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RESEARCH ARTICLE

Biological activity assessment and phenolic compounds characterization from the fruit pericarp of *Litchi chinensis* for cosmetic applications

Mayuree Kanlayavattanakul[1], Dusadee Ospondpant[1], Uracha Ruktanonchai[2], and Nattaya Lourith[1]

[1]*School of Cosmetic Science, Mae Fah Luang University, Chiang Rai, Thailand and* [2]*National Nanotechnology Center, Pathumthani, Thailand*

Abstract

Context: *Litchi chinensis* Sonn. (Spindaceae) is an important economic fruit of Thailand. Therapeutic effects of the fruits are contributed by anti-inflammatory phenolics.

Objective: To extract the litchi fruit pericarp in order to identify biologically actives substances with potential for cosmetic application.

Materials and methods: The litchi pericarp was macerated by 70% ethanol (EtOH) and partitioned using *n*-hexane and ethyl acetate (EtOAc). *In vitro* antioxidant activities were assessed by 1, 1-diphenyl-2-picrylhydrazyl (DPPH), ABTS and ferric reducing ability of plasma (FRAP) assays including tyrosinase inhibitory effect. Cellular radical scavenging capacity was monitored in a normal human fibroblast cell culture (NHF). Total phenolic content was determined and characterized by HPLC.

Results: The EtOAc fraction was a significant antioxidant, stronger than ascorbic acid ($p < 0.01$), as assessed by ABTS (IC_{50} = 7.137 ± 0.021 μg/mL), DPPH (IC_{50} = 2.288 ± 0.063 μg/mL) and FRAP ($EC_{1mMFeSO4}$ = 8013.183 ± 58.804 μg/mL) assays. It demonstrated an antityrosinase effect (IC_{50} = 197.860 ± 1.230 μg/mL) and showed no cytotoxic activity toward Vero and NHF cells, at a maximum tested concentration (50 μg/mL), with cellular antioxidant activity. Total phenolic content was highest in the most potent antioxidant fraction. Quercetin, rosmarinic and gallic acids were found. Total phenolic content is highly related to FRAP, antityrosinase, and ABTS activities.

Discussion and conclusion: Pericarp from litchi fruit can be obtained abundantly from agricultural waste, and the strong antioxidant activity demonstrated in this report may have application in topical cosmetic products. This ecological antioxidant can be prepared using a feasible method resulting in less waste and increased agro-industrial profitability.

Keywords: Antioxidant, cellular antioxidant, tyrosinase inhibitor, cosmetics

Introduction

Free radicals, such as reactive oxygen species and reactive nitrogen species, are normal cellular metabolism products. Overproduction and accumulation of free radicals, combined with deficiencies in enzymatic and non-enzymatic self-defense antioxidants, negatively affect biological systems (Wickens, 2001). Cellular oxidative damage is encountered in many diseases, including cancer (Paz-Elizur et al., 2008), Alzheimer's (Moreira et al., 2005), aging (Wickens, 2001), inflammation (Beal, 2003), diabetes (Naito et al., 2006), Parkinson's (Beal, 2003) and AIDS (Sepulveda & Watson, 2002). Therefore, antioxidants are important for preventing the negative effects of radicals. They are capable of slowing down or preventing the oxidation of other molecules. Well-known natural antioxidants, such as vitamin C and E and plant phenolic compounds and flavonoids (Kroon & Williamson, 1999; Shahidi & Chandrasekara, 2010), are gaining much interest. There have been several studies showing that they may be important for the promotion of health.

Address for Correspondence: Mayuree Kanlayavattanakul, School of Cosmetic Science, Mae Fah Luang University, Chiang Rai 57100, Thailand. Tel.: +66-53-916-832, Fax: +66-53-916-831. E-mail: mayuree@mfu.ac.th

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Litchi [*Litchi chinensis* Sonn. (Sapindaceae)] is a fruit with a characteristic flavor and taste that, based on its biological profile, has many health benefits. It could be useful for weight control, by controlling blood glucose, and for lowering cholesterol, triglycerides, free fatty acids and insulin, which is promising for the treatment of diabetes. It also has an anti-inflammatory effect. Furthermore, its polysaccharides were potentially scavenged free radical in a dose-dependent manner (Devalarja et al., 2011), in addition to its phenolic constituents that strongly contribute to obesity-associated metabolic syndrome. The leading litchi fruit extract is prepared and commercialized in Japan (Sakurai et al., 2008).

Economically, litchi is an important fruit for Thailand and has been widely cultivated in the Northern provinces, in particular Chiang Rai. Litchi is exported as fresh fruit, canned fruit in syrup, juice, and dried fruit. The amount of fruit processed for exportation tends to increase every year. Consequently, waste from this process, mainly the pericarp and seed, is increasing and leading to environmental problems since it is easily microbial deteriorated (Schieber et al., 2001). Integration of the ecological concerns and the cosmetic consumer's need for organic and/or green cosmetics, challenges us to find a healthcare application for the fruit residue. Litchi fruit pericarp exhibited greater biological activity than the seed (Huang et al., 2010). However, cellular antioxidant activity of litchi cultivated in Chiang Rai has never been reported and, in particular, never for the Emperor cultivar, which has a characteristically giant fruit size and a sophisticated taste and flavor. Therefore, the litchi fruit pericarp was a subject to study. The extraction method was modified to be economically feasible. Antioxidant capability was evaluated by means of DPPH, ABTS and FRAP assays in parallel with cellular antioxidative prevention and oxidative-damage treatment. Melanogenesis, causing plant browning and human skin darkening, was evaluated by the tyrosinase inhibitory effect. The sun protection efficacy of the extracts was also studied to determine if the fractions had multiple functions that might broaden their application. To standardize the fractions, the total phenolic content was determined and the phenolics were characterized by means of HPLC. Biological activities were correlated with the active principles and the phenolics to offer the accurate and concise quality control of litchi fruit pericarp fractions.

Materials and methods

Litchi fruit pericarp extraction

Litchi of the Emperor cultivar was purchased from a local market located in Chiang Rai during December 2009. This plant material was identified by botanist Dr. Nijsiri Ruangrungsri, Faculty of Pharmaceutical Sciences, Department of Pharmacognosy, Chulalongkorn University, Bangkok, Thailand. The voucher specimen (MKLC 0811) was deposited for further reference at our laboratory herbarium at Mae Fah University, Chiang Rai. Those with blemishes or evidence of disease were discarded. Prior to separation of the pericarp, and seed from the fruit pulp, the fruit was washed in tap water and wiped dry. The pericarp was oven dried (at 50°C) and ground into powder. All of solvents were from Sigma-Aldrich (MO, USA). The extraction procedure was modified from Lourith et al. (2009). Litchi fruit pericarp powder (36.717 g) was extracted with 70% ethanol (EtOH) (250 mL) and vigorous shaking for 20 min. The extraction was repeated until the extract was colorless. The resulting extracted solutions were combined and concentrated to dryness under a reduced pressure, and at 40°C. The 70% EtOH extract was reconstituted (50 mL) and partitioned with *n*-hexane (150 mL × 3). The organic layers were combined, washed by saturated brine and concentrated to dryness, yielding the *n*-hexane fraction. The aqueous phase was further extracted with ethyl acetate (EtOAc) (150 mL × 3). The work-up procedure was done as usual, giving an EtOAc fraction. The remaining aqueous phase was then lyophilized, resulting in an aqueous (Aq.) fraction.

DPPH radical scavenging activity

Antioxidant activity was assessed by means of the DPPH assay (Lourith et al., 2009). Samples and an ascorbic acid standard (Fluka, MO, USA) were prepared in absolute EtOH (Merck, Darmstadt, Germany) at concentrations of 1.0–10.0 μg/mL and 1.0–8.0 μg/mL, respectively, and a calibration curve was generated ($r > 0.999$). A portion of the test sample was mixed with DPPH, purchased from Fluka, at a concentration of 6×10^{-5} M in absolute EtOH and reacted for 30 min under ambient conditions in a light protection vessel. A microplate reader (ASYS/UVM340, UK) was used to monitor the reduction of $DPPH^{\cdot}$ at 517 nm. The concentration resulting in 50% inhibition (IC_{50}) of $DPPH^{\cdot}$ was compared with the standard. All measurements were done in triplicate.

ABTS radical scavenging activity

The ability of the extracts to scavenge 2,2′–azino-bis(3-ethylbenzothaiazoline)-6-sulfonic acid radicals or $ABTS^{\cdot+}$ was determined (Pongpunyayuen & Lourith, 2011) in parallel with the DPPH assay. The stock solution containing 7 mM of ABTS (Fluka) and 2.450 mM potassium persulfate (Fluka) was incubated under ambient temperature for 16 h in a light protection vessel. Before use, the solution was diluted in EtOH to obtain an absorbance of 0.700 ± 0.200 at 750 nm with the microplate reader. The samples (1–100 μg/mL, 20 μL) were mixed with the ABTS solution (180 μL) for 5 min prior to absorbance measurement at 750 nm. The ability to scavenge $ABTS^{\cdot+}$ then was calculated. The IC_{50} of a reference compound, ascorbic acid, was used for comparison to the IC_{50} value of the extracts. The measurements were done in triplicate.

FRAP

The reducing power of the litchi fruit pericarp fractions was examined using a modified FRAP method

(Tachakittirungrod et al., 2007). FRAP reagent was prepared in a 2,4,6-tri(2-pyridyl)-*S*-triazine (TPTZ) solution (10 mM, 2.5 mL) purchased from Fluka in a solution containing 40 mM HCl, $FeCl_3$ (20 mM, 2.5 mL) and acetate buffer, pH 3.6 (0.3 M, 25 mL), that were supplied by Fluka. The test samples (3 μg/mL, 20 μL) in EtOH were reacted with the FRAP reagent (180 μL). The absorbances were recorded at 595 nm using a microplate reader. $FeSO_4$ (Fluka), at known concentrations (50–500 μM), was used for the calibration curve. The reducing power was expressed as an equivalent concentration (EC) to that of 1 mM $FeSO_4$ and ascorbic acid was used as the positive control. The reducing power was determined in triplicate.

Tyrosinase inhibitory effect

The tyrosinase inhibitory activity of the fractions was determined using a modified dopachrome method with L-DOPA (Aldrich) as the substrate (Kanlayavattanakul & Lourith, 2010). All samples were prepared at different concentrations using absolute EtOH. Forty μl of each sample was added to 80 μL of 20 mM phosphate buffer (pH 6.8) supplied by Ajax Finechem (New South Wales, Australia) and 40 μL of mushroom tyrosinase (240 unit/mL, Aldrich) and pre-incubated at 25°C for 10 min. L-DOPA (0.85 mM, 40 μL) was then added and the reaction was incubated at 25°C for another 20 min. There was a corresponding blank for each sample and this blank contained all components except for the mushroom tyrosinase. The absorbance was measured at 490 nm, using kojic acid (Fluka) as a positive control. The enzyme deactivation efficacy of each fraction was screened preliminary at 1000 μg/mL. Each assay was performed in triplicate and the IC_{50} of the fraction containing the most ability to inhibit tyrosinase was further evaluated.

Cytotoxicity against Vero cells

Cytotoxicity against Vero cells was analyzed using the Green Fluorescent Protein detection method, and comparing it to elliptine (Iwase & Tsutsui, 2007).

Cytotoxicity against a normal human fibroblast (NHF) cell culture

Cytotoxicity in NHF cells was determined using a method described by Dash et al. (2008). NHF cell lines obtained from the National Nanotechnology Center were incubated at 37°C in a chamber containing 5% CO_2. The cells were grown in Dulbecco's modified Eagle's medium (Gibco, NY, USA) supplemented with 10% fetal bovine serum (Gibco), 1% L-glutamine (Gibco) and 1% penicillin G/streptomycin (Gibco). Cultured-cells at passage 11–16 were used in this study. Cytotoxicity assessments were performed using MTT (USB, OH, USA) or the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. NHF cells (8×10^3 cells/well) were seeded on a 96-well plate and incubated overnight at 37°C in an incubator containing 5% CO_2. The cells were treated with the fractions at concentrations from 0.1 to 50 μg/mL and incubated for another 24 h. The untreated cells were used as controls. Following incubation, media in each well was replaced with fresh media containing the MTT solution (5 mg/mL, 20 μL) and further incubated for 4 h. Thereafter, the media containing MTT was removed and 200 μL of DMSO (Sigma, MO, USA) was added to solubilise the formazan crystals formed in the viable cells. Absorbance was measured at 550 nm using the microplate reader. The extent of thiazolyl tetrazolium was implied for cell viability checking. Cell viability (%) was calculated and, those with a value greater than 75% of the control (100% cell viability) were interpreted as non-cytotoxic.

Cellular oxidative damage treatment

Damage to NHF cells was induced with H_2O_2 (Merck). NHF cells (8×10^3 cells/well) were seeded and incubated as usual. Cytotoxicity was induced by the addition of 50% H_2O_2 (1×10^{-3} to 1×10^3 μg/mL) and incubated for 24 h. Untreated cells were used as a control. The treated cells were then washed with MTT solution and the formazan crystals were dissolved in DMSO. Cell viability was then determined by the same microplate reader at 550 nm and the concentration of H_2O_2 that resulted in cell damage was obtained. This concentration was then applied to NHF cells (8×10^3 cells/well) for 24 h. After treatment, the cells were incubated with the extracts (5 and 10 μg/mL) for another 24 h. Determination of cell viability was conducted as described in cytotoxicity against a NHF cell culture section (Dash et al., 2008).

Prevention of cellular oxidation

The extracts (5 and 10 μg/mL) were mixed with H_2O_2 at the concentration that was determined to induce cellular toxicity. The incubation and treatment were performed using the standard protocol and cell viability measured as previously described (Dash et al., 2008).

UV protection efficiency

The extracts were monitored using a UV spectrophotometer (Libra S22, Biochrom, UK) in the range of 250–400 nm (Rancan et al., 2002) and these results were compared with the results from UV absorbers in commercialised sun protection cosmetics, benzophenone (BP-3) and octyl methoxycinnamate (OMC), supplied by Noveon (OH, USA).

Determination of total phenolic content

The total phenolic content in the peel extracts was determined as previously described (Lourith et al., 2009). Samples were mixed with Folin-Ciocalteu reagent (4 μL, each) purchased from Fluka in a 96-well plate, followed by the addition of Na_2CO_3 (7.5%, 80 μL) provided by Fluka. The volume of the mixture was adjusted to 200 μL with water. The solution was then incubated at ambient temperature for 1 h. Later, absorbencies were measured using the microplate reader at 750 nm with gallic acid (Fluka) as a standard. The total phenolic content in each extract was compared with the standard curve and

expressed as milligrams of gallic acid equivalents per 1 g of crude extract (g GAE/g crude). The procedure was repeated in triplicate.

Characterisation of phenolics

Phenolics in litchi peel extracts were characterised using HPLC (Waters 2695, Agilent, CA, USA) with a photodiode array detector (Waters 2996, Agilent) at 250 nm (Kanlayavattanakul & Lourith, 2010). Samples were successively separated on a reversed phase column (Alltech, Prevail C_{18} 5 μm, 250 × 4.6 mm) equipped with a guard column (Alltech, Prevail all-guard cartridge C_{18} 5 μm, 7.5 × 4.6 mm) and eluted with a solvent system consisting of AcCN (A) and 3% aq. AcOH (B) with the following gradient: 0–3 min 100% B, 3–5 min 85% B, 5–10 min 80% B, 10–15 min 75% B, 15–20 min 70% B, and 20–30 min 50% B at a flow rate of 1 mL/min. The HPLC components were controlled by Waters Empower II software (Agilent). All solvents and standards were of HPLC grade. Gallic acid, rosmarinic acid and quercetin (Fluka) concentrated at 1–500 μg/mL in AcCN (Labscan, Ireland), were used as standards. The calibration curves had linear correlation coefficients of more than 0.999 ($r > 0.999$). Characterization of phenolics in the fractions (1000 μg/mL) delineated by absorption wavelengths and retention times was done in three cycles. The analyzed content was calculated into g/kg crude.

Statistical analysis

Data were presented as the means ± SD and a one-way ANOVA test was used to evaluate the difference between groups using the program SPSS version 16.0. The level of significance was at $p < 0.05$.

Results and discussion

Litchi fruit pericarp was firstly extracted by 70% EtOH. The 70% EtOH extract was further extracted by liquid–liquid extraction with different solvents with increasing polarities to afford *n*-hexane, EtOAc and Aq. fractions, respectively. Antioxidant activity was first assessed using a single-electron transfer mechanism. $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ scavenging activities of the EtOAc fraction were found to be significantly superior ($p < 0.05$) to the Aq., 70% EtOH and *n*-hexane fractions, respectively (Table 1). Furthermore, this fraction was shown to have more potent antioxidant activity than ascorbic acid ($p < 0.05$). In addition, the electron donor efficiency of the extracts determined by the FRAP assay were consistent with the DPPH and ABTS assays. The FRAP reducing power indicates the potential of the litchi pericarp fractions to reduce the oxidized intermediates of the lipid peroxidation process. These natural antioxidant can function as primary and secondary antioxidants (Tachakittirungrod et al., 2007). Similar to the DPPH and ABTS assays, the antioxidant capability of the EtOAc fraction was superior to the ascorbic acid positive control. Therefore, litchi pericarp, particularly the EtOAc fraction, is a potential antioxidant candidate for skin wrinkle and aging treatments, as confirmed by three assays of free radical scavenging activity.

In an attempt to widen this potent antioxidant application, the skin-lightening efficacy of the fractions was evaluated *in vitro*, based on the relationship between antioxidant and antityrosinase activities (Kim & Uyama, 2005). A measurement of the inhibitory effect against tyrosinase, which is the key enzyme in melanin production, was conducted. The most potent antioxidant extract (EtOAc) was able to significantly ($p < 0.05$) deactivate the enzyme (Table 1). This finding is in agreement with a previous report (Kim & Uyama, 2005) confirming the relevance of antioxidant activities with skin-lightening effect, as the antioxidant efficacy of the litchi fruit pericarp fraction was consistent with the tyrosinase inhibitory effect. The EtOAc extract from litchi fruit pericarp was further evaluated by its IC_{50} against tyrosinase. Although this fraction showed the greatest antityrosinase activity when compared to the others, its efficiency was less than kojic acid, the tyrosinase inhibitor generally used in commercialised skin-lightening cosmetics. Therefore, further purification would enhance the enzyme deactivation capacity of this fraction. However, this fraction is adequate for skin-lightening applications when the degree of hyperpigmentation is low.

Before using the antioxidant activity recovered from the litchi residue in cosmetic applications, safety should be ensured. Cellular cytotoxicity in mammalian cells was preliminary evaluated in Vero cells, which are the recommended mammalian cell lines for toxicity testing (Eisenbrand et al., 2002). The antioxidant EtOAc fraction was non-cytotoxic at the maximum test concentration (50 μg/mL), which is much greater than its IC_{50}. Safety of

Table 1. Antioxidant and antityrosinase activities of litchi fruit pericarp fractions.*

	Antioxidant			Antityrosinase		
Sample	DPPH (IC_{50}, μg/mL)	ABTS (IC_{50}, μg/mL)	FRAP (EC)	At 1000 μg/mL (%)	IC_{50} (μg/mL)	Total phenolic content (mg GAE/g crude)
n-Hexane	90.374 ± 0.580	49.500 ± 0.287	693.503 ± 2.446	—	—	—
70% EtOH	8.980 ± 0.110	17.950 ± 0.054	1285.311 ± 50.926	27.525 ± 0.437	—	90.547 ± 3.603
EtOAc	2.288 ± 0.063	7.137 ± 0.021	8013.183 ± 58.804	89.740 ± 0.470	197.860 ± 1.230	357.891 ± 1.640
Aqueous	3.564 ± 0.025	14.131 ± 0.115	2099.812 ± 43.150	10.320 ± 0.310	—	94.227 ± 5.581
Ascorbic acid	3.404 ± 0.025	6.347± 0.087	6214.689 ± 28.249	—	—	—
Kojic acid	—	—	—	—	36.500 ± 0.600	—

*Data were presented as means ± SD.

this fraction in mammalian cells was further confirmed in NHF cells that biosynthesise collagen, protein responsible for skin elasticity. Collagen production is decreased with age, eventually resulting in skin wrinkles and aging. The litchi pericarp EtOAc fraction was non-cytotoxic in NHF cell lines in the range of concentrations tested (0.1–50 μg/mL).

Cellular antioxidant activity was studied further to broaden and confirm the potential health benefits of the extract. The ability to treat and to prevent oxidative cell damage was measured. H_2O_2 mediated NHF cell death at 3.8 pg/mL (Table 2). This concentration was used once again to measure cellular antioxidant activity and in the cytotoxicity treatment study. The EtOAc fraction increased the viability of NHF cell lines following H_2O_2-induced cell damage (Table 3). Following treatment with 5 and 10 μg/mL EtOAc fraction, NHF cell viability was 79.1 ± 14.0 and 80.0 ± 12.3%, respectively, that significantly higher than the positive control ($p < 0.05$). Additionally, the ability for the litchi fruit pericarp EtOAc fraction to prevent oxidative cell damage was assessed. Similar to the cytotoxic treatment capacity, the ability for the fraction to prevent oxidative damage was dose-dependent (Table 3). Viability of the NHF cell lines was significantly ($p < 0.05$) increased to 95.7 ± 4.4% with 5 μg/mL of the EtOAc fraction and enhanced to 97.8 ± 0.8% when the cells were treated with 10 μg/mL of the EtOAc fraction. From these antioxidant activities, it can be concluded that litchi fruit pericarp can be used as an efficient antioxidant promising for cosmetics.

To increase the fractions' potential in cosmetics, particularly the products relevant to anti-wrinkle, the ability for the extract to protect from the sun and its relevance to photoaging was confirmed in a comparison with two common commercially-available UVB (290–320 nm) and UVA (320–400 nm) absorbers, BP3 and OMC, as shown in Figure 1. Litchi fruit pericarp fractions, in particular the EtOAc extract, exhibited the greatest UV protection efficacy followed by Aq., *n*-hexane and 70% EtOH fractions, respectively. Thus, the antioxidant-rich EtOAc fraction of the litchi peel would be useful for its ability to protect against photooxidative damage by UVB (Saija et al., 2000).

The active molecules of the litchi fruit pericarp were further subjected to quality control, and standardisation. The total phenolic content was measured and was compared with the top three potent biologically active fractions. The content of phenolics is in agreement with the antioxidant and antityrosinase activities, as the EtOAc extract, which was the most potent fraction, contained the highest phenolic content (Table 1).

Analysis of phenolics in the fractions was further performed by means of HPLC. Hydroxycinnamic acids

Table 2. NHF cell damage induced by H_2O_2.*

	H_2O_2 concentration (μg/mL)										
	0.0001	0.0038	0.0153	0.0610	0.2441	0.9765	3.9062	15.6250	62.5000	250.0000	1000.0000
Cell viability (%)	61.3 ± 3.4	60.7 ± 6.1	53.9 ± 2.9	35.4 ± 0.8	22.4 ± 0.2	22.5 ± 0.1	20.7 ± 0.2	20.6 ± 0.2	21.1 ± 0.4	21.7 ± 0.4	22.1 ± 0.5

*Data are presented as means ± SD.

Table 3. Cellular antioxidant activity of litchi fruit pericarp EtOAc fraction.*

	Cellular oxidative treatment		Prevention of cellular oxidation	
Litchi fruit pericarp EtOAc fraction (μg/mL)	Cell viability (%)	*p*	Cell viability (%)	*p*
5	79.1 ± 14.0	0.031	95.7 ± 4.4	0.009
10	80.0 ± 12.3	0.006	97.8 ± 0.8	0.004

*Data are presented as means ± SD.

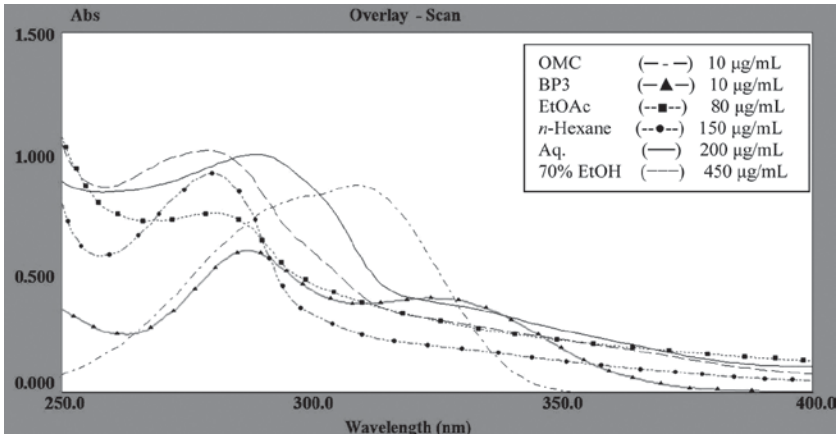

Figure 1. UV protection efficacy of litchi peels extracts and commercial sunscreen agents.

Table 4. Phenolics in litchi fruit pericarp fractions.*

	Phenolics (g/kg crude)		
Fraction	Gallic acid	Rosmarinic acid	Quercetin
70% EtOH	0.277 ± 0.008	0.002 ± 0.001	0.010 ± 0.000
EtOAc	0.224 ± 0.006	0.145 ± 0.040	0.150 ± 0.025
Aq.	0.794 ± 0.114	0.437 ± 0.152	0.836 ± 0.008

*Data are presented as means ± SD.

Table 5. Correlation between biological activities and actives.

	Relation coefficient (r)			
Assay	Total phenolic content	Gallic acid	Rosmarinic acid	Quercetin
DPPH	0.4310	0.0655	0.3870	0.2316
ABTS	0.8865	0.0623	0.0255	8×10^{-5}
FRAP	0.9902	0.2271	0.0071	0.0629
Antityrosinase	0.9526	0.5298	0.1535	0.2930

are becoming of interest as biologically active agents in healthcare products, including pharmaceutical and cosmetic products (Kroon & Williamson, 1999; Shahidi & Chandrasekara, 2010). Gallic acid (Zhang et al., 2000) and epicatechin (Zhao et al., 2006) are hydroxycinnamic derivatives reported in litchi fruit pericarp. However, another hydroxycinnamic acid of cosmetic important has sacredly been presented in the residue. In the present study, gallic and rosmarinic acids and quercetin were therefore characterized by means of HPLC. These biologically actives were detected (Table 4) in litchi pericarp by the developed extraction method described. These findings report rosmarinic acid and quercetin in litchi fruit pericarp for the first time.

Interestingly, the total phenolic content was highly related to the FRAP, antityrosinase and ABTS results as shown in Table 5. Determination of the active molecule is therefore economic and feasible in order to utilize the litchi peel extracts, in particular the EtOAc fraction, which is a non-cytotoxic and efficient antioxidant beneficial for human health.

Conclusions

The litchi pericarp EtOAc fraction was the most potent biologically active extract. Its antioxidant activity was significantly more potent than ascorbic acid as evaluated in ABTS, DPPH and FRAP assays. This recovered antioxidant from litchi non-edible part also exhibited *in vitro* tyrosinase inhibitory effect, although at a less capacity than the standard kojic acid. This biologically active fraction was non-cytotoxic in Vero and normal human fibroblast cells. In addition, it posed cellular antioxidant activity. The active principle was determined consequently. Total phenolic content was found highest in the EtOAc fraction of which HPLC analysis revealed that quercetin, gallic and rosmarinic acids were contained in litchi pericarp. Correlation between total phenolic content and activities was offered as a feasible method for standardization of litchi pericarp extract.

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Declaration of interest

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