Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 109 (2008) 54–63
Food Chemistry
www.elsevier.com/locate/foodchem

Rind of the rambutan, *Nephelium lappaceum*, a potential source of natural antioxidants

Uma Palanisamy [a,*], Cheng Hwee Ming [b], Theanmalar Masilamani [a], Thavamanithevi Subramaniam [a], Ling Lai Teng [b], Ammu K. Radhakrishnan [c]

[a] *SIRIM Bhd, 1 Persiaran Dato Menteri, 40911 Shah Alam, Selangor Darul Ehsan, Malaysia*
[b] *Universiti Malaya, Faculty of Medicine, University of Malaya, 59100 Kuala Lumpur, Malaysia*
[c] *International Medical University, No. 126 Jalan 19/155B, Bukit Jalil, 57000 Kuala Lumpur, Malaysia*

Received 13 August 2007; received in revised form 29 October 2007; accepted 5 December 2007

Abstract

The rind of rambutan, which is normally discarded was found to contain extremely high antioxidant activity when assessed using several methods. Although having a yield of only 18%, the ethanolic rambutan rind extract had a total phenolic content of 762 ± 10 mg GAE/g extract, which is comparable to that of a commercial preparation of grape seed extract. Comparing the extract's pro-oxidant capabilities with vitamin C, α-tocopherol, grape seed and green tea, the rind had the lowest pro-oxidant capacity. In addition, the extract at 100 μg/ml was seen to limit oxidant-induced cell death (DPPH at 50 μM) by apoptosis to an extent similar to that of grape seed. The extracts were not cytotoxic to normal mouse fibroblast cells or splenocytes while the powderised rind was seen to have heavy metals contents far below the permissible levels for nutraceuticals. Our study for the first time reveals the high phenolic content, low pro-oxidant capacity and strong antioxidant activity of the extract from rind of *Nephelium lappaceum*. This extract, either alone or in combination with other active principles, can be used in cosmetic, nutraceutical and pharmaceutical applications.

Keywords: *Nephelium lappaceum*; Rambutan; Free radical-scavenging activity; Phenolic content; Pro-oxidant; Nutraceuticals

1. Introduction

The powerful antioxidant properties of vitamins C, E and β-carotenes are well known (Krinsky & Johnson, 2005; Scheibmeir et al., 2005). However, there is another group of naturally-occurring inhibitors of oxidation that are receiving a lot of attention lately, namely the phenolics and polyphenolics, which are secondary plant metabolites. Studies have shown that polyphenols are better scavengers of free radicals than vitamins C and E (John, Jianmei, Joseph & Yukio, 2003). For instance, green tea is thought to be particularly effective at protecting against free radical damage in the gastrointestinal tract (Orner, Dashwood, & Dashwood, 2004). Other antioxidant herbs include bilberry (Valentová, Ulrichová, Cvak, & Šimánek, 2006), grape seed extract (Hwang et al., 2004), pomegranate (Rosenblat, Hayek, & Aviram, 2006) and milk thistle (Jacobs, Dennehy, Ramirez, Sapp, & Lawrence, 2002.

Much of the interest in plant phenolics are in flavonoids. Flavonoids are some of the most powerful and effective antioxidant compounds available to humans; and since we are unable to produce flavonoids ourselves, we must get them from the food we eat and from supplements. Several studies

Abbreviations: DPPH, 1,1-diphenyl-2-picrylhydrazyl; ABTS, 2,2-azinobis 3-ethylbenzothiazoline 6-sulfonate; GAE, gallic acid equivalent.

* Corresponding author. Tel.: +60 3 55446972; fax: +60 3 554469887.
E-mail addresses: umadevi@sirim.my (U. Palanisamy), chengh-m@ummc.edu.my (H.M. Cheng), theanmalar_masilamani@sirim.my (T. Masilamani), thava@sirim.my (T. Subramaniam), linglaiteng@gmail.com (L.T. Ling), ammu_radhakrishnan@imu.edu.my (A.K. Radhakrishnan).

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.12.018

have shown that normal intake of flavonoids through eating a range of fruits and vegetables is not enough to keep modern levels of free radicals at bay (Clarkson & Thompson, 2000). Food companies are increasingly promoting the health benefits of antioxidants contained in fruits and vegetables. The trend has been to investigate a variety of plants as new potential sources of antioxidants (Miliauskas, Venskutonis & van Beek, 2004; Peschel et al., 2006). Besides vitamins, a defined range of herbal extracts represents the most potential in the antioxidant market. Companies must be careful to have substantial documentation of antioxidant evaluation of their natural ingredients. The antioxidant market is set to grow over the foreseeable future; however what is often lacking is good science that shows the antioxidant claims of these compounds.

Native to Southeast Asia, rambutan *(Nephelium lappaceum* L.) belongs to the same family (Sapindaceae) as the sub-tropical fruits lychee and longan (Marisa, 2006). This fruit is an important commercial crop in Asia, where it is consumed fresh, canned, or processed, and appreciated for its refreshing flavour and exotic appearance (Ong, Acree, & Lavin, 1998). In Malaysia, the dried fruit rind has been employed in local medicine.

2. Materials and methods

2.1. Chemicals and reagents

Ammonium thiocyanate, L-ascorbic acid, concanavalin A, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), Folin-Ciocalteu reagent, gallic acid, linoleic acid, NBT (tetrazolium salt), potassium persulfate, superoxide dismutase, tert-butylphenol (BHT), xanthine and xanthine oxidase were purchased from Sigma Chemicals (MO). Emblica™ was obtained from EMD (Darmstadt, Germany); and grape seed extract was purchased from Vitis AG (Trittenheim, Germany). DL-α-tocopherol and tocotrienol from Fluka Biochemika (Buchs, Switzerland), trichloroacetic acid from Merck (Darmstadt, Germany), ABTS diammonium salt from Amresco Inc. (Solou, OH). All cell cultures were purchased from American Tissue Culture Collection (Manassas, VA). The cell death detection ELISAPLUS kit and cell proliferation kit II was from Roche Applied Science (Mannheim, Germany). RPMI 1640 with L-glutamine, sodium pyruvate, foetal bovine serum, penicillin-steptomycin, HEPES buffer solution and typan blue stain were obtained from Invitrogen New Zealand Ltd. (Auckland), phosphate buffered saline from ICN Biochemicals Inc. (Aurora, OH) and accutase from Innovative Cell Technologies, Inc. (San Diego, CA).

2.2. Plant collection

Fresh fruit pulp, rind, seed and leaves of *N. lappaceum* L. were obtained from Kuala Lumpur. The plant was authenticated by the Herbarium of the Forest Research Institute of Malaysia (FRIM), Kepong, Malaysia.

2.3. Preparation of plant extracts

The fruit pulp, seed, rind and leaves were washed with copious amounts of water and distilled water. These were then allowed to air dry at room temperature for 2–3 h. Following this, plant parts were placed in a circulating oven at 40 °C until completely dry. The dried fruit pulp, seed, rind and leaves were powdered using a Waring blender or milled using the Fritsch dry miller.

Deionised water or ethanol (analytical grade) at 1:10 (w/v) concentrations were added to the powdered pulp, seed, rind and leaves. Water extraction was carried out at 40 °C while solvent extraction was performed at room temperature for 24 h in an orbital shaker. The suspension thus obtained was filtered using a 114 Whatman filter paper and the filtrate collected. Aqueous filtrate was concentrated using a freeze drier while solvent filtrate was concentrated using a rotary evaporator. The method of supercritical extraction (SFE) was used only on the rind of *N. lappaceum*.

2.4. Supercritical fluid extraction

Extraction was carried out using the supercritical fluid extraction system Thar SFE 500 from Thar Technologies (Pittsburgh, PA). Eighty grams of powdered rind or leaves of *N. lappaceum* L. were placed in the extraction vessel, and CO_2 at a pressure of 300 bar and temperature of 50 °C was passed through the vessel for 2 h. The gas flow rate was set at 30 g/min. The components (oils and actives) soluble in liquid CO_2 precipitated at the end of the run were collected. The product thus obtained was a completely pure extract with no liquid organic solvents. Ethanol however, was used to wash down the lines and the precipitate was further concentrated on a rotary evaporator. The residue remaining after the SFE extraction was re-extracted using ethanol as solvent. A ratio of 1:20, that is 100 g of powderised rind to 2000 ml of ethanol, was used for further extraction and processed as described for the ethanol extraction.

2.5. Antioxidant assays

Five different free radical-scavenging assays were performed.

2.5.1. Scavenging activity onto DPPH radicals

Scavenging activity of DPPH (1,1-diphenyl-2-picrylhydrazyl) free radicals by the extracts was assessed, according to the modified method reported by Chang et al. (2002). An aliquot total of 950 μl of 0.004% DPPH ethanolic solution was added to 50 μl of an extract of different concentrations and the mixture was allowed to react at 37 °C for 10 min. Then, absorbance was measured at 515 nm with a Cary 50 Bio UV–visible spectrophotometer (Varian, Inc., Palo Alto, CA). All measurements were done in triplicate.

2.5.2. Scavenging activity onto galvinoxyl radicals

Galvinoxyl, another stable phenoxyl radical can be reduced by hydrogen-donating free radical scavengers. The concentrations of extracts and standards required to achieve 50% phenoxyl radical-scavenging activity were determined, according to the method of Shi, Noguchi, and Niki (2001). An aliquot of 900 μl of galvinoxyl methanol working solution (5 mM) was added to 90 μl of a sample of different concentrations to make a final volume of 990 μl and the mixture was allowed to react at 37 °C. After 20 min, the absorbance value was measured at 428 nm. All measurements were done in triplicate.

2.5.3. Scavenging activity onto ABTS radicals

ABTS (2,2-azinobis 3-ethylbenzothiazoline 6-sulfonate) is oxidised to the coloured nitrogen centred radical cation $ABTS^{+}$ in a persulfate system. The radical-scavenging capacities of extracts and positive controls were assessed, according to the modified method reported by Re et al. (1999). Two hundred microlitres of the working solution (5 mM, pH 7.4) were mixed with 20 μl of extract in a microtitre plate to a final volume of 220 μl, and the mixture was allowed to react for 6 min, before measuring its absorbance at 690 nm using an AsYS microplate reader UVM-340 (ASYS Hitech GmbH, Eugendorf, Austria). The positive controls used in all the scavenging assays were L-ascorbic acid and commercial grape seed extract (Vitis vital™). Scavenging activity was expressed as IC_{50}, which represents the final concentration of the extract (μg/ml) in the reaction mixture required to inhibit 50% of the free radical-scavenging activity. All measurements were done in triplicate.

2.5.4. Scavenging activity onto superoxide anions

An enzyme system (xanthine/xanthine oxidase) forms the superoxide anions which react with NBT (tetrazolium salt) to form a red formazan. An antioxidant substance absorbs or destroys superoxide anions, thereby reducing the formation of formazon. The results are presented as percent inhibition and compared to the positive control superoxide dismutase, using the modified method of Chang, Lin, Chuang, and Chiang (1996). Stock solutions of NBT (0.2 M Tris-HCl, 5 mM $MgCl_2$, 0.75 mM NBT), xanthine (66 mM xanthine in 0.5 M NaOH) and 27 mM EDTA were prepared. An NBT working solution was prepared by adding stock solutions of the above to give a final concentration of 0.15 mM NBT, 3 mM xanthine, 0.108 mM EDTA and 0.05 M Na_2CO_3. The final pH of the working solution was pH 10–10.2. The assay was conducted in 96-well plates where 25 μl of extracts were added to 200 μl of NBT working solution and 25 μl of xanthine oxidase (0.3 units/ml). The reaction was allowed to proceed for 5 min after which its absorbance at 560 nm was recorded using an AsYS microplate reader UVM-340. Superoxide dismutase, L-ascorbic acid and grape seed served as the positive controls. All measurements were done in triplicate.

2.6. Inhibition of induced lipid autooxidation

Linoleic acid undergoes autoxidation under specified conditions. Autoxidative activity is determined by the ability to inhibit the peroxidation of linoleic acid. The results are presented as percent inhibition and compared to positive controls: 1% *tert*-butylphenol (BHT) and α-tocopherol (vitamin E). The modified method of Lee and Lim (2000) was followed, where a mixture containing extract, 5% linoleic acid in 0.05 M phosphate buffer (pH 7.0, containing 0.5% Tween-80) was incubated for 16 h at 40 °C in the dark. One hundred microlitres of this incubated mixture was aspirated and mixed with 3 ml of 70% ethanol, 100 μl of 0.3 g/ml ammonium thiocyanate and 100 μl of 2.45 mg/ml ferrous chloride in 3.5% hydrochloric acid. The addition of acid–alcohol solution stopped the autoxidation reaction and hydroperoxides formed complexed with the ferric thiocyanate to produce an absorbance at 500 nm, which was measured after 3 min. Extract concentrations used were; rind at 0.1 and 0.4 mg/ml and leaves at 0.3 and 0.7 mg/ml. All measurements were done in triplicate.

2.7. Pro-oxidant assay

Reducing power of iron ion was measured according to the method of Bing and Yuejin (2005), where equal volumes of extract and 1% potassium ferricyanate $[K_3Fe(CN_6)]$ were incubated at 50 °C for 20 min. An equal volume of 10% trichloroacetic acid was added and the mixture centrifuged at 3000*g* for 10 min. The upper layer of the solution (1.0 ml) was mixed with 1 ml of distilled water and 0.2 ml of 0.1% ferric chloride ($FeCl_3$) and its absorbance at 700 nm was recorded. Ethanol or distilled water was used as the negative control while Emblica™ (a commercial antioxidant with very low pro-oxidant activity) was used as the positive control. Comparison of pro-oxidant capabilities was performed with Vitamin C, grape seed, tocotrienol, tocopherol and green tea. Results are expressed in comparison with positive controls at concentrations ranging from 0.1 mg/ml to 0.5 mg/ml. All measurements were done in triplicate.

2.8. Heavy metal content determination

Lead, arsenic and mercury content of the powdered rind of *N. lappaceum* was determined against international standards. Inductively coupled plasma-optical emission spectroscopy (ICP-OES) was used for the determination of arsenic and lead, and atomic absorption spectroscopy (AAS) for the determination of mercury. All measurements were done in triplicate.

2.9. Phenolic content determination

Total phenolics were determined using the Folin-Ciocalteu method, modified according to Miliauskas et al. (2004), which is based on a colorimetric oxidation and

reduction reaction. A 1-ml aliquot of the extract at defined concentrations was added to 5 ml of Folin-Ciocalteu reagent; 4 ml of 7.5% Na_2CO_3 solution was added to the mixture after 3–5 min and was thoroughly mixed. The absorbance at 765 nm was recorded after 1 h. A linear dose-response regression curve was generated using the absorbance reading of gallic acid at the wavelength of 765 nm after 30 min. Total phenolic content of the sample was calculated. The content of phenolic compounds in a respective sample was expressed in mg/g of extract, gallic acid equivalents (GAE). All measurements were done in triplicate.

2.10. Measurement of cell proliferation of cultured cells by XTT assay

Confluent cells (3T3 mouse fibroblasts cells and 4T1 mouse mammary gland tumor cells) were harvested and counted before being plated onto 96-well plates at 5×10^3 cells/well. Following this, known concentrations of aqueous and ethanol extracts of the rind of *N. lappaceum* were added into each of the 96 wells. The plates were then incubated for 24 h in a humidified CO_2 incubator (Nuaire, Plymouth, MN) at 37 °C. At the end of the incubation period, the 96-well culture plates were removed from the CO_2 incubator at the end of the culture periods and cell proliferation was determined using the XTT cell proliferation kit, as recommended by the manufacturers. The XTT labelling and electron-coupling reagents were thawed using a water bath set at 37 °C. Five millilitres of labelling and electron-coupling reagents were mixed with 0.1 ml of the coupling reagent, and the concoction mixed thoroughly, to obtain a clear solution. Fifty microlitres of the XTT labelling mixture was pipetted into each of the 96 wells at 50 μl/well. Following this, the culture plates were returned to the humidified 5% CO_2 incubator and incubated for a further 6 h. The absorbance of the test samples was read at a wavelength of 450 nm, using an ELISA plate reader (Expert Plus ELISA Reader; ASYS Hitech GmbH). The reference wavelength used was 650 nm. The XTT labelling reagent alone was used as blank. All measurements were done in triplicate.

2.11. Measurement of cell death by apoptosis in cells cultured in extracts of the rind of N. lappaceum

Confluent cells (3T3 or 4T1 cells) were harvested and counted before being plated onto 96-well plates at 5×10^3 cells/well. Following this, known concentrations of aqueous and ethanol extracts of the rind of *N. lappaceum* were added into each of the 96 wells. The extracts were appropriately diluted in complete RPMI 1640 medium to obtain final concentrations of 50 μg/ml, 100 μg/ml and 500 μg/ml. One hundred microlitres of the diluted extracts were added into the wells containing the cultured cells. Wells containing cells in medium without any added extracts were used as negative control. Grape seed extract used in the same concentrations was used as the positive control. The plates were then incubated in a humidified 5% CO_2 incubator at 37 °C for 24 h. Cell death due to apoptosis in cells cultured in the presence and absence of the extracts was determined using the cell death detection kit, as recommended by the manufacturers. All measurements were done in triplicate.

2.12. Measurement of oxidant-induced cell death by apoptosis in cells cultured in the extracts of the rind of N. lappaceum

Confluent cells (3T3 or 4T1 cells) were harvested and counted before being plated out in 96-well plates at 5×10^3 cells/well. Following this, 100 μl of known concentrations (50 μg/ml, 100 μg/ml and 500 μg/ml) of aqueous and ethanol extracts of the rind of *N. lappaceum* were added into each of the 96 wells. Grape seed extract used in the same concentrations was used as the positive control, while cells in medium without any added extracts were used as negative control. The plates were then incubated in a humidified 5% CO_2 incubator at 37 °C for 1 h. Following this, 100 μl of known concentration of DPPH were added to all the wells. The culture plates were incubated for 24 h in a humidified 5% CO_2 incubator at 37 °C. Wells containing cells and oxidants alone served as positive control, i.e., where maximum cell death was expected, while wells containing cells, oxidants and grape seed extract were used as negative control where only low levels of cell death were expected. Oxidant-induced cell death by apoptosis in cells cultured in the presence and absence of the extracts was determined using the cell death detection kit, as recommended by the manufacturers. All measurements were done in triplicate.

2.13. Effect of culturing murine splenocytes in the presence of extracts of the rind of N. lappaceum

A seven-week old male BALB/c mouse was painlessly killed with ether. Once the mouse was dead, it was dipped in 70% alcohol to sterilise the animal, prior to taking it into the clean area for culture purposes. The spleen from the sacrificed animal was removed aseptically. The spleen was placed in a sterile petri dish containing complete RPMI 1640 media. The splenocytes were then gently teased out from the splenic capsule. The splenocyte suspension was then transferred into sterile 15 ml tubes and centrifuged at 500*g* for 5 min at 4 °C. After centrifugation, the supernatant was discarded and 5 ml of complete RPMI 1640 medium was added to the tube. The pellet was resuspended in the medium by gentle mixing. The mixture was left on ice while the number of white blood cells were determined using a haemocytometer. The splenocyte suspension was appropriately diluted in complete RPMI 1640 medium, to obtain a final cell concentration of 1×10^5 cells/ml. One hundred microlitres of the diluted splenoyctes were then pipetted into the wells of sterile 96-well tissue culture plates

(1×10^4 cells/well). Following this, known concentrations (10 μg/ml, 50 μg/ml, 100 μg/ml and 125 μg/ml) of the aqueous and ethanol extracts of the rind of *N. lappaceum* were added into the wells containing the splenocytes, and cultured for 24 h, 48 h and 72 h in a humidified 5% CO_2 incubator at 37 °C. Wells containing cells in culture medium alone with no added extracts were taken as the negative control, while wells that contained 25 μg/ml concanavalin A served as the positive control. At the end of the specified culture periods (24 h, 48 h and 72 h), the plates were removed from the incubator and cell proliferation was determined using the XTT cell proliferation determination kit as described above. All measurements were done in triplicate.

2.14. Statistical analysis

Results were expressed as mean ± standard error. Statistical comparisons between groups were performed with Student's *t*-test for independent observations. Differences were considered significant at $p < 0.05$.

3. Results and discussion

3.1. Extraction yields

Water and ethanol extraction of the fruit pulp, seed, rind and leaves of the *N. lappaceum* was carried out as described earlier. The extraction yields of aqueous and ethanol are given in Table 1. Extraction with ethanol displayed a higher yield, while fruit pulp followed by the seed, rind and finally the leaves displayed the highest solids extracted in the ethanolic extraction. Aqueous extraction, however, saw fruit pulp followed by rind, leaves and finally the seed.

Supercritical fluid extraction (SFE) was carried out on the rind only and the yield of dry matter was 0.42%. The residue after SFE when re-extracted with ethanol gave a yield of 16.3% of dry matter, very similar to the yield when direct extraction with ethanol was performed (see Table 1). Therefore, SFE prior to ethanolic extraction does not increase yield any further. As such the simple and direct extraction with ethanol is recommended to process the extracts.

3.2. Antioxidant assays

3.2.1. Scavenging activity onto DPPH radicals

All extracts were evaluated for their DPPH-scavenging ability and their activity is shown in Fig. 1. As observed in Fig. 1, the rind and leaves of *N. lappaceum* displayed the highest DPPH radical-scavenging ability, with the ethanolic rind extracts having the highest $1/IC_{50}$ value. The activity exhibited by the ethanolic rind extracts is comparable to Vitamin C and far better than grape seed extracts. The SFE extracts of the rind of *N. lappaceum* did not exhibit any DPPH-scavenging activity (results not shown), however the residue when re-extracted with ethanol exhibited activity similar to ethanolic extracts. Further studies on the antioxidant ability of *N. lappaceum* were carried out using the rind and leaves only.

3.2.2. Scavenging activity onto galvinoxyl and ABTS radicals

Rind and leave extracts were evaluated for its galvinoxyl and ABTS-scavenging ability and their activities are shown in Table 2. Both galvinoxyl and ABTS assays show that the ethanolic extracts of the rind of *N. lappaceum* have far better radical-scavenging activity than Vitamin C and grape seed.

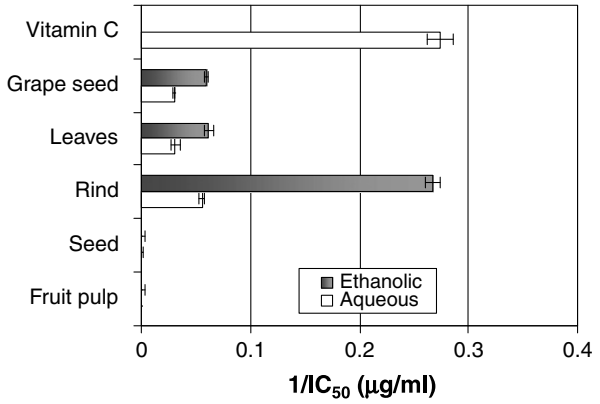

Fig. 1. DPPH-scavenging activity, expressed as $1/IC_{50}$, of the aqueous and ethanolic extracts of fruit pulp, seed, rind and leaf of *Nephelium lappaceum,* compared with grape seed and vitamin C.

Table 1
Yields of the aqueous and ethanolic extracts of the fruit pulp, seed, rind and leaves of *Nephelium lappaceum*

Extract	Extraction yield (%)[a]	
	Aqueous	Ethanolic
Fruit pulp	18.6 ± 0.2	22.2 ± 0.3
Seed	4.0 ± 0.1	20.0 ± 0.2
Rind	13.2 ± 0.2	17.8 ± 0.2
Leaf	10.2 ± 0.2	14.8 ± 0.1

[a] Values are the mean ± standard deviation ($n = 5$).

Table 2
Galvinoxyl- and ABTS-scavenging activity of the rind and leaf of *Nephelium lappaceum*

Extract	Galvinoxyl[a] (IC_{50}, μg/ml)		ABTS[a] (IC_{50}, μg/ml)	
	Aqueous	Ethanolic	Aqueous	Ethanolic
Rind	19.4 ± 0.3	1.7 ± 0.1	16.5 ± 0.2	1.7 ± 0.1
Leaf	26.1 ± 0.3	13.7 ± 0.2	24.5 ± 0.3	12.2 ± 0.2
Grape seed	21.2 ± 0.2	7.5 ± 0.3	32.8 ± 0.3	2.7 ± 0.1
Vitamin C	3.7 ± 0.1	Not relevant	7.7 ± 0.1	Not relevant

[a] Values are the mean ± standard deviation ($n = 5$).

3.2.3. Scavenging activity onto superoxide anions and inhibition of induced lipid autooxidation

Rind and leave extracts were evaluated for their scavenging activity onto superoxide anions and inhibition of induced lipid autooxidation and their activities are shown in Tables 3 and 4. It was observed that at the concentrations tested (50 μg/ml), the aqueous and ethanolic extracts of the rind of *N. lappaceum* exhibited similar scavenging ability for superoxides as superoxide dismutase (5 μg/ml) and aqueous grape seed extracts. Concentrations of rind and leaf of *N. lappaceum* and grape seed tested had the following IC_{50} values; aqueous rind 0.4 mg/ml; ethanolic rind 0.1 mg/ml; aqueous leaf 0.7 mg/ml; ethanolic leaf 0.3 mg/ml; aqueous grape seed 0.7 mg/ml; ethanolic grape seed 0.3 mg/ml.

The inhibition of induced lipid autooxidation by the aqueous and ethanolic extracts of rind and leaf of *N. lappaceum* at various concentrations is shown in Table 4. Aqueous extracts of both the rind and leaf managed to protect lipid autooxidation by about 50% at the very low concentrations of 0.4 mg/ml and 0.7 mg/ml, respectively. The concentration of the ethanolic extracts of rind tested was 0.1 mg/ml, which may be too low for effective protection against lipid autooxidation.

3.3. Pro-oxidant assay

A pro-oxidant is defined as a substance that can produce oxygen byproducts of metabolism, which can cause damage to cells. It is known that vitamin C at higher concentrations tends to behave like a pro-oxidant. The interaction of vitamin C with 'free', catalytically active metal ions could contribute to oxidative damage, through the production of hydroxyl and alkoxyl radicals. However it is uncertain whether these mechanisms occur *in vivo*. We have evaluated the rind and leaf of *N. lappaceum* for its pro-oxidant capability and compared it against vitamin C, grape seed and Emblica™, a commercially available plant extract used in cosmetics and as a nutraceutical, known for its very low pro-oxidant capacity (see Fig. 2).

Vitamin C showed the highest pro-oxidant activity, induced by transition metals. Among the positive controls Emblica™, followed by tocotrienol, green tea (results not shown) and finally α-tocopherol showed lower pro-oxidant capacity. Aqueous rind of *N. lappaceum* exhibited the lowest pro-oxidant capacity, even lower than Emblica™ while the ethanolic rind extract had lower pro-oxidant capacity than vitamin C, tocotrienol and ethanolic grape seed extract. The ethanolic rind extract was seen to have comparable pro-oxidant profiles to aqueous grape seed extract, green tea and tocotrienol.

Table 3
Superoxide-scavenging ability of the aqueous and ethanolic extracts of the rind and leaf of *Nephelium lappaceum*

Extracts	Superoxide scavenging activity (%)[a]	
	Aqueous	Ethanol
Rind	47.5 ± 0.5	41.4 ± 0.5
Leaf	28.2 ± 0.3	6.6 ± 0.2
Grape seed	41.1 ± 0.4	21.4 ± 0.2
Superoxide dismutase (5 μg/ml)	46.9 ± 0.5	NA

Concentration of extracts in reaction mixture was 50 μg/ml.
[a] Values are the mean ± standard deviation ($n = 5$).

Table 4
Inhibition of induced lipid autoxidation of the aqueous and ethanolic extracts of rind and leaf of *Nephelium lappaceum*

Extracts	Inhibition of autoxidation[a] (concentration used in parentheses, mg/ml)	
	Aqueous	Ethanol
Rind	49.5 ± 0.5 (0.4)	0 ± 0.0 (0.1)
Leaf	50.5 ± 0.5 (0.7)	4.5 ± 0.2 (0.3)
Grape seed	15.3 ± 0.4 (0.7)	11.6 ± 0.3 (0.3)
BHT	NA	80.0 ± 0.7
Tocopherol	NA	73.0 ± 0.5

BHT and tocopherol used at 1% concentration.
[a] Values are the mean ± standard deviation ($n = 5$).

3.4. Heavy metal content

Lead, arsenic and mercury contents of powdered *N. lappaceum* were determined against international standards, using the ICP-OES, for the determination of arsenic and lead, and AAS for the determination of mercury. The levels of these heavy metals in the rind and leaf of the powdered *N. lappaceum* were far below the maximum permissible levels for nutraceuticals (see Table 5).

3.5. Total phenolic content

The total phenolic content of the extracts were determined using the Folin-Ciocalteu method. Although the total phenolic content of the ethanolic extracts of the rind of *N. lappaceum* is comparable to grape seed ethanol extracts, its DPPH-scavenging activity is far higher than that of grape seed (see Fig. 3). It has been shown that phenolic content tends to correspond with scavenging activity (Auger et al., 2004; Bagchi et al., 2006), however this does not hold true when comparing with the commercial grape seed extract. On the other hand, the rind and leaf extracts of *N. lappaceum,* having lower total phenolic content, are seen to have correspondingly lower scavenging activity.

3.6. Measurement of cell death by apoptosis in cells cultured in the presence of N. lappaceum extracts

Confluent 3T3 and 4T1 cells were incubated in the presence and absence of aqueous and ethanolic extracts of the rind of *N. lappaceum.* The enrichment factor, which refers to the amount of mono- and oligonucleosomes in the cytoplasm of the apoptotic cells is presented in Fig. 4a and b. Apoptosis levels in cells treated with *N. lappaceum* and grape seed extracts were lower compared to the cells

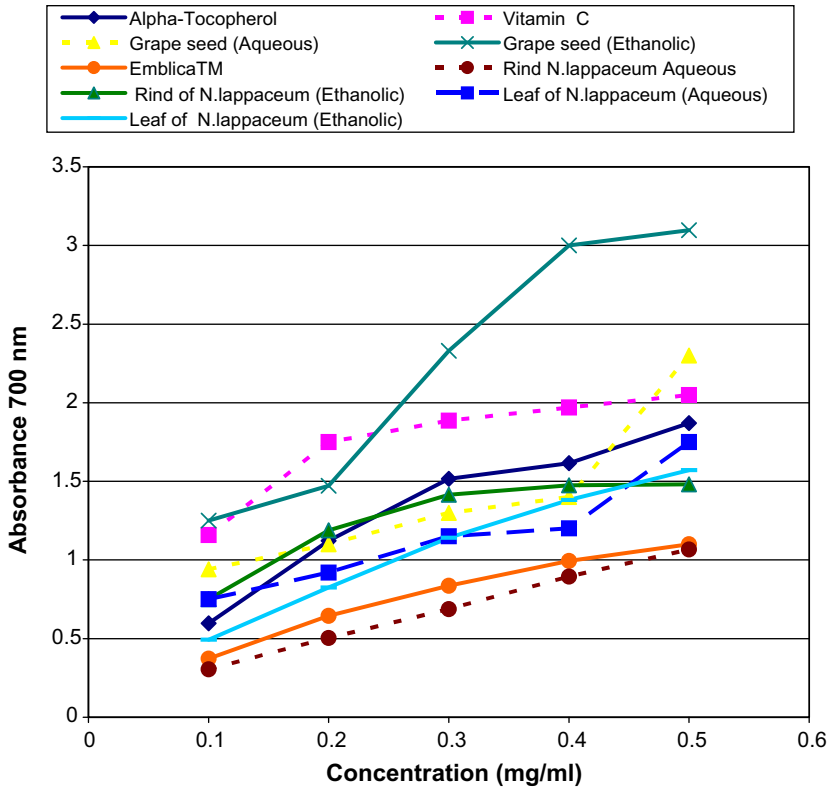

Fig. 2. Pro-oxidant capacity of the rind and leaf of *Nephelium lappaceum,* compared with vitamin C, α-tocopherol, grape seed and Emblica™, over a range of concentrations.

Table 5
Lead, arsenic and mercury determination of the rind and leaf of powdered *N. lappaceum*

Heavy metal[a] (mg/kg)	Rind of *N. lappaceum*	Leaf of *N. lappaceum*
Lead (Pb)	0.36 ± 0.03	0.15 ± 0.01
Arsenic (As)	<0.01	<0.01
Mercury (Hg)	<0.02	<0.02

[a] Values are the mean ± standard deviation ($n = 5$).

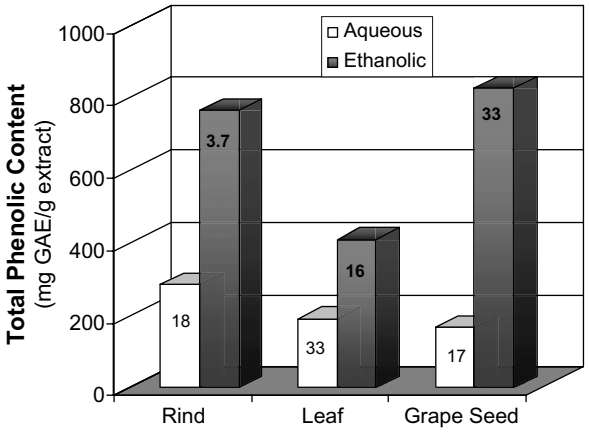

Fig. 3. Total phenolic content and DPPH-scavenging activity (IC_{50} as data labels in μg/ml) of extracts, compared with grape seed.

cultured in the absence of plant extracts in both 3T3 and 4T1 cells, at the concentration tested (100 μg/ml). The aqueous and ethanolic extracts of the rind of *N. lappaceum* appear to protect 3T3 cells from cell death due to apoptosis by about 60%. A similar protection from cell death due to apoptosis was observed in 4T1 cells. However, the level of protection of 4T1 cells from apoptotic death by *N. lappaceum* extracts appear to be lower than that observed for normal 3T3 cells. The lower levels of apoptosis in 4T1 cells may be due to the defective apoptotic pathways in tumour cells where there is an over-expression of the inhibitor of apoptotic proteins, which suppresses the activity of apoptotic activators (Yang et al., 2003). Therefore, owing to such low levels of apoptosis in untreated cancer cells, it may not be possible to find a significant difference in the apoptotic rate between treated and untreated cancer cells.

3.7. Apoptosis levels in cells exposed to oxidants in the presence of N. lappaceum extracts

This study suggests that reactive oxygen species (ROS), such as DPPH, can induce significant apoptotic cell death. The rate of apoptotic cell death due to the free radical DPPH was observed to increase significantly in both normal 3T3 mouse fibroblast and murine mammary cancer cells (4T1). The rate of apoptotic death in 3T3 cell lines was markedly reduced following the addition of 100 μg/

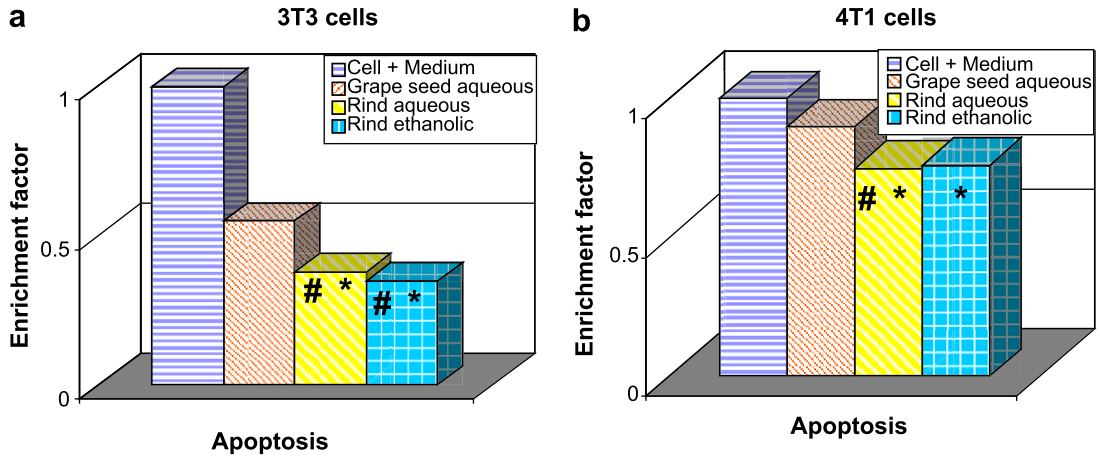

Fig. 4. The effect of aqueous and ethanolic extracts (100 μg/ml) of the rind of *Nephelium lappaceum* on 3T3 cells (a) and 4T1 cells (b) without the addition of oxidants. * Designates a significant difference from cell ($p < 0.05$). # Designates a significant difference from grape seed ($p < 0.05$).

ml *N. lappaceum* extracts when 5 μM and 50 μM DPPH was used (see Fig. 5a and b).

The extracts may work in two ways, firstly as a direct free radical scavenger of the oxidant DPPH, which can reduce the activation of the apoptotic pathway (Miller & Lefkowitz, 2001), or secondly the extracts can increase the levels of physiological antioxidants in these cells.

When using 5 μM DPPH, *N. lappaceum* extracts at the concentration tested were able to limit oxidant-induced cell death by apoptosis to a similar degree to grape seed

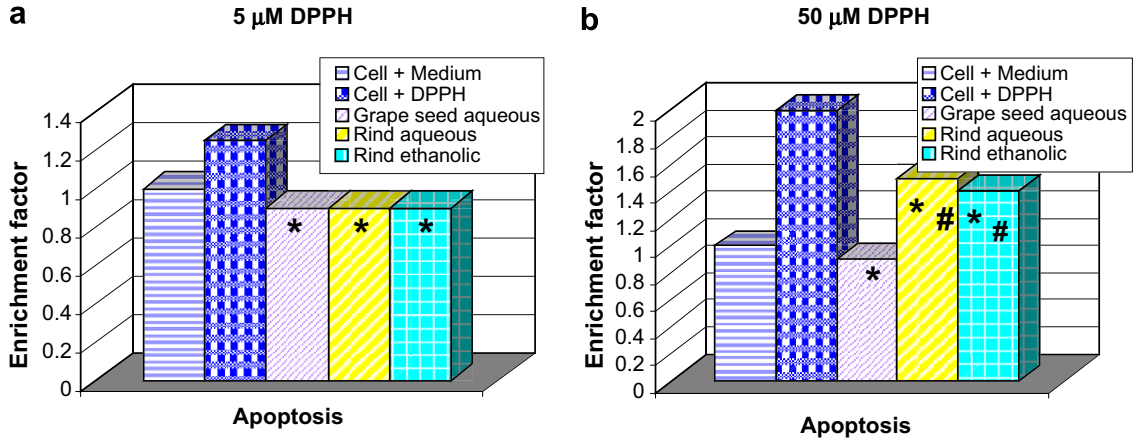

Fig. 5. The effect of aqueous and ethanolic extracts of the rind of *Nephelium lappaceum* on 3T3 cells with the addition of 5 μM (a) and 50 μM (b) DPPH. * Designates a significant difference from cell alone ($p < 0.05$). # Designates a significant difference from grape seed ($p < 0.05$).

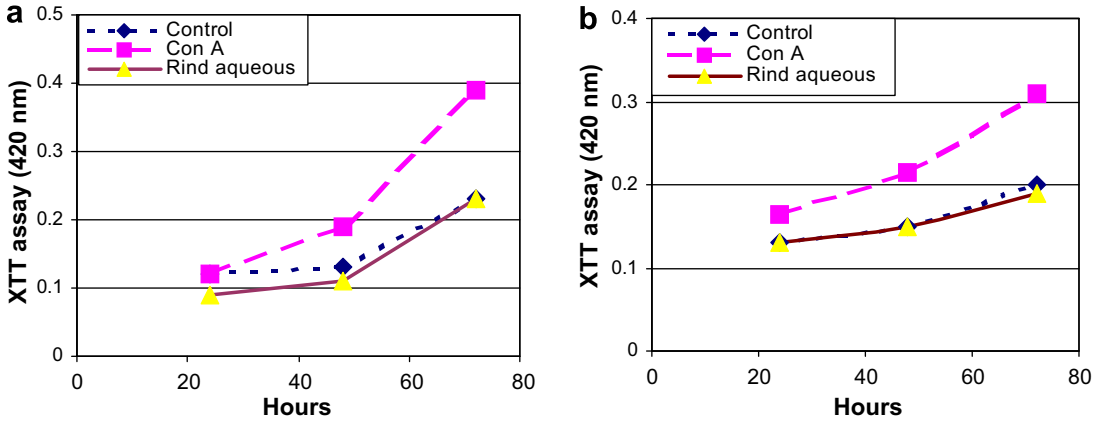

Fig. 6. Proliferation curves for murine splenocytes cells cultured in 125 μg/ml (a) aqueous and (b) ethanol extracts of the rind of *Nephelium lappaceum*. Con A: concanavalin A.

extract. However, at 50 μM DPPH, grape seed extract was seen to provide a more significant protection against apoptosis. The grape seed extract used in this study is from a commercial source and may contain a higher concentration of active compounds, compared to the *N. lappaceum* extracts used here.

3.8. Effect of culturing murine splenocytes in the presence of N. lappaceum extracts

Splenocytes obtained from BALB/c mice, were cultured in the presence of aqueous and ethanol extracts from the rind of *N. lappaceum* for either 24 h, 48 h or 72 h in a humidified 5% CO_2 incubator. At the end of each incubation period, cell proliferation was determined and its findings are depicted in Fig. 6a and b. Proliferation observed when the splenocytes were cultured in the presence of concanavalin A was used as positive control, while splenocytes cultured in medium without addition of plant extracts served as the negative control. At the concentrations (10 μg/ml, 50 μg/ml, 100 μg/ml and 125 μg/ml) studied, the extracts did not affect the splenocytes proliferation over a period of 72 h.

4. Conclusion

The ethanolic extract of the rind of *N. lappaceum* has a high free radical-scavenging activity, comparable to that of vitamin C and much higher than that of grape seed. In addition, it has been shown to have superoxide-scavenging ability of 30%, in contrast to SOD (superoxide dismutase), which has a 50% scavenging ability. The aqueous extract also reduces autoxidation of linoleic acid by 50%. It is an ideal antioxidant, as it has no pro-oxidant activity induced by transition metals, unlike vitamin C at higher concentrations. The ethanolic rind extract has a phenolic content in the range of 762–822 mg/g GAE. In addition, when using cultured cells, the extract was seen to possess a significant amount of antioxidative property and limits cell death by apoptosis. More importantly, rambutan extracts did not show any inhibitory effects on cultured normal mouse fibroblast cells or splenocytes, suggesting that the extract does not contain compounds that are cytotoxic to normal cells.

Acknowledgments

This work was supported by the Grant No. 01-01-01-0083 EA001 of the Ministry of Science, Technology and Innovation, Malaysia. The authors would like to thank Ms Jamuna A. for her help in preparing this manuscript.

References

Auger, C., Gerain, P., Laurent-Bichon, F., Portet, K., Bornet, A., Caporiccio, B., et al. (2004). Phenolics from commercialised grape extracts prevent early atherosclerotic lesions in hamsters by mechanisms other than antioxidant effect. *Journal of Agricultural and Food chemistry, 52*(16), 5297–5302.

Bagchi, D., Roy, S., Patel, V., He, G., Khanna, S., Ojha, N., et al. (2006). Safety and whole-body antioxidant potential of a novel anthocyanin-rich formulation of edible berries. *Molecular and Cellular Biochemistry, 281*(1-2), 197–209.

Bing, T., & Yuejin, H. (2005). Concentration-dependance of prooxidant and antioxidant effects of aloin and aloe-emodin on DNA. *Food Chemistry, 91*, 413–418.

Chang, C. W., Kim, S. C., Hwang, S. S., Choi, B. K., Ann, H. J., & Lee, M. Y. (2002). Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay guided comparison. *Plant Science, 63*(6), 1161–1168.

Chang, W. S., Lin, C. C., Chuang, S. C., & Chiang, H. C. (1996). Superoxide anion scavenging effects of coumarins. *American Journal of Chinese Medicine, 24*(1), 11–17.

Clarkson, P. M., & Thompson, H. S. (2000). Antioxidants: What role do they play in physical activity and health? *American Journal of Clinical Nutrition, 72*(2), 637–646.

Hwang, I. K., Yoo, K.-Y., Kim, D. S., Jeong, Y.-K., Kim, J. D., Shin, H.-K., et al. (2004). Neuroprotective effects of grape seed extract on neuronal injury by inhibiting DNA damage in the gerbil hippocampus after transient forebrain ischemia. *Life Sciences, 75*(16), 1989–2001.

Jacobs, B. P., Dennehy, C., Ramirez, G., Sapp, J., & Lawrence, C. A. (2002). Milk thistle for the treatment of liver disease: A systematic review and meta-analysis. *American Journal of Medicine, 113*, 506–515.

Krinsky, N. I., & Johnson, E. J. (2005). Carotenoids actions and their realation to health and disease. *Molecular Aspects of Medicine, 26*(6), 459–516.

John, S., Jianmei, Y., Joseph, E. P., & Yukio, K. (2003). Polyphenolics in grape seeds-biochemistry and functionality. *J. Medicinal Food, 6*(4), 291–299.

Lee, J. C., & Lim, K. T. (2000). Effects of cactus and ginger extracts as dietary antioxidants on reactive oxidant and plasma lipid level. *Food Science and Biotechnology, 9*(2), 83–88.

Marisa, M. W. (2006). Ascorbic acid and mineral composition of longan (*Dimocarpus longan*), lychee (*Litchi chinensis*) and rambutan (*Nephelium lappaceum*) cultivars grown in Hawaii. *Journal of Food Composition & Analysis, 19*, 655–663.

Miliauskas, G., Venskutonis, P. R., & van Beek, T. A. (2004). Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food Chemistry, 85*(2), 231–237.

Miller, W. E., & Lefkowitz, R. J. (2001). Arrestins as signaling molecules involved in apoptotic pathways: A real eye opener. *Science's STKE, 69*, 1–10.

Ong, P. K. C., Acree, T. E., & Lavin, E. H. (1998). Characterization of Volatiles in Rambutan Fruit (*Nephelium lappaceum* L.). *Journal of Agricultural Food Chemistry, 46*, 611–615.

Orner, G. A., Dashwood, W.-M., & Dashwood, R. H. (2004). Free radicals: The pros and cons of antioxidants. tumor-suppressing effects of antioxidants from tea. *Journal of Nutrition, 134*, 3177S–3178S.

Peschel, W., Sánchez-Rabaneda, F., Diekmann, W., Plescher, A., Gartzía, I., Jiménez, D., et al. (2006). An industrial approach in the search of natural antioxidants from vegetable and fruit wastes. *Food Chemistry, 97*, 137–150.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26*, 1231–1237.

Rosenblat, M., Hayek, T., & Aviram, M. (2006). Anti-oxidative effects of pomegranate juice (PJ) consumption by diabetic patients on serum and on macrophages. *Atherosclerosis, 187*(2), 363–371.

Scheibmeir, H. D., Christensen, K., Whitaker, S. H., Jegaethesan, J., Clancy, R., & Pierce, J. D. (2005). A review of free radicals and antioxidants for critical care nurses. *Intensive and Critical Care Nursing, 21*, 24–28.

Shi, H., Noguchi, N., & Niki, E. (2001). Galvinoxyl method for standardizing electron and proton donation activity. *Methods in Enzymology, 335*, 155–166.

Valentová, K., Ulrichová, J., Cvak, L., & Šimánek, V. (2006). Cytoprotective effect of a bilberry extract against oxidative damage of rat hepatocytes. *Food Chemistry, 101*(3), 912–917.

Yang, L., Mashima, T., Sato, S., Mochizuki, M., Sakamoto, H., Yamori, T., et al. (2003). Predominant suppression of apoptosome by inhibitor of apoptosis protein in non-small cell lung cancer H460 cells: Therapeutic effect of a novel polyarginine-conjugated Smac peptide. *Cancer Research, 63*(4), 831–837.