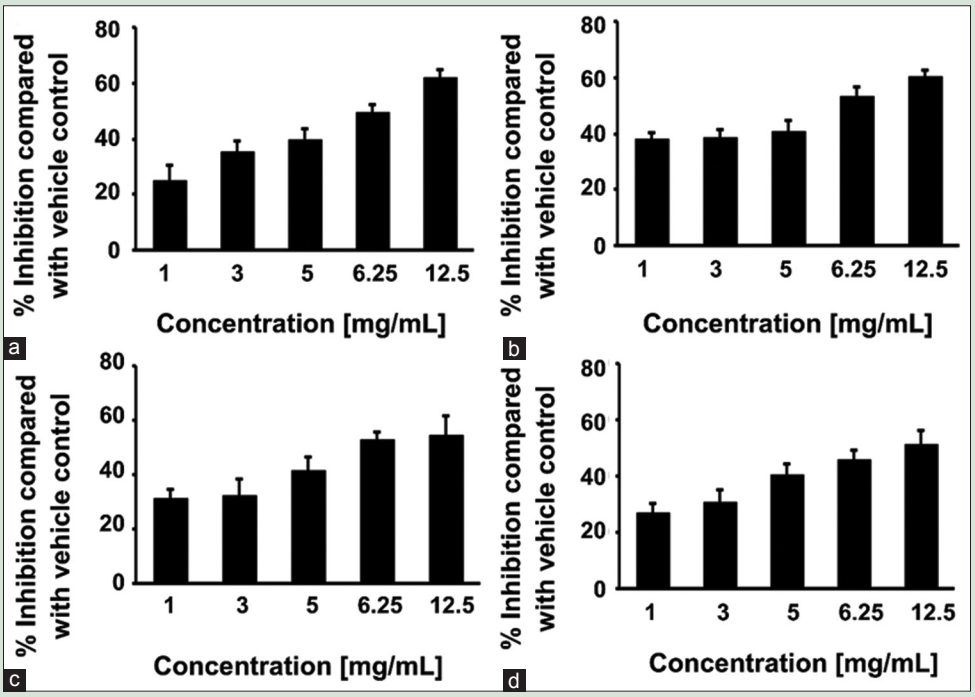

Figure 4: Effect of different concentrations of the DS extracts on lipid peroxidation inhibition. (a) EE, (b) CE, (c) EAE, and (d) AE. DS: *Derris scandens*; EE: Ethanolic extract; CE: Chloroform extract; EAE: Ethyl acetate extract; AE: Aqueous extract

sphaerobioside, genistein, scandenin A, scandenin, and lupalbigenin were found in whole DS that was extracted with CE.[28] Variation of season, location, and environmental conditions led to phytochemical contents' variation in this plant.

Phytochemicals are potential substances for preventing oxidative stress and chronic diseases related to free radical. DPPH and ABTS radical were selected because ther are easy, rapid and reliable protocol. Furthermore, antilipid peroxidation assay was selected for comparing the antioxidative behavior of each of the DS extracts. Genistein and luteolin 3',4'–di – O – glucuronide contained in the DS extract are potentially ABTS radical scavenging and antilipid peroxidation.[29,30] Genistein, luteolin, and gallic acid act as hydrogen donor, antioxidant, and lipid peroxidation suppression.[31-33] The DS extracts had low ability to scavenge DPPH radical because high genistein concentration could

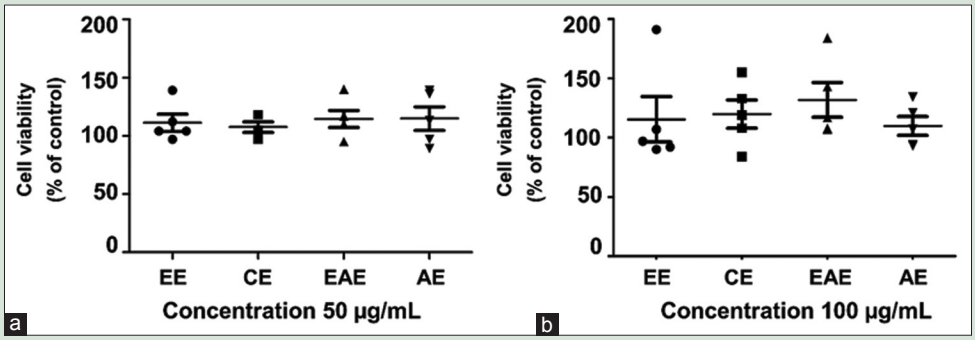

Figure 5: The cytotoxicity of (a) 50 µg/mL and (b) 100 µg/mL EE, CE, EAE, and AE on human peripheral blood mononuclear cells. Percent inhibition compared with vehicle control. EE: Ethanolic extract; CE: Chloroform extract; EAE: Ethyl acetate extract; AE: Aqueous extract

Table 3: Cytotoxicity effects of the *Derris scandens* extracts on Caco-2 cells

Fraction	4 h		48 h	
	IC_{20} (µg/mL)	IC_{50} (µg/mL)	IC_{20} (µg/mL)	IC_{50} (µg/mL)
EE	18.64±1.38[a]	37.81±0.51[d]	9.50±2.69[g]	26.45±3.57[j]
CE	21.21±0.05[b]	41.31±1.61[e]	11.80±1.33[h]	36.36±6.87[k]
EAE	52.44±3.45[c]	127.35±11.19[f]	42.86±15.40[i]	124.88±13.02[l]
AE	>400	>400	>400	>400

IC_{20} and IC_{50} values are expressed as mean±SEM (n=3). Small capital letters are significant difference from the other group at P<0.05. EE: Ethanolic extract; CE: Chloroform extract; EAE: Ethyl acetate extract; AE: Aqueous extract; SEM: Standard error of mean

Table 4: Hemolysis induction of the *Derris scandens* extracts on human red blood cells

Fraction (µg/mL)	Treatments	Percentage hemolysis
	0.05% triton X–100	100
	Vehicle control	0
EE	50	3.79±0.99
	100	6.13±2.86
CE	50	3.81±0.99
	100	5.04±1.56
EAE	50	3.51±0.87
	100	3.67±0.76
AE	50	3.41±0.59
	100	3.43±0.84

EE: Ethanolic extract; CE: Chloroform extract; EAE: Ethyl acetate extract; AE: Aqueous extract

act as pro-oxidant.[34] Free radicals can damage cellular membranes, lipoproteins, and other molecules. The reaction consisted of three major steps: initiation, propagation, and termination. Lipid peroxidation can occur generally both enzymatically and nonenzymatically. Human liver microsome was used as a source of lipid and was attached with free radicals to generate malondialdehyde.[35] The major active compounds contained in the DS might receive one electron from ferrous ion, and ferrous ion was changed to ferric ion leading to inhibit initiation step. The results suggested that phytochemical profiles in the DS extracts exhibited antioxidative ability. The partial extractions did not only provide the data of antioxidant compounds but also could suggest the preparation and usage of the DS as a nutraceutical or dietary supplement.

The Caco-2 cell is one of the good *in vitro* models for prediction of drugs and xenobiotics' absorption. In this study, we selected two different incubation times including 4 h for acute toxicity and 48 h for half-life of genistein in the human body. The EE and CE significantly decreased the cell viability in time-dependent manner. Genistein and luteolin are toxic substances to cancer cells.[36,37] Moreover, genistein combined with luteolin was more cytotoxic than monomeric compound.[38] The AE was low toxic to the cell that means the DS consumption by boiling might be safe than ethanol maceration.

PBMCs' model reflects to different feeding conditions in organs/tissues involved in energy homeostasis. Both 50 and 100 µg/mL were treated on Caco-2 cell, and the results showed that the DS extracts did not display toxic effect on the PBMCs. Genistein at high concentration was not cytotoxic to the PBMCs[39] whereas luteolin could reduce the proliferation of the PBMCs in dose-dependent manner.[40] This study suggested that genistein was a major bioactive compound in the DS grown in Southern Thailand and it might be safe to human normal cells.

Hemolysis assay is a reliable model to study the pharmaceutical safety of new drugs or compounds before intravenous administration in animals or clinical experiments recommended by the US Food and Drug Administration.[41] Our results showed that the DS extracts had low hemolytic activity (lower than 7%) when treated at 100 mg/mL. Hemolysis depend on plant concentration, chemical composition, sex, and individual susceptibility.[42] Thus, the consumption of the DS as therapeutic agents in traditional medicine did not induce RBC hemolysis. Chronic or high- dose consumption of the DS and its products should be avoided.

CONCLUSIONS

The DS was a good natural source of phenolic and flavonoid compounds and had antioxidant power, especially the EE. The EE and CE extracts were highly toxic to cancer cell (Caco2), but no adverse effect to normal cells (PBMCs and RBCs). However, it is necessary to study its toxicity in animal models and clinical study.

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Conflicts of interest

There are no conflicts of interest.

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