COMPARISON THE BIOACTIVE COMPOUNDS AND THEIR ACTIVITIES BETWEEN LONGAN AND LITCHI SEEDS EXTRACTS

Pitikan Wisitsak[1]*, Junniphaphorn Nimkamnerd[1], Natthawut Thitipramote[1,2], Nisakorn Saewan[1,2], Phanuphong Chaiwut[1,2] and Punyawatt Pintathong[1,2]

[1]Excellent Center for Cosmetics and Wellness, Mae Fah Luang University, Chiang Rai, 57100, Thailand
[2]School of Cosmetic Science, Mae Fah Luang University, Chiang Rai, 57100, Thailand
*e-mail address: excellent_center@mfu.ac.th

Abstract

This study aimed to compare the bioactive compounds and their antioxidant and antimicrobial activities between longan (*Dimocarpus longan* Lour.) and litchi (*Litchi chinensis* Sonn.) seeds extracts. The longan seed (cultivar "*Edor*"; LS) and litchi seeds (cultivar "*Kim-Cheng*"; KC and "*Chakkrapat*"; CP) were extracted with 95% ethanol with ratio of 1:10 (sample: solvent, w/v) using shaking extraction at 150 rpm, room temperature for 4 hours. Extraction yield, bioactive compounds (extractable phenolic and flavonoid content), antioxidant activities including 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radicals scavenging assay, 2,2'-Azino-bis(3-ethylbenzothialine-6-sulfonic acid) (ABTS) radicals scavenging assay and linoleic acid peroxidation inhibition assays) and antimicrobial activities (inhibition of *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus*) of these LS, KC and CP extracts were investigated. The results showed that LS extract composed the high contents of protein and carbohydrate while both litchi seed (KC and CP) had high fiber and fat. The extraction yield, the total phenolic and flavonoid contents were found in LS extract (5.24% dry basis, 3.550 mg GAE/g DW and 0.268 mg QE/g DW, respectively) (p<0.05). Consistently, LS extract had high antioxidant activities (DPPH and ABTS radical scavenging) with IC_{50} values of 0.755 and 0.301 mg/ml, respectively. However, KC and CP litchi seed extracts showed high activity of lipid peroxidation inhibition (IC_{50} 9.925 and 10.205 mg/ml, respectively). Moreover, LS and KC extracts also had an antimicrobial activity against only the Gram positive strain, *S. aureus*. Nevertheless, longan and litchi seed extracts did not inhibit the Gram negative bacteria, *P. aeruginosa* and *E. coli*. This research demonstrated that longan and litchi seed extracts could be the alternative sources of natural antioxidant and antimicrobial substances.

Keywords: antimicrobial activity, antioxidant activity, bioactive compound, litchi seed extract, longan seed extract

Introduction

Antioxidant is a group of substances which prevents the oxidation reaction of free radicals (Prior *et al*. 2005). The synthetic antioxidant including butylated hydro-xyanisole (BHA) and butylated hydroxytoluene (BHT) are frequently used in food and cosmetic industry. However, BHA and BHT may have side-effect to the human health in the long term treatment (Moure et al. 2001). Therefore, the natural antioxidant becomes an alternative choice. Longan and litchi are classified in Sapindaceae family. Longan cultivar "*Edor*" and litchi cultivars

"*Kim-Cheng*" and "*Chakkrapat*" are the mostly cultivated and consumed in Northern Thailand including Chiang Rai province. In year 2011, the cultivated yield of longan and litchi were approximately 47,580 and 24,135 tons, respectively in Chiang Rai province. In fruits industrial processing and fresh consumption, these seeds become as the by-product (~10-15% w/w of whole fruit). Thus, the aim of this research was to compare the active compounds and their activities (antioxidant and antimicrobial) from longan and litchi seed extracts. Moreover, the utilization of fruit processing industry by-products as a material for value-added product preparation would be useful.

Methodology

Sample preparation
The longan (cultivar "*Edor*"; LS) and litchi (cultivar "*Kim-Cheng*"; KC and "*Chakkrapat"*; CP) fruits were collected from Chiang rai province. The seeds were separated, dried (60°C, 24 hours), blended to powder and kept at –20°C until used.

Extraction
The 10 g of dried seed powder was extracted with 100 ml of 95% ethanol with ratio of 1:10 (sample : solvent, w/v) by shaking extraction (at 150 rpm, room temperature for 4 hours). The supernatants were filtered through filter paper Whatman no. 1, vacuum evaporated and kept at -20°C until use.

Proximate analysis of longan and litchi seed
The seeds were ground using a blender. Moisture, ash, crude protein, crude oil, crude fiber and ash content were determined in accordance with the standard methods of AOAC (2000).

Determination of extraction yield
Extraction yield was calculated following the equivalent of %yield = (weight of extract/weight of dried sample)x100.

Determination of bioactive compounds
Extractable phenolic content (EPC) was carried out according to Folin-Ciocalteu's reagent method with slightly modifications from Singleton and Rossi (1965). The 20 µl of extracts (1 mg/ml) were mixed with 100 µl of Folin's reagent and incubated for a minute before added 80 µl of 7.5% (w/v) Na_2CO_3. The reaction was leave static for 30 min at an ambient temperature and measure at 765 nm. Extractable phenolic content was reported as mg of gallic acid/g of dried sample (mg GAE/g DW).

Flavonoid content was determined by aluminum chloride colorimetric method (Chang et al. 2002). The 25 µl of extracts (1 mg/ml) were mixed with 75 µl of 95% EtOH, 5 µl of 10% $AlCl_3$, 5 µl of 1 M KOAC and 140 µl of DI water. The mixture was incubated for 30 min at an ambient temperature and measure at 415 nm. Flavonoid content was reported as mg of quercetin/g of dried sample (mg QE/g DW).

Determination of antioxidant activities

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity
The $DPPH^{\bullet}$ scavenging activity of longan extract was assayed according to Thaipong et al. (2006) with some modifications. The 10 µl of extracts (1 mg/ml) were mixed with 190 µl of 0.1 mM $DPPH^{\bullet}$ solution. The mixture was incubated for 30 min in the dark chamber and

measured at 515 nm. The result expressed as the concentration of extract which inhibited $DPPH^{\bullet}$ by 50% (IC_{50}) (mg/ml).

2,2'-Azino-bis(3-ethylbenzothialine-6-sulfonic acid) (ABTS) scavenging activity
The $ABTS^{\bullet+}$ scavenging activity of longan extract was assayed by Thaipong et al. (2006). The 10 µl of extracts (1 mg/ml) were mixed with 190 µl of 7.0 mM $ABTS^{\bullet+}$ solution (ABTS solution was oxidized with 2.45 mM $K_2S_2O_8$ and incubated for 15h in the dark room). The mixture was incubated for 15 min in the dark chamber and measured at 734 nm. The result expressed as the concentration of extract which inhibited $ABTS^{\bullet+}$ by 50% (IC_{50}) (mg/ml).

Linoleic acid peroxidation inhibition activity
The linoleic acid peroxidation inhibition activity of seed extracts were carried out with slightly modifications (Choi et al. 2002). Linoleic acid emulsion was prepared with linoleic acid (0.3 g) and Tween 20 (0.3 g) in 50 ml of phosphate buffer (0.1 M, pH 7.0). A reaction solution containing 500 µl of linoleic acid emulsion, 30 µl of 0.1 M phosphate buffer, 10 µl of 20 mM ascorbic acid and 100 µl of extract. Added 10 µl of $Fe_2SO_4.7H_2O$ to start up the reaction and then incubated at 37°C for 30 min. Thereafter, 40% (w/v) TCA and 1% (w/v) TBA were added and heated at 95°C for 10 min. The mixture was centrifuged at 3,500 rpm for 5 min and measure at 532 nm. The result expressed as the concentration of extract which inhibited $LOO^{\bullet}$ by 50% (IC_{50}) (mg/ml).

Determination of antimicrobial activities

Bacterial culture and preparation of inoculum
Microorganisms used in this study consisted of three strains including *E. coli*, *S. aureus* and *P. aeruginosa*. All bacterial strains were prepared in Trypticase Soy Broth (TSB) at 37°C for overnight and the bacterial suspension was measured the optical density of 0.08-0.10 at 625 nm by spectrophotometer and obtained a final concentration approximately 10^6-10^8 cfu/ml.

Paper disc diffusion method
The screening of antimicrobial activities of the extracts was carried out by paper disc diffusion method by slightly modifications (Rangkadilok et al. 2012). The suspension was swabbed by using a sterile cotton wound on the surface of media (Trypticase Soy Agar; TSA). The seed extracts were dissolved in DMSO (1 mg/ml). A sterile paper disc (Whatman No.1 about 5 mm in diameter) was soaked with 5 µl of seed extract solution (500 µg/disc). Gentamicin (10 µg/disc) and DMSO used as the positive and negative control, respectively. The discs were placed on the agar surface and incubated at 35°C for 18-24h. Thereafter, the diameters of the inhibition zones were measured in millimeter.

Results

Proximate analysis of longan and litchi seeds
The chemical components of longan and litchi seed were shown in Table 1. LS seed contained the highest contents of protein and carbohydrate while the moisture fat and fiber contents showed the lowest value. Furthermore, both litchi seeds (KC and CP) had high fat and fiber.

Table 1 Proximate composition of longan (LS) and litchi (KC and CP) seeds (%)

Parameter	Longan seed (LS) (%)	Litchi seed	
		KC (%)	CP (%)
Moisture	7.40±0.27[b]	12.65±0.31[a]	12.29±0.11[a]
Ash	1.73±0.02[a]	1.53±0.07[b]	1.70±0.07[a]
Protein	7.17±0.12[a]	4.56±0.05[b]	3.98±0.06[c]
Fat	0.23±0.04[b]	0.40±0.03[a]	0.34±0.08[ab]
Fiber	7.89±0.09[b]	9.98±0.22[a]	8.32±0.32[b]
Carbohydrate	75.57±0.28[a]	70.88±0.45[c]	73.36±0.42[b]

Values (Mean ± S.D.; n=3) with in the roll followed by a difference superscript differ statistically (ANOVA; $p<0.05$)

Extraction yield and phenolic contents (extractable phenolic and flavonoid)

The extraction yield was higher in LS extract (5.24% dry basis) than these of KC extract (3.76% dry basis) and CP extract (3.26% dry basis), respectively (Figure 1). Consistently, LS extract showed the highest contents of phenolic and flavonoid (3.550 mg GAE/g DW and 0.268 mg QE/g DW, respectively) ($p<0.05$). Comparing between litchi cultivars, KC extract contained higher phenolic and flavonoid contents (2.729 mg GAE/g DW and 0.156 mg QE/g DW, respectively) than CP extract (1.982 mg GAE/g DW and 0.072 mg QE/g DW, respectively).

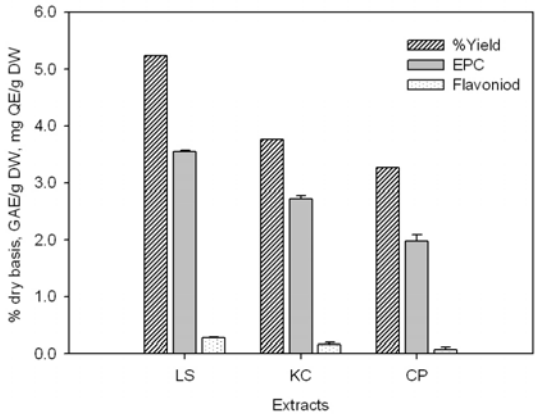

Figure 1 The extraction yield, extractable phenolic (EPC) and flavonoid content of longan (LS) and litchi (KC and CP) seed extracts

Antioxidant activities

DPPH radical scavenging activity

The results showed the DPPH radical scavenging activity of longan and litchi seed extracts (Table 2). LS extract exhibited the higher scavenging activity (IC_{50} 0.755 mg/ml) than those of KC and CP extracts (IC_{50} 0.809 and 0.963 mg/ml, respectively). However, the scavenging activity of LS extracts was 6 times less than trolox as a standard.

ABTS scavenging activity

The ABTS scavenging activity data showed accordingly with DPPH scavenge activity. LS extract was higher ABTS scavenging activity (IC_{50} 0.301 mg/ml) than KC and CP extracts (IC_{50} 0.320 and 0.444 mg/ml, respectively). The scavenging activity of trolox was 2.2 times higher than LS extracts.

Linoleic acid peroxidation inhibition activity

Both litchi seed extracts (KC and CP) had high activity of lipid peroxidation inhibition (IC_{50} 9.925 and 10.205 mg/ml, respectively) that were greater than LS extract (IC_{50}= 30.186 mg/ml). However, trolox had the highest activity of lipid peroxidation inhibition (IC_{50}= 0.147 mg/ml).

Table 2 Radical scavenging activities (DPPH and ABTS) and lipid peroxidation inhibition ($LOO^{\bullet}$) of longan (LS) and litchi seed extracts (KC and CP)

Samples	IC_{50} (mg/ml)		
	$DPPH^{\bullet}$	$ABTS^{\bullet+}$	$LOO^{\bullet}$
LS	0.745 ± 0.012^{b}	0.305 ± 0.004^{b}	30.186 ± 0.802^{c}
KC	0.828 ± 0.015^{c}	0.322 ± 0.003^{c}	9.925 ± 0.137^{b}
CP	0.950 ± 0.004^{d}	0.460 ± 0.015^{d}	10.205 ± 0.150^{b}
Trolox	0.125 ± 0.002^{a}	0.138 ± 0.003^{a}	0.147 ± 0.007^{a}

Values (Mean ± S.D.; n=3) with in the column followed by a difference superscript differ statistically (ANOVA; $p<0.05$)

Antimicrobial activities

The longan and litchi seed extracts were tested against three pathogen bacteria (*E. coli*, *S. aureus* and *P. aeruginosa*) by using paper disc diffusion method (Table 3). LS and KC extracts could inhibit only *S. aureus* but not in case of CP extract. The inhibition zone diameter against *S. aureus* of LS and KC extracts were 6.83 and 6.33 mm, respectively. Antimicrobial activity of LS and KC extracts were 3 times less than gentamicin as the positive control (19.00 mm).

Table 3 Antimicrobial activities of seed extracts and antibiotic against bacterial strain

Samples	Antimicrobial activities (diameter of inhibition, mm)		
	E. coli	***S. aureus***	***P. aeruginosa***
LS	*nd*	active (6.83±0.29)	*nd*
KC	*nd*	active (6.33±0.57)	*nd*
CP	*nd*	*nd*	*nd*
Gentamicin	active (13.00±0.00)	active (19.00±0.00)	active (21.50±0.50)

Mean±S.D.; n=3; *nd* as not detectable the clear zone

Discussion and Conclusion

The results showed that longan and litchi seed extracts contained the bioactive compounds, according to the previous studies that longan and litchi seed extracts composed of corilagin, gallic acid, ellagic acid, procyanidin A and B2 and quercetin (Rangkadilok et al. 2005; Rangkadilok et al. 2007; Prasad et al. 2009; Zheng et al. 2009; Wang et al. 2011). Therefore, the seed extracts which contained high level of phenolic content may increase the scavenging activity the free radicals. Antioxidants can deactivate radicals by two major mechanisms including hydrogen atom transfer (HAT) and single electron transfer (SET). Lipid peroxidation inhibition assay was grouped by SET mechanism while DPPH and ABTS assay had both HAT and SET mechanisms (Prior et al. 2005). This result indicated that LS extract showed high activity of DPPH and ABTS free radicals scavenging due to the hydrogen

donation mechanism. According to Rangkadilok et al (2007) suggested that dried longan seed extract was a potent scavenger of reactive oxygen species; ROS (X/XO and ORAC assay). KC and CP extracts had the higher ability of lipid peroxidation inhibition than LS extract for 3 times. Furthermore, litchi fruit pericarp strongly inhibited linoleic acid peroxidation and scavenged DPPH radical (Duan et al. 2007). However, linoleic acid peroxidation activity had not been reported in litchi seed extract.

This study demonstrated that LS extract showed the inhibitory effect against *S. aureus*. Besides, the longan seed extract demonstrated the antifungal (*Candida* sp. and *Cryptococcus neoformans*) activity due to the polyphenolic compound in the seed (Rangkadilok et al. 2012). For litchi seed extracts, KC extract could inhibit the growth of *S. aureus* but this bacterium was not inhibited by CP extract. Nonetheless, both longan and litchi seed extracts could not effect on the growth of *E. coli* and *P. aeruginosa*. This study indicated that LS and KC extracts had the inhibitory activity against the Gram positive bacterium, *S. aureus*. It may be possible that phenolic compound contained hydroxyl groups which related to their relative toxicity to microorganisms. The mechanisms could be responsible for phenolic toxicity to microorganisms including substrate deprivation and membrane disruption (Cowan 1999).

In conclusion, longan seed extract (LS) had the highest bioactive compounds (phenolic and flavonoid content) which exhibited high antioxidant activities (DPPH and ABTS radical scavenging). Both litchi seed extracts (KC and CP) showed high activity of lipid peroxidation inhibition. Furthermore, LS and KC extracts had an antimicrobial activity against *S. aureus*. This research indicated that longan and litchi seed extracts had antioxidant and antimicrobial activities. Therefore, it was suggested that these seed extracts could be applied as the active ingredient in cosmetic products. In addition, these by-products from fruit processing industry will be utilized as value-added.

Acknowledgements

This research was supported by Excellent Center for Cosmetics and Wellness (ECCW), School of Cosmetic Science, Mae Fah Luang University and Scientific and Technological Instruments Center, Mae Fah Luang University.

References

1. A.O.A.C. (2000) Official Methods of Analysis. Association of Official Analytical Chemist. EUA.
2. Chang C, Yang MH, Wen HM, Chern JC (2002) Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal 10:178-182.
3. Choi CW, Kim SC, Hwang SS, Choi BK, Ahn HJ, Lee MY (2002) Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay-guided comparison. Plant Sci 163:1161-1168.
4. Cowan MM (1999) Plant products as antimicrobial agents. Clin Microbiol Rev 12: 564–582.
5. Duan X, Jiang Y, Su X, Zhang Z, Shi J (2007) Antioxidant properties of anthocyanins extracted from litchi (*Litchi chinenesis* Sonn.) fruit pericarp tissues in relation to their role in the pericarp browning. Food Biochem 101:1365-1371.

6. Prasad KN, Yang B, Yang S, Chen Y, Zhao M, Ashraf M, Jiang Y (2009) Identification of phenolic compounds and appraisal of antioxidant and antityrosinase activities from litchi (*Litchi chinensis* Sonn.) seeds. Food Chem 116:1-7.
7. Moure A, Cruz JM, Franco D, Domínguez JM, Sineiro J, Domínguez H, Núñez MJ, Parajó JC (2001) Review: Natural antioxidants from residual sources. Food Chem 72:145-171.
8. Prior RL, Wu X, Schaich K (2005) Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. J Agric Food Chem 53:4290-4302.
9. Rangkadilok N, Sitthimonchai S, Worasuttyangkurn L, Mahidol C, Ruchirawat M, Satayavivad J (2007) Evaluation of free readical scavenging and antityrosinase activities of standardized longan fruit extract. Food Chem Toxicol 45:328-36.
10. Rangkadilok N, Tongchusak S, Boonhok R, Chaiyaroj SC, Junyaprasert VB, Buajeeb W, Akanimanee J, Raksasuk T, Suddhasthira T, Satayavivad J (2012) In vitro antifungal activities of longan (*Dimocarpus longan* Lour.) seed extract. Fitoterapia 83:545-553.
11. Rangkadilok N, Worasuttyangkurn L, Bennett RN, Satayavivad J (2005) Identification and quantification of polyphenolic compounds in longan (*Euphoria longana* Lam) fruit. J Agri Food Chem 53:1387-92.
12. Singleton VL, Rossi JA (1965) Colorimetric of total phenolics with phosphomolybdic-phosphotungstic acid reagent. Am J Enol Vitic 16:144-158.
13. Thaipong K, Boonprakob U, Crosbyb K, Cisneros-Zevallosc L, Byrne DH (2006) Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. J Food Compos Anal 19:669–675.
14. Wang L, Lou G, Ma Z, Liu X (2011) Chemical constituents with antioxidant activities from litchi (*Litchi chinensis* Sonn.) seeds. Food Chem 126:1081-1087.
15. Zheng G, Xu L, Wu P, Xie H, Jiang Y, Chen F, Wie X (2009) Polyphenols from longan seeds and their radical-scavenging activity. Food Chem 116:433-436.