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Research Article

Antioxidant activity and cytotoxicity of Rang Chuet (*Thunbergia laurifolia* Lindl.) extracts

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Abstract: *Thunbergia laurifolia* Lindl. or Rang Chuet (RC) is widely described in Thai traditional medicine for protection against dietary and environmental toxicants with little substantiation. To better access the potential of RC as a medicinal plant, extracts were prepared by infusion with water, ethanol and acetone.

Antioxidant activities and total phenolic content of RC extracts were evaluated using free radical scavenging, ferric reducing antioxidant power assay (FRAP) and the Folin-Ciocalteu method. It was found that water extraction of phenolic compounds was the most efficient (2433.9mg GAE/100g) compared to ethanol and acetone extraction which had phenolic contents of 565 and 142.1mg GAE /100g, respectively. In addition, RC water extract possessed the highest antioxidant activities using free radical scavenging at the EC_{50} values of 0.13 mg GAE/mL, whereas ethanol and acetone extract showed EC_{50} at 0.26 and 0.61 mg GAE/mL respectively. Finally, the water extract also showed the highest total antioxidant activity using FRAP assay at 0.93 mmol/g followed by ethanol and acetone extracts (0.18 and 0.04 mmol/g).

Extracts were subsequently investigated for their cytotoxicity. Cytotoxicity of RC crude extracts were investigated in L929, BHK(21)C13, HepG2 and Caco-2 cell lines. The toxicity was indicated at high concentrations over 100 μg/mL for all extracts, which would be the index for further recommended concentration use.

Keywords: cytotoxicity, antioxidant activity, biochemistry, FARP

Introduction

Thunbergia laulifolia Lindl., commonly known in Thai as Rang Chuet (RC), belongs to the botanical family of Acanthaceae. It has been used in Thailand as a natural remedy for centuries. RC is commonly consumed as herbal tea. Various parts of RC are used, for example, aqueous extracts of fresh leaves, dried leaves, dried root and bark as an antidote for insecticide, ethyl alcohol, arsenic and strychnine poisoning; the dried root is also used as anti-inflammatory and antipyretic agents (Thongsaard and Marsden., 2002). In addition, it has been reported that RC leaves change body temperature of rats by centrally acting at the thermo regulating centre and/or cause vasodilatation and thereby increase heat dissipation (Chamreondararassame, 2003).

As Rang Chuet has been used in traditional medicine, researchers are interested in studying the compounds in RC leaf extracts. Kanchanapoom *et al.* (2002) reported two iridoid glucosides, 8-epi-grandiforic and 3'-O- β-glucopyranosyl-stibericoside isolated from the aerial parts of *Thunbergia laurifolia*, along with seven known glucoside compounds. The flowers of *Thunbergia laurifolia* were also reported to contain Delphinidin 3:5-di-o-β-D-glucopyranoside, apigenin and apigenin-7-o-β-D-glucopyranoside (Purnima and Gupta, 1978). In addition, the plant was reported to contain flavonoids such as apigenin, casmosiin, delphinidin-3-5-di-O-β-D-glucoside and chorogenic acid (Thongsaard and Marsden., 2002).

The pharmacological properties of Rang Chuet water crude extracts have been reported as an antidote for insecticide poisoning, treatment for drug addiction, reducing toxicity of insecticide (Folidol) and antimicrobial activity, as well as antioxidant activity (Tejasen and Thongthapp, 1979; Rengyutthakan, 1980; Thongsaard and Marsden, 2002; Khunkitti et al., 2003; Srida et al., 2002). It has also been reported that extracts of RC leaves have a protective effect on ethanol-induced hepatotoxicity using hepatic lipid peroxidation, blood ethanol concentration as well as hepatic alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) as indicators (Chanawirat et al., 2000). Treatment of alcoholism is also claimed using its aqueous extract. Alcohol and hexane extracts from *Thunbergia laurifolia* also posses anti-inflammatory activity against carageenin-induced paw edema in mice (Charumanee et al., 1998).

In this work, the free radical scavenging activities of the plant extracts were followed via their reaction with the stable DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical and their ferric ions reducing antioxidant activity potential (FRAP) assay. In addition, cytotoxicity of Rang Chuet crude extracts were investigated in L929, BHK(21)C13, HepG2 and Caco2 cell lines. Results from this study will provide a better understanding of the antioxidant properties and cytotoxicity of this plant and possibly lead to the identification of plants with high antioxidant activity for further investigation and development into value-added food products and neutraceuticals. Furthermore, IC_{50} value of extracts will provide indicators of safe level use for the future.

Materials and Methods

Plant materials

The medicinal plant Rang Chuet, *Thunbergia laurifolia* Lindl. (Acanthaceae) used in this experiment was collected in December 2005 - Febuary 2006 from local areas in Nakhon Ratchasima Province, Thailand. Leaves were air dried at 60^{o}C for 6 h, after which they were ground in a blender (National, MX-T2GN, Taiwan) to fine powder and stored in vacuum packages at 4^{o}C until use. Leaf powder such as this is typical of raw material utilized for manufacture of extracts and/or herbal tea products from RC.

Chemicals and standards

All chemicals of analytical grade including: 1,1-diphenyl-2-picryhydrazyl free radical (DPPH), 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), Ferric, chloride-6-hydrate, Ferrous, sulphate 7-hydrate, acetate buffer pH 4.6, Gallic acid, Folin-Ciocalteu phenol reagent and anhydrous sodium carbonate (Na_2CO_3) were obtained from Sigma-Aldrich Co. (St. Louis, USA). Solvents including acetone, ethanol, hydrochloric acid and methanol were purchased from Mallinckrodt-Baker (Phillipsburg, NJ, USA). Cell culture, Media, MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], DMSO, Sorensen's Glycine buffer, were purchased from American Type Culture Collection; ATCC (Manassas, VA, USA).

Preparation of RC Extracts

- **Extraction for antioxidant activity test and total phenolics**

Leaf powder of about 100 mg was extracted with three 12 ml portions of boiling water (100^{o}C), ethanol and acetone in a shaking water bath at 25^{o}C for 15 min. Centrifugation at 3000 g (Thermo IEC, Waltham, MA) was applied for 3 min between extraction three times. The filtrates obtained after vacuum filtration were combined and the volumes were adjusted to 50 ml with the same solvent. Aliquots of 2 ml were transferred to culture test tubes (VWR, Wilmington, NC) and dried in a vacuum dryer (Rapid Vap® Vacuum Evaporation Systems, Labconco Corp., Kansas City, MO). Samples were stored at -20^{o}C until use.

- **Solvent extraction for cytotoxicity test**

The powder (5g) was homogenized with water (200 ml), ethanol (200ml) and petroleum ether (200 ml) and homogenate was extracted in a shaker at 55^{o}C for 72 h. and then filtered using Whatman No.1 paper. The filtrate was concentrated using vacuum evaporator (BUCHI Rotavapor R-114, Switzerland) and kept at 4^{o}C until use (Kanchanapoom et al., 2002 with some modifications).

- **Supercritical Fluid Extraction for cytotoxicity test**

Powder was sieved at 425 microns before extraction using supercritical fluid extraction. A supercritical fluid extractor SFE was used for extraction. In this study, extraction was

performed by filling 8-ml volume extraction vessel with 3.0 g of the sieved leaf powder. The leaf powder was then extracted with supercritical CO_2 under 250 atm pressure and 50^{o} C temperature for 5 min static followed by 15 min dynamic. A Duraflow manual variable restrictor was used in SFE system to collect the extracted analytes. The supercritical CO_2 flow rate through the Duraflow restrictor was approximately 0.3-0.4 ml/min (compressed). The analytes were collected in a 3 ml of petroleum ether (Yamini et al., 2002 with some modification).

Total phenolics

Total soluble phenolic constituents of the extracts (water, ethanol and acetone) were determined employing the method of Waterhouse (2002) using the Folin-Ciocalteu reagent with gallic acid as standard. Twenty µl of freshly prepared RC extract was added to a 1.5 ml cuvette, to which 1.58 m of dd water and 100 µl Folin-Ciocalteu reagent was added. The sample was thoroughly mixed and incubated for 5 min at room temperature. Following incubation, 300 µl of the $NaCO_3$ (2% w/v) solution was added and the mixture was allowed to stand at room temperature for 2 hr. Absorbance was measured at 765 nm. Results were expressed as gallic acid equivalents.

DPPH free radical scavenging assay

The DPPH free radical scavenging activity of RC extracts (acetone, ethanol and water), BHT and ascorbic acid were determined using DU 800 Spectrophotometer (Beckman Coulter, CA) according to the method described by Ferruzzi *et al*. (2002), in terms of hydrogen donating or radical scavenging ability. Briefly, 0.1 mm solution of DPPH in methanol was prepared. The initial absorbance of the DPPH in methanol was measured at 515 nm and did not change throughout the period of assay. An aliquot (100 µl) of an extract diluted at the concentration range of 0.01-0.15 mg GAE/ml was mixed with 1.9 ml of methanolic DPPH solution. The change in absorbance at 515 nm was measured at 15 min. The percentage of scavenging was calculated as the ratio of the absorption of the sample relative to the control DPPH solution, without the extracts. The BHT and ascorbic acid in MeOH solution were used as positive controls. The EC_{50} of the extracts was calculated at the 15 min scavenging value using nonlinear regression of Sigma Plot 9.1 (Systat Software Inc, Illinois). Inhibition of free radical DPPH (%) was calculated according to the formula:

$$\text{Inhibition } \% = (A_{blank}-A_{sample}/A_{blank}) \times 100$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the tested compound.

Exact concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted of %inhibition against extract concentration. Tests were carried out in triplicate. The synthetic antioxidant BHT and ascorbic acid were included in experiments as positive controls (Schlesier et al., 2002).

Ferric-reducing antioxidant power (FRAP) assay

The method of Wong, *et al*.(2006) was utilized. Briefly, the FRAP reagent was prepared from acetate buffer (pH 3.6), 10 mmol TPTZ solution in 40 mmol HCl and 20 mmol ferrous chloride solution in the proportion of 10:1:1 (v/v), respectively. The FRAP reagent was fresh and daily prepared and was warmed to 37^{o}C in a water bath prior to use. The extract of 50 µl was added to 1.5 ml of the FRAP reagent. The absorbance of the reaction mixture was then recorded at 593 nm after 4 min. The standard curve was constructed using ferric sulphate solution (100-2000 µm) and the results were expressed as µmol equivalents of ferric per g dry weight of plant materials. All measurements were taken in triplicate and the mean values were calculated.

Cell culture

The target cells were L929 (mouse connective tissue ECACC Cat. No. 85011425), BHK(21)C13 (baby hamster Syrian kidney ECACC Cat. No. 85011433), HepG2 (human liver hepatocarcinoma ATCC Cat. No. HB-8065) and Caco2 (a human colon adenocarcinoma ATCC Cat. No. HTB-37, ATCC, USA). The L929 cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum and 2mM L-glutamine. The BHK(21)C13 cells were grown in Glasgow Modified Eagle's Medium (GMEM) supplemented with 5% fetal bovine serum, 1.5 g/l tryptose phosphate broth and 2mm L-glutamine. The HepG2 and Caco2 were grown in Minimun Essential Medium (MEM) supplemented with 10% fetal bovine serum, 0.1mm MEM non-essential amino acid, 1.0 mm sodium pyruvate and 2 mm L-glutamine. All cells were incubated at 37^{o}C in a fully humidified incubator with 5% CO_2 : 95% air atmosphere.

Cytotoxicity

L929, BHK(21)C13, HepG2 and Caco2 were seeded in a 96-well plate with 500 cells/well for L929 and 2000 cells/well for the others, and incubated for 48 h. All the RC dried crude extracts were dissolved in ethanol to make stock solution then diluted to various extract concentrations in media from 1.56 ug/ml to 200 ug/ml. The extracts were added to the wells and incubated for 24 h. The test samples were removed from the cell cultures and the cells were re-incubated for a further 24 h in fresh medium and then tested with MTT.

Briefly, 50 μl of MTT in PBS at 5 mg/mL was added to the medium in each well and the cells were incubated for 4 h. Medium and MTT were then aspirated from the wells and formazan solubilized with 200 μl of DMSO and 25 μl of Sorensen's Glycine Buffer, pH10.5. The optical density was read with a plate reader at a wavelength of 570 nm. The data were analyzed with the SoftMax Program (Molecular Devices) to determine the IC_{50} for each extract sample. Two controls were set up, one with medium as reagent control and the second with the ethanol 1% as solvent control.

A dose-response curve was derived from 8 concentrations in the test range of 200-1.56 μg/ml using 4 wells per concentration to determine the mean of each point. Within each

experiment 2-dose response curves were obtained. Results of toxic compounds were expressed as the concentration of sample required to kill 50% (IC_{50}) of the cells compared with controls.

Statistical analysis

Descriptive statistics including mean and standard error of mean (SEM) were calculated for each extract antioxidant activity (n=3).

Results and Discussion

Antioxidant activity measurement

The generation of radical oxidative species involves either radical processes or different potential redox systems. The soluble properties of antioxidant compounds determine their effective antioxidant activities in either aqueous or lipid systems (Sanchez-Gonzalez, *et al*., 2005). Therefore, two aqueous-based models were chosen to assess the antioxidant activity of the RC extracts, one measuring radical-scavenging activities and the other measuring total reducing power.

Total phenolic contents

The phenolic contents of the aqueous, ethanol and acetone extracts of RC were tested using the Folin-Ciocalteu reagent. The yield and total phenolics in RC extracts are presented in Table 1. The phenolic contents of the water extract were found to have highest phenolic contents (2433.9 mg GAE/100g) followed by ethanol (565 mg GAE/100g), and acetone (142.1 mg GAE/100g). Comparing the efficiency of extraction with boiling water, ethanol and acetone, the method using boiling water showed the highest efficiency in the extraction of phenolic compounds. Therefore, RC water extract would be expected to have the highest antioxidant activity. The acetone extracts gave the highest yield of extract at 36.6% (g/g leaf powder x100) but this extract showed the lowest phenolic contents.

Extraction method is also critical to the recovery of antioxidant phytochemicals. The nature of both plant materials and the bioactive components should be considered in order to achieve good extraction efficiency. Lipophilicity or hydrophilicity affects the solubility of a phytochemical in the extracting solvent and, conversely, polarity of a solvent also has an impact on the extraction efficiency. Many different extractions methods exist for antioxidant phytochemicals, but most of them are based on solvent extraction using water, organic solvent or liquefied gas, or combinations of these under different temperature and pressure, although other methods such as physical press, filtration, stream distillation and solid absorption have been used (Tsao and Deng, 2004).

Polar antioxidants such as phenolic acids and glycosides of many flavonoids are generally extracted using water, alcohol or a mixture of water and alcohols. Our results (Table 1.)

are consistent with these previous notions. For antioxidants such as aglycones of some flavonoids, non-aqueous solvents are used (Tsao and Deng, 2004). The efficiency of the boiling water in extracting compounds producing antioxidant activity is higher than that of methanol extract (Wong et al., 2006).

Table 1. Yield and total phenolics of Rang Chuet using various extraction solvents.

Solvent	**Yields** (g/g *100)	**Total phenolic contents** (mg GAE/100g)
Water	32.6	2433.9 ± 57.7
Ethanol	23.3	565.0 ± 7.9
Acetone	36.6	142.1 ± 10.6

1,1-Diphenyl-2-picrylhydrazyl radical-scavenging

DPPH is a stable nitrogen-centered free radical. The colour changes from violet to yellow upon reduction by either the process of hydrogen or electron-donation. Substances which are able to perform this reduction can be considered as antioxidants and therefore radical scavengers (Hinneburg et al., 2006). The hydrogen atoms or electrons donation ability of the corresponding extract will be measured from the bleaching of violet colored methanol solution of DPPH.

The studied extracts exhibited the scavenging activity of various strengths and were dose dependent in all extracts. In addition, positive controls with BHT and ascorbic acid were tested for their DPPH radical scavenging. The calculated EC_{50} for 15 min incubation time are reported in Table 2.

Table 2. DPPH scavenging activity of RC extracts.

Rang Chuet Extracts	EC_{50}
Water	0.129 ± 0.01 (mgGAE/mL)
Ethanol	0.261 ± 0.04 (mgGAE/mL)
Acetone	0.607 ± 0.06 (mgGAE/mL)
BHT	0.278 ± 0.04 (mg/mL)
Ascorbic acid	0.103 ± 0.02 (mg/mL)

DPPH radical scavenging activity of water, ethanol, and acetone crude extracts of RC revealed antioxidant potency when considering the EC_{50} values. A lower value of EC_{50} indicates a higher antioxidant activity. Water extract has the highest scavenging activity

amongst all extracts. Moreover, scavenging activity of water extract and ascorbic acid are closely comparable and there appears to be high correlation between scavenging activity and phenolic content of RC extracts.

The difference in antioxidant activities amongst the RC extracts is due to multiple factors, including concentration of the extracts and qualitative profile of extracts. Water extract had primarily components as apigenin and phenolic acids as caffeic acid and gallic acid as well as acetone and ethanol had main components as chlorophyll derivatives and lutein (Data not shown).

Antioxidant activity

The antioxidant potential of the RC extracts were estimated from their power to reduce the TPTZ-Fe(III) complex to TPTZ-Fe(II) complex (FRAP assay), which is simple, fast and reproducible (Wong et al., 2006). The FRAP is versatile and can be readily applied to both aqueous and alcohol extracts of different plants. The results are expressed as mol ferrous equivalents per gram of sample.

Table 3 shows the antioxidant activity of RC extracts including positive controls of BHT. For the RC extracts, the antioxidant activity ranged from 0.044 to 0.928 mmol Fe(II)/g. Water extract showed the highest antioxidant activity (0.928 mmol Fe(II)/g), followed by the ethanol extract (0.079 mmol Fe(II)/g) and the acetone extract (0.044 mmol Fe(II)/g). Wong et al. (2006) classified categories of medicinal plants based on their antioxidant activities: extremely high (> 500 μmol Fe(II)/g), high (100 – 500 μmol Fe(II)/g), medium (10 – 100 μmol Fe(II)/g), and low (<10 μmol Fe(II)/g). Under this classification, the water extract exhibited extremely high antioxidant activity, while ethanol and acetone extracts showed medium antioxidant activity. In this study, RC was extracted with boiling water similar to the traditional Thai method used for medicinal herbs. The other samples were extracted with a more modern method, using ethanol and acetone.

Table 3. Antioxidant activity of water, ethanol and acetone RC extracts using FRAP assay.

Rang Chuet Extracts	Total antioxidant activity (mmol/g)
Water	0.928 ± 0.050
Ethanol	0.079 ± 0.002
Acetone	0.044 ± 0.005
BHT	1.421 ± 0.593

It should be added that the leaves of RC are available at a low cost and thus provide an economic source of potential natural antioxidants for use as food supplements or food additives. The results combined with phytochemical profiling showed that RC leaves contain phenolic acid as gallic acid, caffeic acid, protocatechuic acid, other than that it contains flavonoid as apigenin and apigenin glucosides (Data not shown). Caffeic acid has been reported to have antioxidant activity (Son and Lewis, 2002) and this phenolic acid

was found in all extracts, being found the highest in the water extract (142.1 mg/100g), thus related to the highest antioxidant activities by both radical scavenging and FRAP assay. Interestingly, it was found that gallic acid is a component in the water extract which related to the highest antioxidant activity. Wong, *et al*. (2006) reported that gallic acid isolated from Chinese medicinal plants had shown strong DPPH radical scavenging activities. However, when compared to standards in FRAP assay, BHT (1.421 mmol Fe(II)/g), ascorbic acid (119.5 mmol Fe(II)/g) and trolox (7.2 mmol Fe(II)/g), all RC extracts showed relatively modest total antioxidant activity.

Antioxidant activity can be explained as reductants, and inactivation of oxidants by reductants can be described as redox reactions in which one reaction species (oxidant) is reduced at the expense of the oxidation of another antioxidant. The FRAP assay measures the antioxidant effect of any substance in the reaction medium as reducing ability. In this assay, the antioxidant activity is determined on the basis of the ability of reduce ferric to ferrous. In the DPPH assay, antioxidants reduce the free radical 2,2-diphenyl-1-picrylhydrazyl, which has an absorption max at 515 nm (Wong, *et al*, 2006).

Water extract showed the highest antioxidant activity both by DPPH and FRAP assay. It could be explained that water extract had the ability to reduce both radicals and ferric ions and also better reduce radicals. Also, all extracts showed antioxidant activity differently, depending on the components in each extract. That the water extract showed the highest antioxidant activity might be because the main constituents are phenolic acids and flavonoids and flavonoids glucosides. Previous researcher reported that flavonoids and phenolic acids are the source of antioxidant activity in plants (Cook and Samman, 1996). The ethanol extract and acetone extract which showed less antioxidant activity was composed of chlorophylls, chlorophyll derivatives and luteins. It could be explained that chlorophylls and chlorophyll derivatives exhibited low antioxidant activity. In addition, the luteins identified in ethanol and acetone extract were in very low amount thus did not demonstrate antioxidant activity.

Hundreds of different polyphenols have been identified in food. The two main types of polyphenols are flavonoids and phenolics. As antioxidants, polyphenols may protect cell constituents against oxidative damage and therefore limit the risk of various degenerative diseases associated with oxidative stress. The phenolic groups in polyphenols can accept an electron to form relatively stable phenoxyl radicals, thereby disrupting chain oxidation reactions in cellular components (Scalbert, *et al*., 2005).

Flavonoids are potent antioxidants, free radical scavengers and metal chelators and inhibit lipid peroxidation. The structural requirement for the antioxidant and free radical scavenging functions of flavonoids include hydroxyl carbon in position three, double bonds between position two and three, a carbonyl group in carbon position four and polyhydroxylation of the A and B aromatic rings (Cook and Samman, 1996). The radical scavenging activities of flavonoids were highly controlled by the number and configuration of phenolic hydroxyl groups in the molecules and also influenced by glycosylation and configuration of other substituents. The flavonoids without any

hydroxyl group (e.g., trans-chalcone, flavone, flavanone, and isoflavone) had no radical scavenging capacity (Cai, *et al*., 2006).

The results from the antioxidant assays show that all extracts can act as radical scavengers to a certain extent. Water extract showed the highest activity in the iron reduction assay and DPPH assay. Yet, the EC_{50} values for these extracts were still lower than those of the tested reference antioxidant, ascorbic acid and BHT. From these results, it could be suggested that RC extracts could be a natural source for antioxidants in food, but the amount required to produce antioxidant activity similar to standard use antioxidants could be large.

Cytotoxicity

Over the last few decades several in vitro assays using mammalian cell cultures have been developed thus avoiding the excessive use of laboratory animals which is expensive, time consuming and often involves ethical problems. Cell culture systems can be more sensitive and more reproducible than tests involving intact animals (Cetin and Bullerman, 2005).

The cytotoxicity of RC extracts were evaluated in BHK, L929, Hep G2 and Caco-2 cell lines with the MTT assay (Mossman, 1983). The IC_{50} value for *Thunbergia laurifolia* is shown in Table 4. In addition, there was no difference between the two controls of medium and ethanol 1%, with no toxicity to the tested cell lines.

Table 4. IC_{50} values for *Thunbergia laurifolia* extracts (water, petroleum ether, ethanol and SFE).

Sample	IC_{50} (ug/mL)			
	L929	BHK(21)C13	HepG2	Caco2
Vehicle control	677±11	689±13	5299±72	2993±27
Water crude extract	182±8	>200	>200	147±15
Petroleum ether crude extract	>200	103±1	140±4	117±9
Ethanol crude extract	>200	134±2	>200	ND
SFE	145±4	118±4	154±1	120

The results showed that the IC_{50}'s were affected differently in each cell line. The petroleum ether extract showed the highest cytotoxicity toward BHK(21)C13 cell line at concentration of 103±1 µg/mL. The water extract showed the lowest cytotoxicity toward BHK(21)C13 and HepG2 cell lines at concentration over 200 µg/ml. Also, petroleum

ether and ethanol extracts showed low cytotoxicity toward L929 at concentration over 200 µg/ml. However, the toxicity for all extracts was indicated at high concentration of 200 ug/ml, which would be the index for further recommended concentration being applied.

All RC extracts exhibited an extremely high value of IC_{50} (>100 µg/ml) against all cell lines tested indicating low cytotoxic to the cells (Okonogi et al., 2006). When compared amongst tested cell lines, BHK(21)C13 cells were found to be sensitive to the cytotoxic effect of RC extracts including petroleum ether, ethanol and SFE extracts, followed by Caco-2 and HepG2 after 24-h exposure, whereas L929 cells showed low response to RC extracts toxicity. The highest cytotoxicity of BHK(21)C13 cells, baby hamster Syrian kidney, toward RC extracts (Petroleum ether, ethanol, and SFE) could lead to understanding that these RC extracts affect kidney cells when concentration at specific values was applied. In petroleum ether extract, cytotoxicity may be caused from the solvent itself and components in extracts which are from non-polar extraction. The use of large amounts of organic solvents poses health and safety risks (Tsao and Deng, 2004).

The water extracts showed low cytotoxicity (> 200 µg/ml) toward BHK(21)C13 and HepG2 cell lines which leads to the suggestion to use this kind of RC extract due to it's low toxicity to kidney cells and human liver cell lines. Water extraction is a non-toxic method for extraction and also the components extracted could be polyphenol parts including flavonoids and phenolic acids which are generally extracted using water (Tsao and Deng, 2004). Quite oppositely, the water extract showed high cytotoxicity at IC_{50} of 147 µg/ml toward Caco-2 cells, which leads to the suggestion that water extract, even though it exhibits low toxicity towards kidney and liver cells, is moderately toxic toward intestinal cells. For Caco-2 cells, the cytotoxicity of RC extracts at IC_{50} of 117, 120, and 147 for SFE, water and petroleum ether extracts respectively. SFE extracts show high cytotoxicity toward human intestinal cells and this suggests careful use is required with specific concentration of this extract. When compared between the cell lines tested, L929 cells showed the lowest cytotoxicity from all RC extracts, which might be due to the type of cell lines (connective tissue) and if compared between types of RC extracts, the water extract had the lowest cytotoxicity for all cell lines tested.

Okonogi, *et al*. (2006) studied antioxidant capacities and cytotoxicities of certain fruit peels in Caco-2 cells and peripheral blood mononuclear cells and reported that the peels of rambutan may be considered potentially useful as a source of natural antioxidants for food or drug products because of their high antioxidant activity and non-toxic properties to normal cells (IC_{50} > 100 ug/ml).

Conclusion

The antioxidant activity and total phenolic contents of RC extracts were evaluated. The RC extracts, in general, showed high antioxidant activities and phenolic content particularly in the water extract, followed by ethanol and acetone extract respectively. Relatively, the antioxidant activity by DPPH and FRAP assay were both highest in water

extract at 0.129 mg GAE/ml and 0.928 mmol Fe (II)/g respectively. In addition, the antioxidant activity values of water extract was less than the positive control (BHT), one fold by DPPH assay and 1.6 fold in FRAP assay. Moreover, there is a relationship between antioxidant activities and total phenolic contents in all extracts. High total phenolic contents showed high antioxidant activity in all extracts. It could be concluded that polyphenol compounds contribute to antioxidant activity in RC extracts.

In addition, the RC crude extracts indicated toxicity to L929, BHK (21)C13, HepG2 and Caco2 over the tested concentration ranges of 200 to 1.56 ug/ml. The IC_{50} could be summarized as low cytotoxicity at concentrations over 100 ug/ml for all crude extracts.

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