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Anti-oxidant properties and anti-hemolytic activity of *Psidium guajava, Pandanous odorus* and *Rhinacanthus nasutus*

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The ethanolic extracts of leaves of *Psidium guajava* (PG), *Pandanous odorus* (PO), *Rhinacanthus nasutus* (RN) contained polyphenolic compounds in the order of PG > RN > PO. All the extracts exhibited 2,2-diphenyl-1-picryhydrazyl (DPPH) and 2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radicals scavenging activities. PG showed a marked antioxidant effect whereas PO and RN were comparable. Interestingly, they were able to bind with ferric (Fe^{3+}) and ferrous (Fe^{2+}) ion and completed within 5 min of incubation times. In accordance with previous results, PG extract could effectively protect erythrocytes from iron induced hemolysis with the activity higher than PO and RN. Based on all results, tested leaf extracts of three Thai medicinal plants might be used for alleviating or preventing harmfulness in many oxidative stress disorders.

Key words: *Psidium guajava*, *Pandanous odorus*, *Rhinacanthus nasutus*, antioxidant, iron binding capacity, anti-hemolysis activity.

INTRODUCTION

Oxygen consumption of cells generates a series of reactive oxygen species (ROS) (Moskovitz et al., 2002). Therefore, aerobic organisms undergo oxidative modification of biomolecules in tissues. Erythrocytes are vulnerable to oxidative stress due to their high content of polyunsaturated lipids and transition metals, especially iron, which is known to catalyze free radicals generation via Fenton reaction (Chiu et al., 1982). In some disorders such as thalassemia and chronic renal disease, ROS production is higher than normal (Nematbakhsh et al., 2013; Shazia et al., 2012). In addition, iron is involved in etiopathology of these patients (Lehmann et al., 2012; Thephinlap et al., 2009; Thephinlap et al., 2011). Iron-mediated oxidative modification of membrane lipid and hemoglobin can cause hemolysis and increase the severities of diseases (Zhu et al., 2002). Although there is powerful defense system for prevention of oxidation, erythrocytes of such patients demonstrate an increased ROS and decreased content of antioxidants than their normal counterparts (Amer et al., 2004; Das et al., 2004; Fibach et al., 2010; Ounjaijean et al., 2008). Therefore, much attention has been focused on the use of substances to inhibit the damage of free radicals. Researches on phytochemicals and their biological effects on health have been intensified. This study aims to investigate the antioxidant properties of three Thai traditional plant extracts including *Psidium guajava* L., *Pandanous odorus* and *Rhinacanthus nasutus*.

Guava (*P. guajava* L.; PG) is found widespread in hot climate countries, including Thailand. In folk medicine, guava leaves extract has been reported to have hypoglycemic activity, antidiarrhea (Lutterodt, 1989),

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antipyretic (Roy et al., 2010), antimicrobial (Jaiarj et al., 1999) and anti-mutagenic agents (Matsuo et al., 1994). *P. odorus* (PO) grows in Southeast Asia. In Thailand, it is known as toei hom, fragrant screw pine. Leaves have been used as a food flavouring and traditional medicine in Philippines, Indonesia and Thailand. Hot water extract of roots and leaves of this plant show hypoglycaemia indications (Peungvicha et al., 1998). *R. nasutus* (RN) has also been used in Thai traditional medicine for the treatment of various diseases including eczema (Visweswara et al., 2010), pulmonary tuberculosis (Visweswara et al., 2013), hepatitis (Bukke et al., 2011), diabetes hypertension and various skin diseases (Visweswara et al., 2012).

In this study, we addressed antioxidant and anti-hemolysis activities of these plants. We employed radical scavenging and iron binding assays as antioxidant activity investigation. Furthermore, hemolysis assay was used as a model of oxidative stress for evaluation of protective effect from free radicals.

MATERIALS AND METHODS

Chemicals

Folin-Ciocalteau reagent, 3-[N-morpholino]propanesulfonic acid (MOPS), hydrogen peroxide, 1,1-diphenyl-2-picryl-hydrazyl, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and gallic acid (GA), were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). A stock of ferric nitrate (AAS iron reagent, 1,000 ppm, in 0.5% HNO_3; APS Finechem, Seven Hills, Australia) was used as the iron source for other preparations. Stock ferric nitrilotriacetate solution was prepared by consecutive mixing of ferric nitrate with the nitrilotriacetic acid (at a 1:5 molar ratio of Fe^{3+} to chelator). Various iron concentrations were freshly prepared in 10 mM MOPS buffer, pH 7.0, before use. All other chemicals and reagents used were of AnalaR grade.

Plant extraction

Leaves of *P. guajava*, *P. odorus* and *R. nasutus* were dried at 50°C for 12 h, finely powdered and used for extraction. The powder (100 g) was mixed with 1,000 ml of ethanol (80%) at 4°C overnight in dark. The mixture was centrifuged at 3,000 × *g*. The supernatant was collected and then filtered through filter paper. The filtrate was added with 10 to 15 g of activated charcoal for 10 min, passed through a filter paper and centrifuged at 3,000 × *g*. The supernatant was collected and filtered through filter paper. The filtrate was concentrated using rotary evaporator further lyophilized. The extracted powder was kept at -20°C until used.

Determination of total phenolic compounds

The content of total phenolic compounds was determined using Folin-Ciocalteau reagent. Briefly, various concentrations of the extracts (300 µl) were incubated with Folin-Ciocalteau reagent (1,500 µl) in dark for 3 min. The mixture were added with sodium carbonate (1,200 µl), then mixed and let to stand in dark for 30 min. Optical density (OD) was read at 730 nm using UV-VIS spectrophotometer. The amount of total phenolic compounds was expressed as mg GAE/g dry extract.

DPPH radical-scavenging assay

Free radical scavenging activity was assessed using stable DPPH radicals. Various concentrations of the tested compounds in final volume of 3.0 ml were mixed with 80 mM of DPPH in methanol. The mixture was shaken vigorously and was allowed to stand for 30 min at room temperature. The OD of resulting solution was measured at 517 nm with spectrophotometer. Percentage of DPPH radical scavenging activity was calculated by the following equation:

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

The half maximal inhibitory concentration (IC_{50}) was defined as the concentration necessary to decrease free radicals by 50% as measured by absorbance of DPPH.

ABTS radical cation decolorization assay

The ABTS radical was generated by reaction of ABTS solution (7 mM) with 2.45 mM potassium persulfate ($K_2S_2O_8$). The mixture was made to stand for 15 min in dark at room temperature. Radical solution was diluted with ethanol to obtain the OD of 0.7 ± 0.2 units at 731 nm. Various concentrations of plant extract (300 µl) were incubated with 2.7 ml of radical solution at room temperature for 8 min in dark. The OD was measured at 731 nm using spectrophotometer. Percentage of ABTS radical scavenging activity was calculated by the following equation:

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

The IC_{50} value was defined as the concentration necessary to decrease free radicals by 50% as measured by absorbance of ABTS.

Measurement of chemical iron binding: Spectral analysis

Extract (1 mg/ml) was prepared by dissolving extracted powder in 50 mM MOPS solution, pH 7.0. The solution (1.0 ml) was subsequently mixed with the ferric nitrate solution (10 µl) in polypropylene tubes to obtain the indicated concentrations. The mixture was incubated at room temperature for 10 min. After incubation, the OD of the extract solution alone and the complex solution were monitored between 400 to 800 nm using the doublebeam UV-VIS scanning spectrophotometer (Shimadzu Corporation, Analytical & Measuring Instruments Division, Kyoto, Japan).

Kinetic formation of iron-binding complex

Various concentrations of ferric nitrate solution were incubated with the 1 mg/ml of the extracts at room temperature for 0 to 15 min. The OD of complex was measured at indicated wavelength as obtained from the spectral analysis. Chemical binding of Fe^{3+} was investigated by incubating the ferric nitrate solution (final concentrations of 0 to 200 µM) with the extract solution (a final concentration of 1 mg/ml at room temperature for 10 min. After incubation, OD of complex was measured at indicated wavelength obtained from above.

Red blood cell suspension

Blood was obtained by venipuncture from healthy male volunteers (18 to 22 years old) collected in heparinized tubes and centrifuged at 3,500 rpm for 15 min. Plasma and buffy coat were removed. Red blood cells (RBCs) were suspended in 10 volumes of 0.9% NaCl

and centrifuged at 2,500 rpm for 5 min. The RBCs were washed three times with the same solution. During the last washing, the packed cells were re-suspended in 10 volumes of phosphate-buffered saline (PBS, pH 7.4) and utilized for the following assay.

Assay for hemolysis

The effect of extracts on ferrous ion-induced hemolysis was evaluated. RBC suspension was incubated with 2.5 µM ferrous sulfate and various concentrations of the extracts at 37°C for 30 min. After incubation, 4 ml of phosphate buffered saline (PBS) was added and further centrifuged at 2,500 rpm for 5 min. Hemolysis was determined by measuring OD of the supernatant at 540 nm. The reaction without extract was used as control sample. Percentage of anti-hemolysis was calculated from following equation:

$$\% \text{ Inhibition} = 100 \times (1 - OD_{sample}) / OD_{control}$$

RESULTS

Polyphenolic content

Polyphenolic content of the RN, PO and PG extracts were determined polyphenolic compounds using Folin-Ciocalteau assay. The amount of polyphenolic compounds is demonstrated in Table 1. A highest content of polyphenolic compounds was observed in PG with a content of 318.00 ± 21.516 mg GAE/g dry extract followed by PO (143.83 ± 11.754 mg GAE/g dry extract) and RN which had the lowest (92.58 ± 1.942 mg GAE/g dry extract).

Free radical scavenging activity

The DPPH radical scavenging activity of analyzed extract was compared to trolox, a water-soluble derivative of vitamin E. As shown in Figure 1, percentage of DPPH radical scavenging activity was increased in a concentration-dependent manner in all three extracts. In comparison, the PG extract exhibited the strongest activity with (IC_{50} = 6.25 µg/ml), whereas the RN and PO extracts showed the comparable activity. The RN and PO extracts at concentration of 1.56 to 20 µg/ml could not decrease DPPH radicals by 50% and unable to determine IC_{50} value. Interestingly, the activity of PG was greater than all concentrations of trolox. The antioxidant activities of plant extracts cannot be evaluated by single assay (Schlesier et al., 2002). Therefore, we investigated antioxidant activities using ABTS radical decolorization assay. As shown in Figure 2, all extracts also showed ABTS radical scavenging activity in the similar pattern to the DPPH assay. The activity of extracts and standard compound exhibited the following order: PG > trolox > RN > PO. The PG extract showed over 50% scavenging activity at concentration of 2.75 µg/ml. Therefore, the PG extract had the highest antioxidant capacity, while the RN and PO extracts rendered the weaker effect.

Table 1. Total phenolic content in the PG, RN and PO extracts.

Sample	Polyphenolic compounds (mg GAE/g dry extract)
RN	92.58±1.942
PO	143.83±11.754
PG	318.00±21.516

Data are obtained from triplicate results of three independent experiments and shown as mean ± standard deviation (SD).

Chemical iron binding activity

Spectral analysis demonstrated that the PG, RN and PO extracts alone exhibited its predominant peak at 272, 334 and 275 nm, respectively (Figure 3A to C). After incubation with ferric ion, they formed colored complex(es) and gave new predominant peak(s). The Fe^{3+}- PG complex(es) exhibited two distinct absorption peaks at 425 and 515 nm. In comparison, the Fe^{3+}- RN complex gave a predominant absorption peak at 405 nm and the Fe^{3+}- PO complex gave the peak at 400 nm. The OD was increased dose-dependently at their specific wavelengths (Figure 4A to C). Kinetic of the complex formation was very fast and reached saturation within 5 min of incubation time (Figure 5). Taken together, it was found that the PG, RN and PO extracts were able to bind ferric ion.

They also bound ferrous ion but exhibited very low absorption intensity (data not shown). PG formed the complex(es) in concentration-dependent manner at low range of dose and tended to be saturated at higher concentrations. Similarly, RN and PO formed to complex(es) in dose-dependent pattern. Apparently, the maximum concentration of ferric ion for the PG binding was 300 µM.

Anti-hemolytic activity

Inhibitory effect of extracts on ferrous ion induced hemolysis was illustrated in Figure 6. Initially, we tested the effect of the extracts on human RBCs and found that they did not show any harmful effect on human RBCs (data not shown). All extracts exhibited satisfactory inhibitory properties against hemolysis at low concentration. The PG extract inhibited hemolysis with 91.5 ± 0.99% as maximum anti-hemolytic activity at 3.125 µg/ml. The maximum anti-hemolytic activities of PO and RN were 83.25 ± 3.66 and 77.88 ± 6.13%, respectively at concentration of 50 µg/ml. Among these three extracts, the PG represented the strongest efficiency followed by the PO and the RN, respectively.

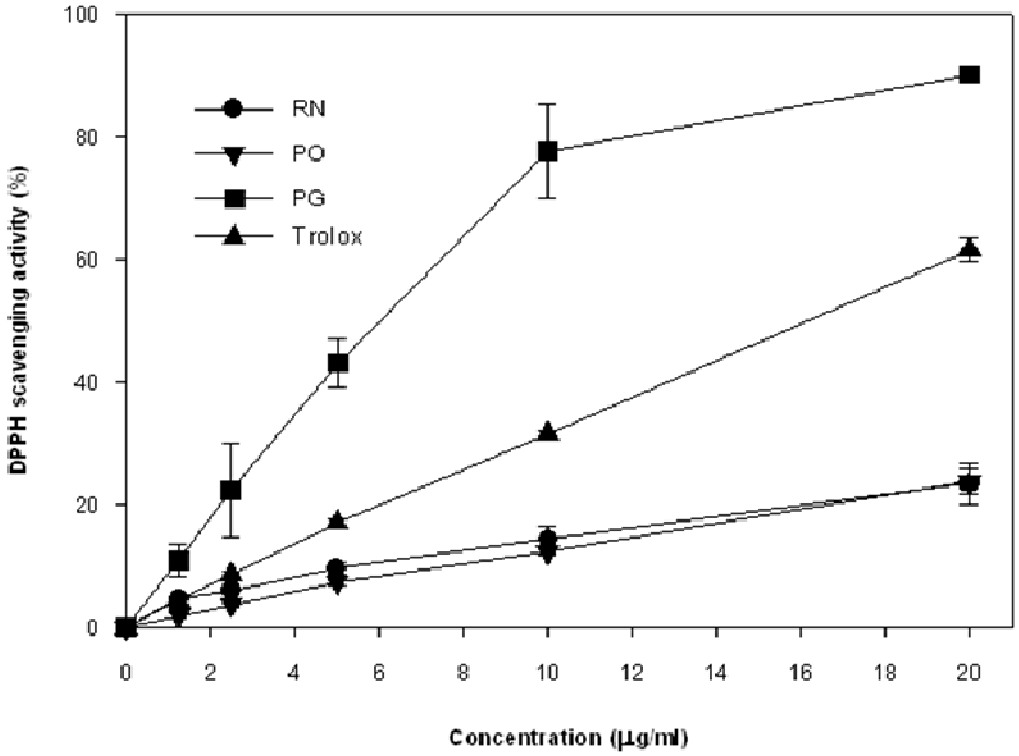

Figure 1. DPPH radical scavenging activity of the PG, RN and PO extracts. Data are obtained from triplicate results of three independent experiments and shown as mean ± standard deviation (SD).

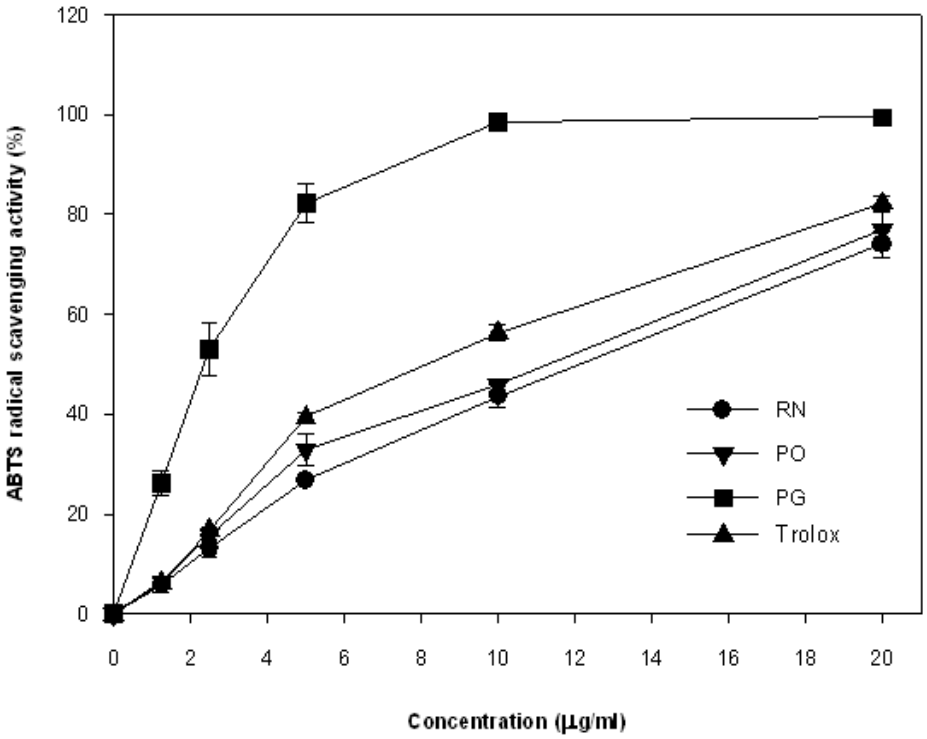

Figure 2. ABTS radical scavenging activity of the PG, RN and PO extracts. Data are obtained from triplicate results of three independent experiments and shown as mean ± standard deviation (SD).

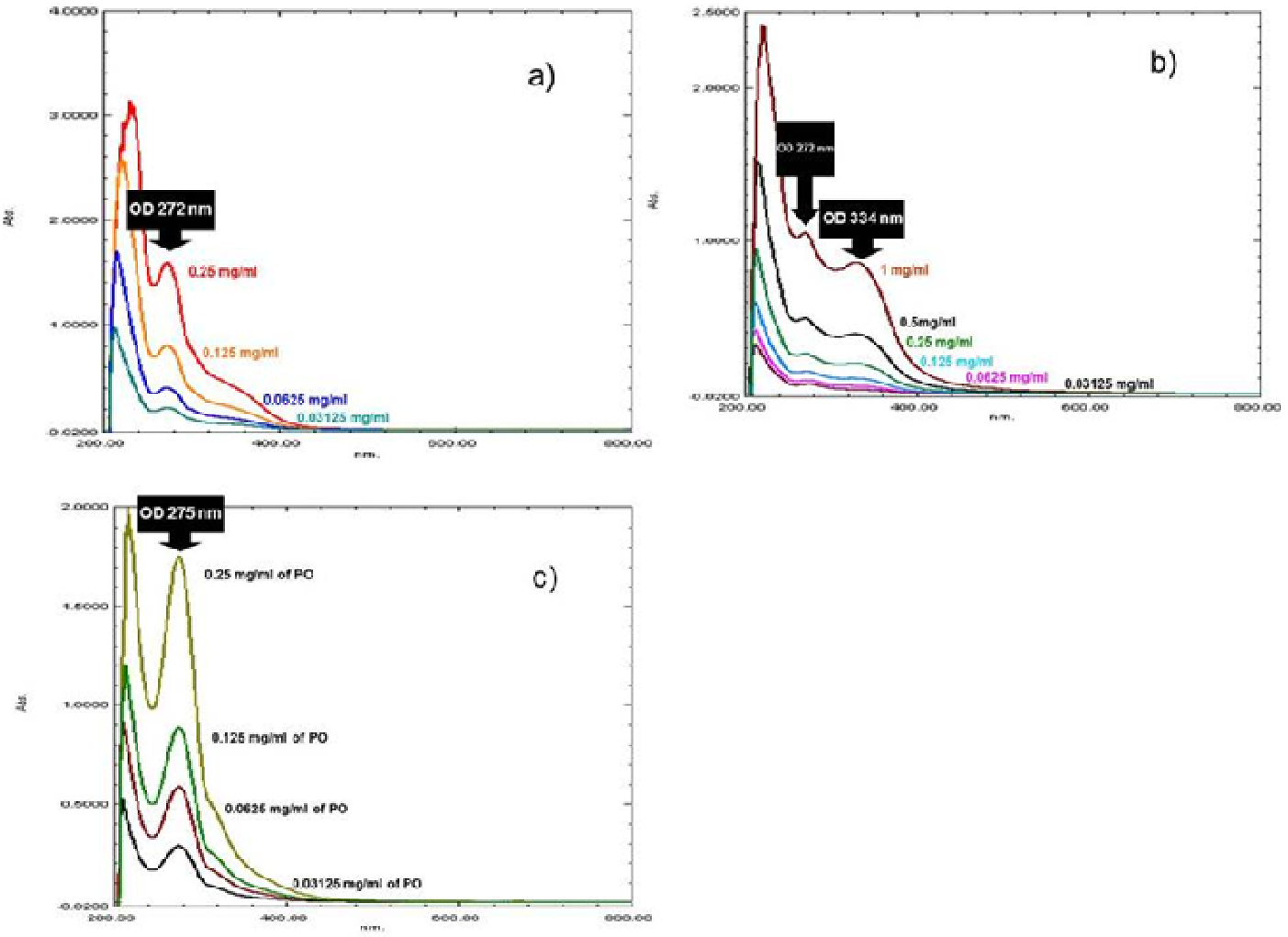

Figure 3. Spectral analysis of various concentrations of PG (a), RN (b) and PO (c) extracts.

DISSCUSSION

Nowadays, use of plant extracts as dietary supplement to improve health is increasing. Many attentions have been focused on polyphenolic compounds. Polyphenols are very important compounds in plants because their hydroxyl groups confer free radical scavenging activity (Yang et al., 2009). Recently, it has been revealed that various polyphenolic compounds such as flavonoids, tannin, catechins, coumarin, xanthones and procyanidin could scavenge radicals (VanderJagt et al., 2002). We have tested biological activities of leaf extracts of three Thai medicinal plants, PG, RN and PO. The amount of such compounds deposited in plants is usually different. PG leaves had the highest polyphenol content followed by PO leaves and RN leaves, which the polyphenol of the PG was 2.21- and 3.43- fold higher than that of PO and RN. Active compounds in RN leaves were widely investigated. The major compounds were naphthoquinone including rhinacanthin (A-D, G-Q), rhinacanthone and lignan group (Gotoh et al., 2004; Panichayupakaranant et al., 2009; Wu et al., 1998).

Siripong and coworkers reported that among rhinacanthins, rhinacanthin-N significantly suppressed tumor growth *in vivo* (Siripong et al., 2006). It has been reported that rhinacanthone isolated from *R. nasutus* roots induced apoptosis in human cervical carcinoma cells (Siripong et al., 2009). Leaves of guava have been reported to contain numerous substances. The phytochemical study of ethanolic extract of guava leaves has demonstrated many components including lipids, carbohydrate, protein, vitamin C, essential oils, tannins, aponins, triterpenic acids and polyphenols (Huang et al., 2004; Nantitanon et al., 2012). Liang et al. (2005) reported that polyphenols in ethanolic extract of guava leaves were gallic acid, quercetin, procatechuic acid, chlorogenic acid, caffeic acid, kaemferol, ferrulic acid, morin and quercetin-3-o-glucopyranoside (Liang et al., 2005; Matsuzaki et al., 2010). Comparative study on antioxidant activity between quercetin, morin and

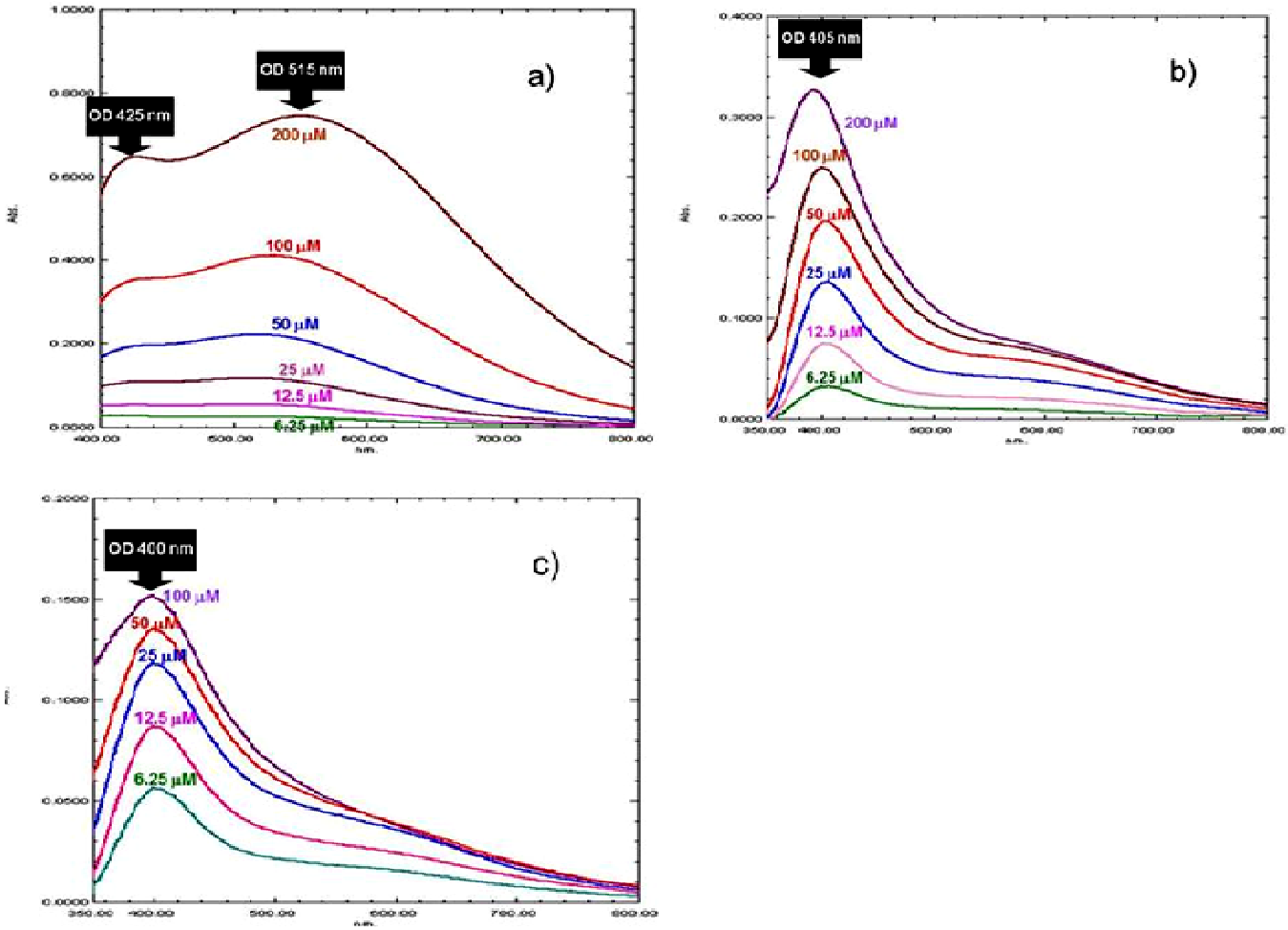

Figure 4. Spectral analysis of the resulting iron complex(es) when PG (a), RN (b) and PO (c) extracts (1 mg/ml) were incubated with various concentrations of ferric ion.

quercetin-3-o-glucopyranoside showed that quercetin was the most active and the synergistic antioxidant activity was obtained when quercetin was mixed with morin (Nantitanon et al., 2012). Therefore, the use of crude extract might render potent antioxidant activity.

In this study, we used DPPH radical scavenging assay, ABTS radical decolorization assay and iron-binding assay to evaluate antioxidant activity of three Thai medicinal plants. DPPH and ABTS assays are the most popular spectrophotometric method because they are simple, rapid, sensitive and reproducible. DPPH radical scavenging assay was used to determine the antiradical power of extracts through donation of hydrogen atom to DPPH radical and form non-radical molecule (Ak et al., 2008). Unlike DPPH, ABTS radicals are more reactive. Its reaction involves an electron-transfer process. Bleaching of ABTS cation has been extensively used to evaluate the antioxidant capacity (Schlesier et al., 2002). We found that the PG, PO and RN extracts possessed both DPPH and ABTS radicals scavenging activity but not the same level. The PG extract showed a marked antioxidant effect with IC_{50} value of 6.25 µg/ml for DPPH and 2.75 µg/ml for ABTS radical scavenging assay. This result was similar to the data of Chen and his coworkers (Hui et al., 2007) that compared the antioxidant activity between four aqueous extracts of nutraceutical herbs including *P. guajava*, *Camellia sinensis*, *Toona sinensis* and *Rosemarinus officinalis* and found that leaves extract of *P. guajava* exhibited the strongest activity.

Our study illustrates that leaves extracts of RN and PO at concentration of 20 µg/ml could not decrease DPPH radicals by 50%. We suggest that the concentration higher than 20 µg/ml might reach 50% scavenging activity. On the other hand, the weak DPPH radical scavenging property might be due to steric inaccessibility of compound and DPPH radical (Yang et al., 2009). The result from ABTS confirmed that the PG extract possessed the strongest free radical scavenging activity with IC_{50} of 2.75 µg/ml and its activity was four times higher than that of PO extract and eight times higher than that of RN extract. It was noticed that the radical scavenging activity of the PG extract was higher than

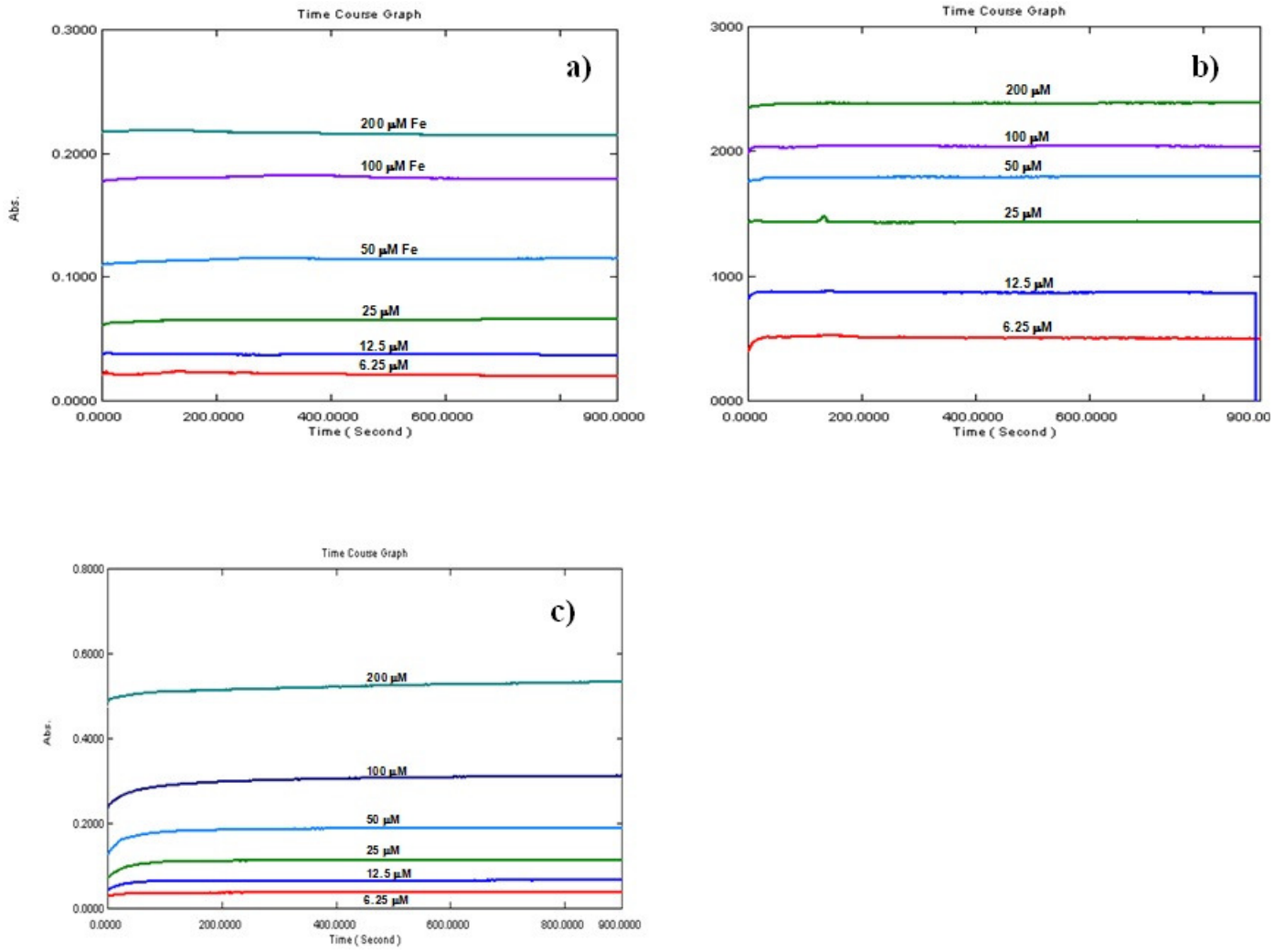

Figure 5. Kinetic of the complex formation between the PG (a), RN (b) and PO (c) extracts (1 mg/ml) and various concentrations of ferric ion.

standard antioxidant, trolox. According to these results, PG was considered to be a good antioxidant.

Since transition metals, especially iron, facilitate the production of ROS via Fenton reaction, the ability of substances to bind iron can be valuable antioxidant property. Interestingly, the PG, PO and RN extracts showed rapid iron binding capacity and completed within 5 min of incubation time. In accordance with Tandan et al (2012), they reported that administration of guava leaves extracts reduced arsenic concentration in blood and tissue and showed metal chelation property. This action might be due to structures of compounds in extracts. Previous evidences reported that the polyphenols would chelate iron with its C=O and C-OH structures (Ak et al., 2008; Rice-Evans et al., 1996; Srichairatanakool et al., 2007). Moreover, the compounds with structure containing two or more of the following groups: -OH, -SH, -COOH, $-PO_3H_2$, C=O, $-NR_2$, -S and -O- in favorable structure-function configuration can show metal chelation (Rice-Evans et al., 1996). Gas phase ligation technique shows that some flavonoids such as kaemferol, quercetin, myricetin and naringenin were able to chelate Cu^{2+} and Fe^{2+} through the functional carbonyl group (Kazazic et al., 2006). In this study, all tested extracts showed effective iron chelating activity and may afford protection against oxidative damage by removing iron that participates in ROS generating Fenton reaction.

Since red blood cell membrane contains high amount of polyunsaturated fatty acids, they are vulnerable to oxidative stress. In patients with β-thalassemia, sickle cells anemia and renal disease, ROS production is higher than normal (Nematbakhsh et al., 2013; Shazia et al., 2012). Moreover, iron overload has been observed in these patients (Finberg, 2012; Nematbakhsh et al., 2013; Taher et al., 2013). Thus, it can increase the susceptibility to peroxidation and induce hemolysis. The abilities of extracts to scavenge free radicals and bind iron were further confirmed by inhibition of ferrous ion-

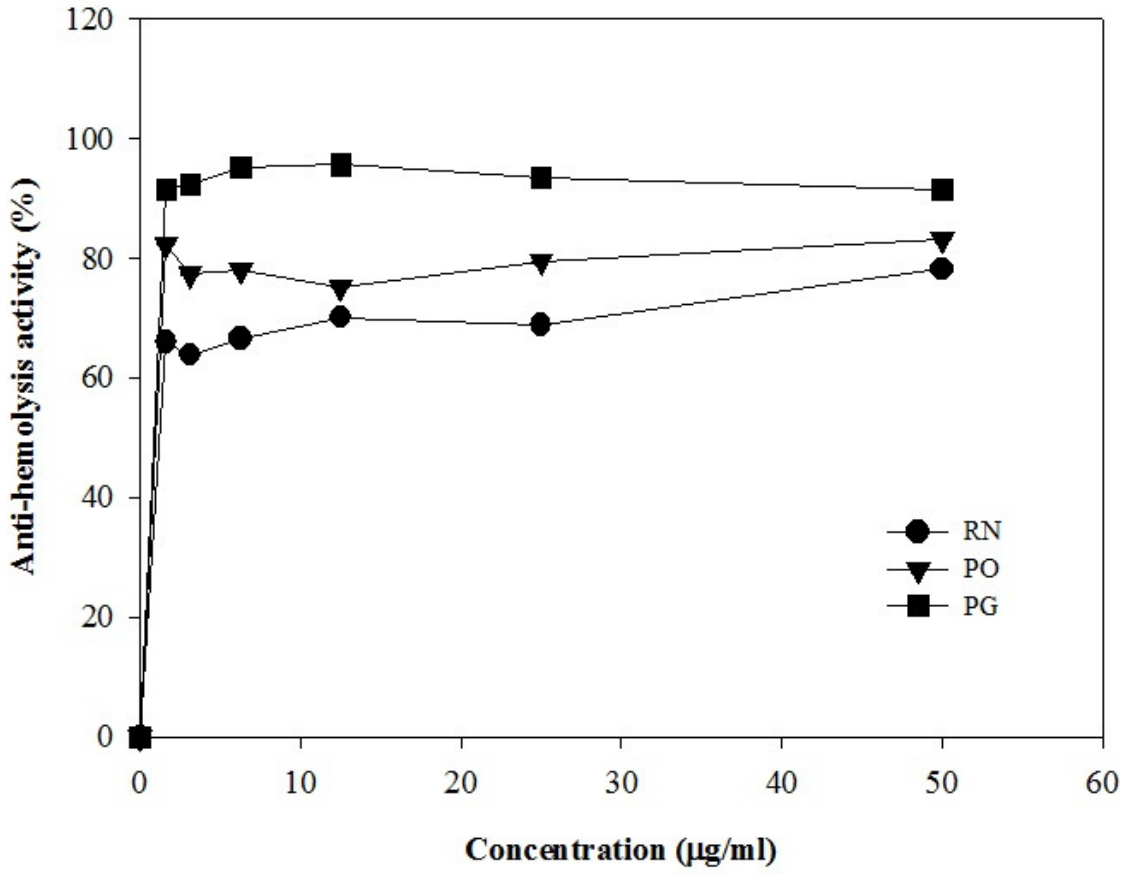

Figure 6. Anti-hemolysis activity of the PG, RN and PO extracts. Data are obtained from triplicate results of three independent experiments and shown as mean ± standard deviation (SD).

induced hemolysis. Regarding the previous results, the PG extract could effectively protect RBCs from iron-induced hemolysis with the activity higher than that of PO and RN extracts. Based on all results, these three Thai plants are sources of polyphenols and might be used for alleviating or preventing harmfulness in many oxidative stress disorders.

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REFERENCES

Ak T, Gulcin I (2008). Antioxidant and radical scavenging properties of curcumin. Chem. Biol. Interact. 174:27-37.

Amer J, Goldfarb A, Fibach E (2004). Flow cytometric analysis of the oxidative status of normal and thalassemic red blood cells. Cytometry A. 60:73-80.

Bukke S, Raghu PS, Sailaja G Thyaga RK (2011). The study on Morphological, Phytochemical and Pharmacological aspects of *Rhinacanthus nasutus*. (L) Kurz. J. Appl. Pharm. Sci. 1:26-32.

Chiu D, Vichinsky E, Yee M, Kleman K, Lubin B (1982). Peroxidation, vitamin E, and sickle-cell anemia. Ann. NY Acad. Sci. 393:323-335.

Das N, Das Chowdhury T, Chattopadhyay A, Datta AG (2004). Attenuation of oxidative stress-induced changes in thalassemic erythrocytes by vitamin E. Pol. J. Pharmacol. 56:85-96.

Fibach E, Tan ES, Jamuar S, Ng I, Amer J, Rachmilewitz EA (2010). Amelioration of oxidative stress in red blood cells from patients with beta-thalassemia major and intermedia and E-beta-thalassemia following administration of a fermented papaya preparation. Phytother. Res. 24:1334-8.

Finberg KE (2012). Striking the target in iron overload disorders. J. Clin. Invest. 123:1424-1427.

Gotoh A, Sakaeda T, Kimura T, Shirakawa T, Wada Y, Wada A, Kimachi T, Takemoto Y, Iida A, Iwakawa S, Hirai M, Tomita H, Okamura N, Nakamura T, Okumura K (2004). Antiproliferative activity of *Rhinacanthus nasutus* (L.) Kurz extracts and the active moiety, Rhinacanthin C. Biol. Pharm. Bull. 27:1070-1074.

Huang J, Zhang Z (2004). Microwave-assisted extraction of quercetin and acid degradation of its glycosides in *Psidium guajava* leaves. Anal. Sci. 20:395-397.

Hui C, Yuh L, Hsieh C (2007). Evaluation of antioxidant activity of aqueous extract of some selected nutraceutical herbs. Food Chem. 104:1418–1424.

Jaiarj P, Khoohaswan P, Wongkrajang Y, Peungvicha P, Suriyawong P, Saraya ML, Ruangsomboon O (1999). Anticough and antimicrobial activities of *Psidium guajava* Linn. leaf extract. J. Ethnopharmacol. 67:203-212.

Kazazic SP, Butkovic V, Srzic D, Klasinc L (2006). Gas-phase ligation of Fe^{+} and Cu^{+} ions with some flavonoids. J. Agric. Food Chem. 54:8391-8396.

Lehmann DJ, Schuur M, Warden DR, Hammond N, Belbin O, Kolsch H, Lehmann MG, Wilcock GK, Brown K, Kehoe PG, Morris CM, Barker

R, Coto E, Alvarez V, Deloukas P, Mateo I, Gwilliam R, Combarros O, Arias-Vasquez A, Aulchenko YS, Ikram MA, Breteler MM, van Duijn CM, Oulhaj A, Heun R, Cortina-Borja M, Morgan K, Robson K, Smith AD (2012). Transferrin and HFE genes interact in Alzheimer's disease risk: the Epistasis Project. Neurobiol. Aging 33(202):e1-13.

Liang Q, Qian H, Yao W (2005). Identification of flavonoids and their glycosides by high-performance liquid chromatography with electrospray ionization mass spectrometry and with diode array ultraviolet detection. Eur. J. Mass Spectrom (Chichester, Eng). 11:93-101.

Lutterodt GD (1989). Inhibition of gastrointestinal release of acetylcholine by quercetin as a possible mode of action of *Psidium guajava* leaf extracts in the treatment of acute diarrhoeal disease. J. Ethnopharmacol. 25:235-247.

Matsuo T, Hanamure N, Shimoi K, Nakamura Y, Tomita I (1994). Identification of (+)-gallocatechin as a bio-antimutagenic compound in *Psidium guava* leaves. Phytochemistry 36:1027-1029.

Matsuzaki K, Ishii R, Kobiyama K, Kitanaka S (2010). New benzophenone and quercetin galloyl glycosides from *Psidium guajava* L. J. Nat. Med. 64:252-256.

Moskovitz J, Yim MB, Chock PB (2002). Free radicals and disease. Arch. Biochem. Biophys. 397:354-359.

Nantitanon W, Okonogi S (2012). Comparison of antioxidant activity of compounds isolated from guava leaves and a stability study of the most active compound. Drug Discov. Ther. 6:38-43.

Nematbakhsh M, Pezeshki Z, Moaeidi BA, Eshraghi-Jazi F, Talebi A, Nasri H, Baradaran S, Gharagozloo M, Safari T, Haghighi M (2013). Protective Role of Silymarin and Deferoxamine Against Iron Dextran-induced Renal Iron Deposition in Male Rats. Int. J. Prev. Med. 4:286-292.

Ounjaijean S, Thephinlap C, Khansuwan U, Phisalapong C, Fucharoen S, Porter JB, Srichairatanakool S (2008). Effect of green tea on iron status and oxidative stress in iron-loaded rats. Med. Chem. 4:365-370.

Panichayupakaranant P, Charoonratana T, Sirikatitham A (2009). RP-HPLC analysis of rhinacanthins in *Rhinacanthus nasutus*: validation and application for the preparation of rhinacanthin high-yielding extract. J. Chromatogr. Sci. 47:705-708.

Peungvicha P, Temsiririrkkul R, Prasain JK, Tezuka Y, Kadota S, Thirawarapan SS, Watanabe H (1998). 4-Hydroxybenzoic acid: a hypoglycemic constituent of aqueous extract of *Pandanus odorus* root. J. Ethnopharmacol. 62:79-84.

Rice-Evans CA, Miller NJ, Paganga G (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Rad. Biol. Med. 20:933-956.

Roy CK, Das AK (2010). Comparative evaluation of different extracts of leaves of *Psidium guajava* Linn. for hepatoprotective activity. Pak. J. Pharm. Sci. 23:15-20.

Schlesier K, Harwat M, Bohm V, Bitsch R (2002). Assessment of antioxidant activity by using different in vitro methods. Free Rad. Res. 36:177-187.

Shazia Q, Mohammad ZH, Rahman T, Shekhar HU (2012). Correlation of oxidative stress with serum trace element levels and antioxidant enzyme status in Beta thalassemia major patients: a review of the literature. Anemia 2012:270923

Siripong P, Hahnvajanawong C, Yahuafai J, Piyaviriyakul S, Kanokmedhakul K, Kongkathip N, Ruchirawat S, Oku N (2009). Induction of apoptosis by rhinacanthone isolated from *Rhinacanthus nasutus* roots in human cervical carcinoma cells. Biol. Pharm. Bull. 32:1251-1260.

Siripong P, Yahuafai J, Shimizu K, Ichikawa K, Yonezawa S, Asai T, Kanokmedakul K, Ruchirawat S, Oku N (2006). Antitumor activity of liposomal naphthoquinone esters isolated from Thai medicinal plant: *Rhinacanthus nasutus* KURZ. Biol. Pharm. Bull. 29:2279-2283.

Srichairatanakool S, Thephinlap C, Phisalaphong C, Porter JB, Fucharoen S (2007). Curcumin contributes to in vitro removal of non-transferrin bound iron by deferiprone and desferrioxamine in thalassemic plasma. Med. Chem. 3:469-474.

Taher AT, Viprakasit V, Musallam KM, Cappellini MD (2013). Treating iron overload in patients with non-transfusion-dependent thalassemia. Am. J. Hematol. 88:409-415.

Tandan N, Roy M, Roy S (2012). Ameliorative Potential of *Psidium* guajava on Hemato-biochemical Alterations in Arsenic-exposed Wistar Rats. Toxicol. Int. 19:121-124.

Thephinlap C, Phisalaphong C, Fucharoen S, Porter JB, Srichairatanakool S (2009). Efficacy of curcuminoids in alleviation of iron overload and lipid peroxidation in thalassemic mice. Med. Chem. 5:474-482.

Thephinlap C, Phisalaphong C, Lailerd N, Chattipakorn N, Winichagoon P, Vadolus J, Fucharoen S, Porter JB, Srichairatanakool S (2011). Reversal of cardiac iron loading and dysfunction in thalassemic mice by curcuminoids. Med. Chem. 7:62-69.

VanderJagt TJ, Ghattas R, VanderJagt DJ, Crossey M, Glew RH (2002). Comparison of the total antioxidant content of 30 widely used medicinal plants of New Mexico. Life Sci. 70:1035-1040.

Visweswara RP, Sivanaryana AG, Sasikala S, Dhananjaya MN (2010). Efficacy of antimicrobial activity of *Rhinacantus Nasutus* (Linn) leaves in different extractions. Int. J. Pharm. Biol. Sci. 1:1-4.

Visweswara RP, Sujana P, Vijayakanth T, Dhananjaya NM (2012). *Rhinacanthus nasutus* - Its protective role in oxidative stress and antioxidant status in streptozotocin induced diabetic rats. Asian Pac. J. Trop. Dis. 2:327-330.

Wu TS, Hsu HC, Wu PL, Teng CM, Wu YC (1998). Rhinacanthin-Q, a naphthoquinone from *Rhinacanthus nasutus* and its biological activity. Phytochemistry 49:2001-2003.

Yang JH, Hu JP, Rena K, Du NS (2009). Structure-activity relationships of phenylethanoid glycosides in plants of Cistanche salsa on antioxidative activity. Zhong Yao Cai 32:1067-1069.

Zhu QY, Holt RR, Lazarus SA, Orozco TJ, Keen CL (2002). Inhibitory effects of cocoa flavanols and procyanidin oligomers on free radical-induced erythrocyte hemolysis. Exp. Biol. Med. (Maywood) 227:321-329.