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Research Article

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Antioxidant activity, total phenolics and flavonoids contents of *Luffa acutangula* (L.) Roxb fruit

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ABSTRACT

A study was performed on the antioxidant activity of the methanol extract of Luffa acutangula (L.) Roxb (ridge gourd) fruit (LAM) and its derived fractions, such as n-hexane (LAH), chloroform (LAC), ethyl acetate (LAE), n-butanol (LAB) and residual aqueous fraction (LAA) and their correlation to the total phenolics and flavonoids contents. The phytochemical screening demonstrated the presence of phenolic and flavonoid compounds in all the fractions except the n-hexane fraction. Carotenoids were present in the LAH and LAC, while absent in the LAE. The antioxidant activity was assessed using β-carotene bleaching method and the results showed that LAM, LAH and LAC have significant antioxidant activities in the comparison with those of BHT and PG. The total phenolics content of the extract/fractions was determined by the Folin-Ciocalteu procedure and ranged from 18.7±0.11 to 105.1±0.08 mg GAE/g of dried weight basis. The flavonoids content of extract/fractions was measured by aluminium chloride colorimetric assay and ranged from 34.9±0.09 to 105.3±0.09 mg QE/g of dried weight basis. The correlation coefficients between the antioxidant activities and the phenolics/flavanoids contents were found to be very small. The highest antioxidant activity was demonstrated by LAH and the highest total phenolics/flavonoids contents were presented by LAE. Thus, phenolics/flavanoids compounds were not the major contributor to the antioxidant activity of this fruit. The other antioxidant secondary metabolites, such as carotenoids, contributed considerably to the total antioxidant activity of the LAH and LAC. The results provided evidence that Luffa acutangula (L.) Roxb fruit could be potential rich source of natural antioxidant for use in food, cosmetic and pharmaceutical products.

Keywords: antioxidant; carotenoid; flavonoid; phenolic; *Luffa acutangula*.

INTRODUCTION

Antioxidants are compounds which possess the ability to protect cells from the damage caused by unstable molecules known as free radicals. Free radicals have been implicated as mediators of many diseases, including cancer, atherosclerosis and heart diseases [1-4]. Antioxidants have the capacity in preventing or slowing the oxidation reactions and have been recognized for their potential in promoting health and lowering the risk for cancer, hypertension and heart disease [5]. Since synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), tertiary butyl hydroquinone (TBHQ) and propyl galate (PG) are suspected to be toxic and have carcinogenic effects, naturally occurring antioxidants has considerably increased for use in food, cosmetic and pharmaceutical products [6-14].

The plant kingdom is endowed with various biologically active compounds, such as phenolics, flavonoids, carotenoids, tocopherols and ascorbate acid (vitamin C) which are known as antioxidant agents. Naturally occurring antioxidants can be found from different part of the plant. Flowers, fruits, leaves, seeds, cereal crops, barks and roots

have been investigated for their antioxidant properties [15-18]. In the search for new antioxidant sources, the present research is focused on fruit of *Luffa acutangula* (L.) Roxb (ridge gourd).

Luffa acutangula (L.) Roxb belongs to Cucurbitaceae family. It is widely found throughout southeastern Asia as a growing vegetative climber. The young fruits usually are taken as vegetables. The plant has been shown to have various medicinal properties such as treatment of jaundice, splenic enlargement and laxative and also proved as CNS depressant used traditionally in insect bites [19]. The plant also has potent α-glucosidase inhibitory effect [20]. The present study was designed to examine the antioxidant activities of the various fractions of methanol extract of *Luffa acutangula* (L.) Roxb fruit, to determine their total phenolics and flavanoids contents and to investigate the correlation between antioxidant activity and phenolics/flavanoids contents.

EXPERIMENTAL SECTION

Materials

Fresh fruit of *Luffa acutangula* (L.) Roxb was purchased from local market in Surakarta, Indonesia. Identification of the species was done at the Department of Biology, Gadjah Mada University, Indonesia. All the chemicals used were of analytical grade, purchased from Merck Chemical Company (Merck, Germany).

Preparation of the methanol extract

The fruit samples were cleaned, dried in the oven at 55°C for 72 h and powdered by Disk Mill FFD model to 40 mesh size. The powdered material (1 kg) were macerated with 95% methanol and allowed to stand for 48 h. The samples were filtered with filter paper and the residue was further macerated twice under the same conditions. The collected filtrates were combined and concentrated using a rotary evaporator (Bibby RE 200) under reduced pressure. The extract then was dried under vacuum dessicator, yielding 237 g of the LAM (23.3%) of greenish brown liquid.

Fractionation of the methanol extract

The methanol extract LAM (150 g) was suspended in 100 mL water and partitioned successively using organic solvents in order of increasing polarity with *n*-hexane, chloroform, ethyl acetate and *n*-butanol. After evaporation of the solvents in a rotary evaporator, the following fractions were obtained, respectively: LAH 6.4 g (4.3%), LAC 5.0 g (2.8%), LAE 3.2 g (2.1%), LAB 2.9 g (2.0%) and LAA 104.4 g (69.1%).

Screening of Phytochemical Components

The plant extract was subjected to qualitative tests adopting standard procedure for the identification of the phyto constituents [21-23].

Test for Phenolics

0.25 g of the extract was dissolved in 10 ml distilled water and filtered. A few drops of 0.1% ferric chloride were added to the filtrate. The appearance of intense brownish-green or a blue-black color indicated the presence of tannins in the test samples.

Test for Flavonoids

Test solution when treated with sodium hydroxide solution, shows increase in the intensity of yellow color which would become colorless on addition of few drops of dilute hydrochloric acid, indicates the presence of flavonoids.

Test for Carotenoids

0.25 g of the extract was extracted with 10 ml of chloroform in a test tube with vigorous shaking. The resulting mixture was filtered and 85% sulphuric acid was added. Blue color at the interface showed the presence of carotenoids.

β-Carotene bleaching method

The experimental procedure was conducted according to Suja *et al.* (2005) [24]. β-Carotene (0.2 mg), 20 mg of linoleic acid and 200 mg of tween 20 were mixed in 1 mL chloroform and the solvent was evaporated in rotary evaporator under vacuum. The residue was diluted with 50 mL oxygenated distilled water. To 4.8 mL of the resulting emulsion, 200 ppm (0.2 mL) of extract was added. A solution with 0.2 mL of ethanol and 4.8 mL of the above emulsion was used as control. Absorbance readings at 463 nm in an UV mini 1240 spectrophotometer

(Shimadzu, France) were carried out at 20 minute intervals during a period of 2 hours, keeping the cuvettes in a water bath at 50°C. Antioxidant activity was calculated as inhibition percentage, relative to the control, using the following equation:

% inhibition of β-carotene oxidation = [(AS(120) – AC(120)) / AC(0) –AC(120))] x 100

where AS(120) and AC(120) is the absorbance at 120 mins of the sample and control, respectively, and AC(0) is the absorbance of the control at zero time.

Total phenolics content

Total phenolics content was determined using the Folin-Ciocalteu method [25]. A calibration curve of gallic acid was prepared and the results were expressed as gallic acid equivalents (mg GAE/g of dried weight basis). In this method, 1 mL of the sample (1 mg/mL) and Folin-Ciocalteu's reagent (1 mL) and were mixed into a test tube. A control sample was prepared at the same time using distilled water (1 mL), and Folin-Ciocalteu's reagent (1 mL). After 4 min, saturated Na_2CO_3 solution (2 mL) was added. Ingredients in test tubes were well mixed using vortex and left in a dark place for 90 minutes. Absorbance was measured at 640 nm using an UV mini 1240 spectrophotometer (Shimadzu, France).

Total flavonoids content

Total flavonoids content was quantified following a method by Park *et al*. (2008) [26] with slight modification using quercetin as standard. In a 10 mL test tube, 0.3 mL of extract/fractions, 3.4 mL of 30% methanol, 0.3 mL of $NaNO_2$ 5% and 0.3 ml of $AlCl_3$ 10% were mixed. After 5 mins, 2 ml of NaOH 4% was added. The solution was mixed well and the absorbance was measured against the reagent blank at 506 nm. The total flavonoids were expressed as milligrams of quercetin equivalents (mg QE/g of dried weight basis).

Statistical Analysis

Experimental results were performed in triplicate and the data are presented as mean ± SD. The results were compared by one-way ANOVA. A difference was considered statistically significant if $p \leq 0.05$.

RESULTS AND DISCUSSION

Phytochemical screening

Phytochemical screening have been performed to evaluate the presence of constituents which are known to show antioxidant properties, such as phenolics, flavonoids and carotenoids. The phytochemical screening of methanol extract LAM and soluble fractions of *Luffa acutangula* (L.) Roxb fruits presented the presence or absence of the phytochemicals tested (Table 1). Phytochemical analysis of the LAM showed the presence of all the phytochemicals tested. Phenolic and flavonoid compounds were present in all the soluble fractions except the LAH. The carotenoids analysis was only be performed for the LAH, LAC and LAE and the results demonstrated the presence of the carotenoids in the LAH and LAC and the absence of the carotenoids in the LAE. The obtained results have shown good agreement with those obtained by Riaz *et al*. (2012) [27]. Phytochemical screening of the methanol derived fractions of *Dodonaea viscosa* Jacq revealed that phenolics and flavonoids were present in the chloroform, ethyl acetate and *n*-butanol fractions, whereas absent in the *n*-hexane fraction.

Table 1. Phytochemical analysis of the methanol extract and derived fractions of the *Luffa acutangula* (L.) Roxb fruit

Fruit extracts	Phytochemicals		
	Phenolics	Flavonoids	Carotenoids
Methanol extract (LAM)	+	+	+
n-Hexane fraction (LAH)	-	-	+
Chloroform fraction (LAC)	+	+	+
Ethyl acetate fraction (LAE)	+	+	-
n-Butanol fraction (LAB)	+	+	n.a
Residual aqueous fraction (LAA)	+	+	n.a

+ = present; - = absent

Antioxidant Activity

The antioxidant activities of the methanol extract and the derived fractions were determined spectrophotmetrically by β-carotene bleaching method. The β-carotene bleaching method is based on the loss of the yellow colour of β-

carotene due to its reaction with radicals which are formed by linoleic acid oxidation in an emulsion. The rate of β-carotene bleaching can be slowed down in the presence of antioxidants [28-29]. Accordingly, the absorbance decreased rapidly in reaction mixtures without antioxidant, whereas in the presence of antioxidant the reaction mixtures retained their colour and thus absorbance for a longer time. This fact is used in the antioxidant activity evaluation of the *Luffa acutangula* (L.) Roxb fruit. Table 2 shows the antioxidant activity of the methanol extract LAM of the *Luffa acutangula* (L.) Roxb fruit in the comparison with those of BHT and PG. The antioxidant power decreased in the order of PG > BHT > LAM. The antioxidant capacities of PG, BHT and LAM were significantly different from each other ($p<0.05$). Although antioxidant activities of the synthetic antioxidants are higher than that of the methanol extract LAM, the results suggested that the methanol extract presented relatively significant antioxidant effect.

Table 2. Antioxidant activity of the methanol extract LAM of the *Luffa acutangula* (L.) Roxb fruit, PG and BHT as assessed with β-carotene bleaching method

Sample	Antioxidant Activity (% inhibition of β-carotene oxidation)
Methanol Extract (LAM)	21.86 ± 1.01
BHT	26.24 ± 0.45
PG	31.29 ± 1.16

Table 3 shows the antioxidant activity of the LAM derived fractions of the *Luffa acutangula* (L.) Roxb fruits. The organic fractions LAH and LAC exhibited considerably good antioxidant activities in the comparison with those of BHT and PG, where the LAH fraction was the most potent antioxidant. The antioxidant power decreased in the order LAH > LAC > PG > BHT > LAE > LAB > LAA. Statistical analysis of antioxidant capacities exposed that LAH was significantly different with PG and BHT ($p<0.05$), whereas LAC, PG and BHT were not significantly different from each other ($p>0.05$). LAE and LAB were statistically undistinguishable from each other ($p>0.05$) but they were significantly different from LAH, LAC, PG and BHT ($p<0.05$).

Table 3. Antioxidant activity of the soluble fractions of the *Luffa acutangula* (L.) Roxb fruit, PG and BHT as assessed with β-carotene bleaching method

Fruit extracts	Antioxidant Activity (% inhibition of β-carotene oxidation)
n-Hexane fraction (LAH)	52.089 ± 2.37
Chloroform fraction (LAC)	36.33 ± 3.58
Ethyl acetate fraction (LAE)	22.09 ± 1.18
n-Butanol fraction (LAB)	19.69 ± 0.46
Residual aqueous fraction (LAA)	15.26 ± 0.22
BHT	30.54 ± 0.35
PG	33.55± 1.33

Total phenolics and total flavonoids contents

Phenolic and flavonoid compounds have been reported as potent antioxidants in β-carotene bleaching assays [30-32]. In this study, total phenolics and total flavonoids contents were estimated by Folin-Ciocalteu method and aluminium chloride colorimetric assay, respectively. Since phytochemical screening demonstrated the absence of phenolic and flavonoids in the LAH, total phenolics and total flavonoids contents estimation was not performed for LAH. Table 4 summarizes that total phenolic compounds in fractions varied widely, ranging from 18.7±0.11 to 105.1±0.08 mg GAE/g dw and the content of flavonoids varied from 34.9±0.09 to 105.3±0.09 mg QE/g dw. The LAC, LAE and LAB fruit fractions contained moderate to high phenolics and flavonoids contents and the LAE exhibited the highest phenolics and flavonoids contents. Statistical analysis of the phenolics content of the fractions were found to be similar to that of flavonoids content. The phenolics and flavanoids contents in LAE were significantly different from those in LAC and LAB ($p<0.05$), however the phenolics and flavanoids contents of LAC and LAB were not significantly different from each other ($p>0.05$). The phenolics/flavanoids contents of LAA was significantly different from those in LAC, LAE and LAB.

Table 4. Total phenolics and flavonoids contents of methanol extract and soluble fractions of *Luffa acutangula* (L.) Roxb fruit

Fruit extracts	Total phenolics (mg GAE/g dw)	Total flavonoids (mg QE/g dw)
Methanol extract (LAM)	24.6±0.05	43.2±0.12
n-Hexane fraction (LAH)	n.a	n.a
Chloroform fraction (LAC)	71.9±0.09	95.1±0.07
Ethyl acetate fraction (LAE)	105.1±0.08	105.3±0.09
n-Butanol fraction (LAB)	82.4±0.09	92.9±0.15
Residual aqueous fraction (LAA)	18.7±0.11	34.9±0.09

A wide variation of total phenolics and flavanoids contents was observed in vegetables, fruits and medicinal plants with high level of antioxidant activity, which supports our findings. Sengul *et al*. (2009) described the antioxidant activity of some medicinal plants and *Crocus sativus* had the highest total phenolic content of 42.29 mg GAE/g dry weight basis with antioxidant activity of 82.23% inhibition of β-carotene oxidation [33]. Alimpic *et al*. (2014) studied antioxidant DPPH free radical-scavenging activity of *Salvia amplexicaulis* Lam. in the whole plant and different parts, leaves, stems and flowers. The ethanol extract of leaves and methanol extract of the whole plant showed the highest activity against the DPPH radical. The ethanol extract of the leaves was the richest in phenols (222.40 mg GAE/g) and flavonoids (49.81 mg QE/g), whereas the methanol extract of the whole plant contained the highest amount of phenolics (180.89 mg GAE/g) and flavonoids (38.15 mg QE/g) [34]. *Torilis leptophylla* L were reported to act as an antioxidant agent due to its free radical scavenging and cytoprotective activity and its total phenolic content of the different fractions were found varied widely, ranging from 49.9±4.1 to 121.9±3.1 mg GAE/g dry weight basis (Saeed, 2012) [35].

Correlation between antioxidant activity and phenolics and flavonoid contents

The correlation coefficient (R^2) between the antioxidant capacity and the phenolics content of the soluble fractions of the *Luffa acutangula* (L.) Roxb fruit was found to be small ($R^2 = 0.16$). This result suggested that 16% of the antioxidant capacity of *Luffa acutangula* (L.) Roxb accessions results from the contribution of phenolic compounds. The correlation coefficient between the antioxidant capacities and the flavonoid contents was also established to be small ($R^2 = 0.26$). Statistical analysis exposed that the total phenolics or flavonoids contents were not correlated to the antioxidant activity ($p>0.05$). Although several studies have reported a good correlation between phenolics content and antioxidant activity of a number of plant species like fruits, vegetables, herbs and medicinal plants [36-40], our result is in agreement with some the other findings [33, 41-42]. Li *et al*. (2007) demonstrated no correlation between total phenolic content and antioxidant activities of 23 selected microalgae [43].

The correlation coefficient between the antioxidant capacities and the phenolic contents was very small could probably be due to the molecular antioxidant response of phenolic compounds. Different types of phenolic compounds have different antioxidant activities, depending on their chemical structure [35, 44]. The antioxidant activity of phenolics is mostly due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers. Only phenolic compounds with a certain structure and particularly hydroxyl position in the molecule can act as proton donating [45-47]. The samples possibly contain different type of phenolic compounds, which have different antioxidant activities.

Although phenolic compounds such as flavonoids, phenolic acids and tannins are considered to be major antioxidant compounds in many plant species like vegetables, fruits and medicinal plants [48-51], they were not a major contributor to the antioxidant capacities in *Luffa acutangula* (L.) Roxb. Thus, the antioxidant activity observed was not merely from the phenolics content and therefore, could not be explained just on the basis of their phenolics content. Some other antioxidant secondary metabolites such as volatile oils, carotenoids, polyunsaturated fatty acids, polysaccharides and vitamins [52-53] in this case contributed to 84% of the total antioxidant activity. The contribution of carotenoids to the total antioxidant activity was supported by the phytochemical screening results which demonstrated the presence of carotenoids in the LAH and LAC fractions possessing potent antioxidant activity.

CONCLUSION

The analysis of antioxidant activity of the *Luffa acutangula* (L.) Roxb fruit suggested that LAM, LAH and LAC can be the potent source of natural antioxidants. Although LAC, LAE and LAB contains considerable amount of phenolic and flavonoid compounds, the statistical analysis revealed the poor correlation between antioxidant activity

and phenolics and flavanoids contents. Thus, phenolic and flavonoid compounds were less important as antioxidants in *Luffa acutangula* (L.) Roxb fruit. The contribution of other antioxidant secondary metabolites to the total antioxidant activity of LAH and LAC was supported by the phytochemical screening results which demonstrated the presence of carotenoids in those fractions. Further work is required to quantify the carotenoids as well as to identify and quantify the other antioxidant secondary metabolites, such as polyunsaturated fatty acids, polysaccharides and vitamins that might have contribution to the high antioxidant activities.

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REFERENCES

[1] G Tirzitis; G Bartosz, *Acta Biochimica Polonica*, **2010**, 57(1), 139-142.
[2] MM Al-Dabbas; T Suganuma; K Kitahara; DX Hou; M Fujii, *J. Ethnopharmacol.*, **2006**, 108, 287-293.
[3] R Tsao; Z Deng; *J. Chromatogr. B*, **2004**, 812, 85-99.
[4] E Hayet; M Maha; A Samia; M Mata; P Gros; H Raida; MM Ali; AS Mohamed; L Gutmann; Z Mighri; A Mahjoub, *World J. Microbiol. Biotechnol.*, **2008**, 24, 2933-2940.
[5] E Souri; G Amin; H Farsam; H Jalalizadeh; S Barezi, *Iran.J. Pharm. Res*., **2008**, 7, 149-154.
[6] P Li; L Huo; W Su; R Lu; C Deng; L Liu; Y Deng; N Guo; C Lu; C He, *J. Serb. Chem. Soc.*, **2011**, 76(5), 709-717.
[7] T Kulisica, A Radonicb, V Katalinicc, M Milosa, *Food Chemistry*, **2004**, 85, 633–640.
[8] AA Hamid; OO Aiyelaagbe; LA Usman; OM Ameen; A Lawal, *Afr. J. of Pure and App. Chem.*, **2010**, 4(8), 142-151.
[9] WM Zhang; B Li B; L Han; HD Zhang, *Afr. J. Biotechnol*., **2009**, 8, 3887-3892.
[10] M Namiki, *Crit. Rev. Food Sci.*, **1990**, 29, 273-300.
[11] O Politeo; M Jukic; M Milos; *Food Chem.*, **2007**, 101, 379-385.
[12] B Tepe; D Daferera; A Sokmen; M Sokmen; M Polissiou, *Food Chem.*, **2005**, 90, 333-340.
[13] CS Ku; SP Mun, *Bioresour. Technol.*, **2007**, 99, 2852-2856.
[14] I Gulcin; ME Buyukokuroglu; M Oktay; OI Kufrevioglu, *J. Ethnopharmacol.*, **2003**, 86, 51-58.
[15] TM Rababah; NS Hettiarachy; R Horax, *J. Agric. Food Chem.*, **2004**, 52, 5183-5186.
[16] N Gámez-Meza; JA Noriega-Rodríguez; L Leyva-Carrillo; J Ortega-García; L Bringas-Alvarado; HS García; LA Medina-Juárez, *J. Food Process. Pres.*, **2009**, 33, 110-120.
[17] G Jiang; Y Jiang; B Yang; C Yu; R Tsao; H Zhang; F Chen, *J. Agric. Food. Chem.*, **2009**, 57, 9293-9298.
[18] M Terashima; I Nakatani; A Harima; S Nakamura; M Shiiba, *J. Agric. Food. Chem.*, **2007**, 55, 165-169.
[19] AV Misar; AS Upadhye, *Indian J. of Pharmaceutical Sci*., **2004**, 66, 4, 463-465.
[20] A Andrade-Cetto; J Becerra-Jimenez; R Cardenas-Vazquez-, *J. Ethnopharcol*., **2008**, 116, 27-32.
[21] JB Harborne. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, 3rd Edition, Chapman and Hall Ltd., London, **1973**; 49-188.
[22] D Krishnaiah; T Devi; A Bono, R Sarbatly, *J. Med. Plants Res.*, **2009**. 3, 67-72.
[23] SF Zohra; B Meriem; S Samira; MSA Muneer, *J. Nat. Prod. Plant Resour.*, **2012**, 2(4), 512-516.
[24] KP Suja; A Jayalekshmy; C Arumugha, *Food Chem*., **2005**, 91(2), 213-219.
[25] VL Singleton; JA Rossi, *Am. J. Enol. Vitic.*, **1965**, 16(3), 144-158.
[26] YS Park; ST Jung; SG Kang; BK Heo; P Arancibia-Avila; F Toledo; J. Drzewiecki; J Namiesnik; S Gorinstein, *Food Chem.*, **2008**, 107, 640–648.
[27] T Riaz; MA Abbasi; AU Rehman; T Shahzadi; M Ajaib; KM Khan, *J. Serb. Chem. Soc*., **2012**, 77 (4), 423-435.
[28] WA Wannes; B Mhamdi; J Sriti; MB Jemia; O Ouchikh; G Hamdaoui; ME Kchouk; B Marzouk, *Food Chem. Toxicol.*, **2010**, 48(5), 1362-1370.
[29] Y Lu; FN Shipton; TJ Khoo; C Wiart; Y Lu; FN Shipton; TJ Khoo; C Wiart, *Pharmacology & Pharm.*, **2014**, 5, 395-400.
[30] P Siddaraju; K Becker, *J. Agric. Food Chem*., **2003**, 50(8), 2144-2155
[31] A Padmashree; N Roopa; AD Semwal; GK Sharma; G Agathian; AS Bawa, *Food Chem*., **2007**, 104(1), 59-66.
[32] GK Jayaprakasha; PS Negi; BS Jena; J Mohan Rao, *J. Food Comp. Anal.*, **2007**, 20(3-4), 330-336.
[33] M Sengul; H Yildiz; N Gungo; B Cetin; Z Eser Z, *Pak. J. Pharm. Sci*., **2009**, 22(1), 102-106.

[34] A Alimpic; M Oaldje; V Matevski; PD Marin; S Delutic-lausevic, *Arch. Biol. Sci., Belgrade*, **2014**, 66 (1), 307-316.
[35] N Saeed; MR Khan; M Shabbir, *BMC Complement. Alter. Med.*, **2012**, 12, 221-233.
[36] C Anesini; GE Ferrano; R Filip, *J. Agric. Food Chem.*, **2008**, 56, 9225-9229
[37] F Song; R Gan; Y Zhang; Q Xiao; L Kuang; H Li, *Int. J. Mol. Sci*., **2010**, 11, 2362-2372
[38] A Bunea; DO Rugina, AM Pintea; Z Sconta; CI Bunea; C Socaciu, *Not. Bot. Horti. Agrobo.*, **2011**, 39(2), 70-76
[39] C Hsu; Y Chan; J Chang, *Biol. Res*., **2007**, 40, 13-21.
[40] JH Yang; HC Lin; JL Mau, *Food Chem*., **2002**, 77, 229-235.
[41] J Javanmardia; C Stushnoff; E Lockeb; JM Vivanco, *Food Chem*., **2003**, 83, 547-550
[42] M Bajpai; A Pande; SK Tewari; D Prakash, *Int. J. Food Sci. Nutrition*, **2005**, 56(4): 287-291.
[43] H Li; K Cheng; C Wong; K Fan; F Chen; Y Jiang, *Food Chem*., **2007**, 102, 771-776
[44] MT Satue-Gracia; M Heinonen; EN Franke, *J. Agric. Food Chem.*, **1997**, 45, 3362-3367.
[45] B Nickavar; M Kamalinejad; H Izadpanah, *Pak. J. Pharm. Sci.*, **2007**, 20(4), 291-294.
[46] A Wojdylo; J Oszmianski; R Czemerys, *Food Chem*., **2007**, 105(3), 940-949.
[47] BGH Shahidi, *Asian J. Sci*., **2004**, 3(1), 82-86.
[48] L Hua-Bin; C Ka-Wing; W Chi-Chun; F King-Wai; C Feng; J Yue, *Food Chem*., **2007**, 102, 771-776.
[49] YZ Cai; Q Luo; M Sun; H Corke, *Life Sci*., **2004**, 74, 2157-2184.
[50] YY Soong; PJ Barlow, *Food Chem.*, **2004**, 88, 411-417.
[51] CC Wong; HB Li; KW Cheng; F Chen, *Food Chem*., **2006**, 97, 705-711.
[52] F Chen, *Trends in Biotechnol*., **1996**, 14, 421-426.
[53] F Chen; HB Li; RN Wong; B Ji; Y Jiang, *J. Chromatography A*, **2005**, 1064, 183-186.