Antioxidant Activity of Phenolic Fractions of *Mallotus philippinensis* Bark Extract

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Abstract

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Phenolic compounds were extracted from *Mallotus philippinensis* bark using methanol. Six fractions (I–VI) were separated from the extract on a Sephadex LH-20 column using ethanol and acetone-water as the mobile phases and were evaluated for their total antioxidant activity, antiradical activity against DPPH$^{\bullet}$ (2,2-diphenyl-1-picrylhydrazyl radical), and reducing power. The total phenolics and tannin contents in the fractions were determined. The content of total phenolics in the fractions ranged from 54 mg/g (fraction I) to 927 mg/g (fraction VI). Condensed tannins were detected in fractions II–VI. Total antioxidant activity (TAA) of phenolic fractions of *Mallotus philipinensis* bark extract ranged from 0.58 mmol Trolox/g (fraction I) to 6.82 mmol Trolox/g (fraction IV). Fraction IV also showed the strongest antiradical activity against DPPH$^{\bullet}$ and reducing power. Several phenolic constituents in the fractions were detected by RP-HPLC using a gradient solvent system with UV-DAD detection.

Keywords: *Mallotus philippinensis*; bark; extract; natural antioxidants; phenolic compounds; tannins; antioxidant activity; antiradical activity

The plants of the genus *Mallotus* are a rich source of biologically active compounds such as phloroglucinols, tannins, terpenoids, coumarins, benzopyrans, and chalcons (Amakura & Yoshida 1996; Tanaka *et al*. 1998; Cheng & Chen 1999; Huang *et al*. 1999; An *et al*. 2001, 2003; Chung *et al*. 2002; Van Kiem *et al*. 2004; Supudompol *et al*. 2004; Wei *et al*. 2004; Ma *et al*. 2004; Likhitwitayawuid & Supudompol 2005). Biological activities of the extracts of the plants belonging to the genus *Mallotus*, or of individual chemical constituents isolated from these extracts, have been reported by several authors. The aqueous extract of *Mallotus pelatus* showed a strong antipyretic effect similar to that of paracetamol (Chattopadhyay *et al*. 2002). Ishii *et al*. (2003) reported that phloroglucinol derivatives of *Malotus japonicus* had been shown to inhibit pro-inflammatory cytokine production. They exhibited the pharmacological ability to suppress prostaglandin E-2 production by activated macrophages (Ishii *et al*. 2002), and reduced nitric oxide production by a murine macrophage-like cell line (Ishii *et al*. 2001). Bergenin, which is the major constituent of *Mallotus japonicus* (Figure 1) was reported as hepatoprotective against hepatotoxicity in rats

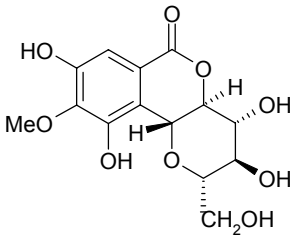

Figure 1. Chemical structure of bergenin

induced carbon tetrachloride (Kim *et al.* 2000). Liphophilic acetylbergenin isolated from *Mallotus japonicus* showed to possess more hepatoprotective activity than the much less lipophilic bergenin (Lim *et al.* 2000, 2001a, b). *Mallotus philippinensis* Muell. (*Euphorbiaceae*), also known as kamala or kamopollaka, was used as a source of a yellow dye (kamala dye), and as an antioxidant for ghee and vegetable oils (Rao & Seshadri 1947)

In our previous study (Arfan *et al.* 2007), several extracts of *Mallotus philippinensis* fruits and bark were prepared and evaluated for their total antioxidant activity, antiradical activity against DPPH$^{\bullet}$ (2,2-diphenyl-1-picrylhydrazyl radical), and reducing power. The extracts prepared from the bark offered the strongest antioxidant activity.

At this moment, the application of botanicals and botanical preparations in the European Union in foods is prohibited (European Food Safety Authority 2004). On the other hand, in Asian countries such as Pakistan and India, the food legislation is not so restricted as in Europe and, therefore, *Mallotus philippinensis* bark extract can find applications as a natural antioxidant for food, an ingredient of functional foods, and nutraceutical.

The present study was aimed at the investigation of the possible *in vitro* antioxidant effects of the phenolic fractions isolated from the bark of *Mallotus philippinensis* using column chromatography.

MATERIALS AND METHODS

Material. All solvents used were of analytical grade. Methanol, acetone, ethanol, acetonitrile, potassium ferricyanide, and trichloroacetic acid were acquired from the P.O.Ch. Company (Gliwice, Poland), (+)catechin, vanillin, Folin & Ciocalteau's phenol reagent, Sephadex LH-20, 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH$^{\bullet}$), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid (Trolox), and trifluoroacetic acid (TFA) were obtained from Sigma Ltd. (Poznań, Poland). *Mallotus philipinesis* bark was collected from the Buner area in N-W.F.P, Pakistan.

Extraction. *Mallotus philippinesis* bark was ground in a coffee mill. Phenolic compounds were extracted from the powdered material using methanol maceration (4 l for 12 h; at room temperature). The extraction was repeated twice more, the supernatants were combined, and methanol was evaporated under vacuum at 45°C using a rotary evaporator. The crude extract prepared (48 g) was stored at –20°C until used.

Column chromatography. A 0.5 g portion of the crude extract was dissolved in 8 ml of ethanol and applied to a chromatographic column (2 × 60 cm) packed with Sephadex LH-20, and subsequently eluted with 95% (v/v) ethanol. The fractions containing low-molecular weight phenolic compounds (5 ml) were collected using a fraction collector and their absorbance was measured at 280 nm. The tannin fraction was eluted from the column with acetone-water (1:1; v/v) (Strumeyer & Malin 1975). The eluates were then pooled into major fractions. Organic solvents were evaporated at 45°C using rotary evaporator and the water solution of the tannin fraction was lyophilised.

Total phenolics. The content of total phenolics in the fractions was determined according to the procedure described by Amarowicz *et al.* (2004b) using the Folin-Ciocalteau's reagent. (+)Catechin was used as the standard in this work.

Condensed tannins. The content of condensed tannins in the fractions was determined according to a modified vanillin method described by Price *et al.* (1978) and expressed as absorbance units at 500 nm per mg of fraction (A_{500}/mg).

The condensed tannins present in the Sephadex LH-20 fractions were also hydrolysed according to the method described by Porter *et al.* (1986). Briefly, into a 10 ml screw cap tube 6 ml of the acid butanol reagent (950 ml of *n*-butanol with 50 ml concentrated HCl), 1.0 aliquot of the extract, and 0.2 ml of the iron reagent (2% ferric ammonium sulfate in 2M HCl) were added and vortexed. The tube was capped loosely and put in a boiling water bath for 50 minutes. The tube was then cooled and the solution was transferred to a volumetric flask and made up to 25 ml with acid butanol. The absorbance at 550 nm was recorded using a Beckman DU 7500 diode array spectrophotometer

Table 1. Contents of total phenolics and condensed tannins in phenolic fractions of *Mallotus philipinensis* bark extract

Fraction	Total phenolics[a] (mg/g)	Condensed tannins[b] (A_{500}/mg)	Condensed tannins[c] (A_{550}/mg)
I	54 ± 1	–	–
II	357 ± 8	0.021 ± 0.001	0.047 ± 0.002
III	719 ± 17	0.972 ± 0.022	0.379 ± 0.002
IV	856 ± 20	1.25 ± 0.027	0.685 ± 0.007
V	794 ± 19	1.113 ± 0.023	1.220 ± 0.014
VI	927 ± 22	1.321 ± 0.030	1.745 ± 0.018

[a]reported as (+)-catechin equivalents; [b]determined using vanillin method; [c]determined after *n*-butanol/HCl hydrolysis

(Beckman Instruments, Fullerton, CA). The results were expressed as absorbance units at 550 nm per 1 mg of fraction (A_{550}/mg).

Total antioxidant activity. The total antioxidant activity (TAA) in the extracts was determined according to the Trolox Equivalent Antioxidant Activity (TEAC) assay described by Re *et al*. (1999). The total antioxidant activity was expressed as mmol Trolox/g of fraction.

Radical-scavenging activity. The capacity of the fractions to scavenge the "stable" free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) was monitored according to the procedure described by Amarowicz *et al*. (2002). Briefly, 0.1 ml of methanolic solution containing different amounts of the fractions was mixed with 2 ml of methanol and then 0.25 ml of 1mM methanolic solution of DPPH radical was added. The mixture was vortexed thoroughly for 1 minute. Finally, the absorbance of the mixture was read at 517 nm after standing at ambient temperature for 30 minutes.

Reducing power. The reducing power of the fractions was determined by the method of Oyaizu (1986). Briefly, the assay medium contained 2.5 ml of the respective fraction in 0.2M phosphate buffer (pH 6.6) and 2.5 ml of 1% (w/v) potassium ferricyanide. After incubation at 50°C for 20 min, 2.5 ml of 10% (w/v) trichloroacetic acid was added to the mixture followed by centrifugation at 1750 g for 10 minutes. The supernatant (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% (w/v) ferric chloride, and the absorbance of the resultant solution was read at 700 nm.

***HPLC analysis*:** Phenolic constituents of fractions I–VI were analysed using a Shimadzu HPLC system (Shimadzu Corp., Kyoto, Japan) consisting of two LC-10AD pumps, SCL 10A system controller, and SPD-M 10A photodiode array detector. Chromatography was carried out using a pre-packed LiChrospher 100 RP-18 column (4.6 × 250 mm, 5 μm; Merck, Darmstad, Germany). The elution for 50 min in a gradient system of 5–40% acetonitrile in water adjusted to pH 2.5 with TFA was employed. The detector was set at 280 nm; the injection volume was 20 μl and the flow rate was 1 ml/minute.

Semi-preparative HPLC. The main phenolic compound from fraction II was separated using a semi-preparative Hibar pre-packed RT column (10 × 250 mm) with Lichrosorb RP-18 (7 μm) (Merck, Darmstad, Germany). The mobile phase water-acetonitrile-acetic acid (88:10:2; v/v/v) (Amarowicz & Weidner 2001) was delivered at a rate of 4 ml/min using an injection volume of 500 μl. The detection was monitored at 280 nm.

ESI-MS. The pure compound separated from the bark extract was dissolved in 80% methanol (v/v) and applied on a column of Shimadzu liquid chromatograph mass spectrometer LC MS–QP 8000 system (Kyoto, Japan). The conditions of analysis were as follows: Luna 3 μ C (2) (2 × 150 mm) (Phenomenex; St. Torrance, CA, USA) column, mobile phase of 80% methanol (v/v), flow rate 0.2 ml/min; injection loop 10 μl; CDL temperature: 230°C; CDL voltage: +85 V; probe voltage: +4.5 kV, defragmentation voltage: +45 V; nebuliser gas flow 4.5 ml/minute.

RESULTS AND DISCUSSION

Six fractions (I–VI) containing phenolic compounds were obtained from the methanol extract of *Mallotus philippinensis* bark using Sephadex LH-20 column chromatography (Figure 2). Five fractions

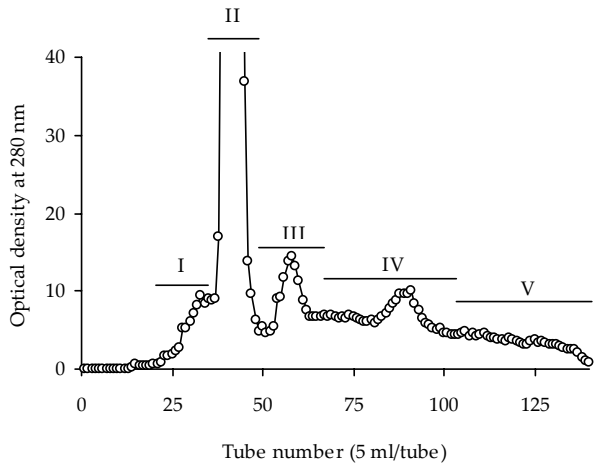

Figure 2. Separation of phenolic compounds from *Mallotus philippinensis* bark extract on a Sephadex LH-20 column with ethanol as the mobile phase

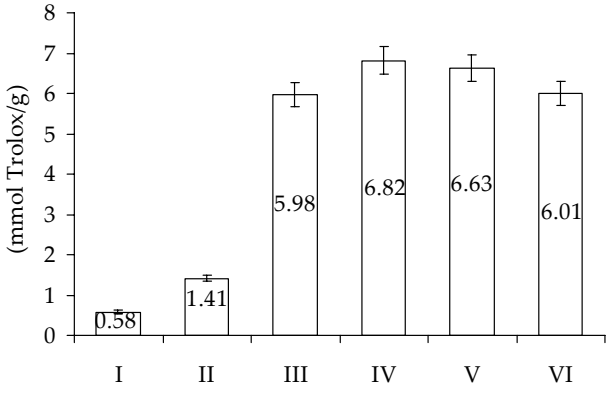

Figure 3. Total antioxidant activity (TAA) of phenolic fractions of *Mallotus philippinensis* bark extract (mmol Trolox/g)

(I–V) were obtained with ethanol as the mobile phase. Fraction VI was eluted from the column with acetone-water (1:1, v/v). The content of total phenolics in individual fractions (Tabel 1) ranged from 54 mg/g (fraction I) to 927 mg/g (fraction VI). The content of total phenolics in the fractions separated from the *Mallotus philippinensis* bark crude extract was high. The results were several times higher than those reported previously for the tannin-rich extracts from leguminous and oil seeds, as well as seed hulls (Amarowicz *et al.* 2000a, b, 2004a). Condensed tannins were detected in fractions II–VI . Fraction VI was characterised by the highest content of condensed tannins. In fraction II, only trace quantities of condensed tannins were detected. The positive results of the vanillin reaction and colorimetric assay after *n*-butanol/HCl hydrolysis in fractions III–V confirm the presence of catechins or dimers/trimers of this compound, which is in accordance with the findings obtained by Sephadex LH-20 column chromatography with ethanol and acetone-water as the mobile phases (Strumeyer & Malin 1975; Amarowicz & Shahidi 1995). The high values found in the two assays used for the detection of condensed tannins are typical for this kind of column chromatography. The polarity of 50% acetone is sufficient to elute from the column condensed tannins with a high degree of polymerisation. The contents of condensed tannins in the fractions separated from the *Mallotus philippinensis* bark crude extract were high. Much lower values were obtained when the extracts of tannin-rich plant materials such as leguminous seeds, canola hulls, and primrose seeds were separated using the same method (Amarowicz *et al.* 2000a, 2004a).

Total antioxidant activity (TAA) of phenolic fractions of *Mallotus philippinensis* bark extract ranged from 0.58 mmol Trolox/g (fraction I) to 6.82 mmol Trolox/g (fraction IV) (Figure 3). The values of TAA in fractions III–VI were several times higher than those reported in the extracts of phenolic compounds or their fractions separated from tannin-rich plant materials such as leguminous seeds, almonds, and hazelnuts (Amarowicz *et al.* 2004a, 2005; Alasalvar *et al.* 2006). In our previous study (Arfan *et al.* 2007), the extract obtained from the *Mallotus philippinensis* fruit using acetone exhibited the total antioxidant capacity of 1.54 mmol Trolox/g.

The fractions separated from the *Mallotus philippinensis* bark crude extract were investigated for the antiradical activity and reducing power. A freshly prepared DPPH$^{\bullet}$ solution exhibits a deep purple colour with an absorption maximum at 517 nm. This colour disappears when an antioxidant is added to the assayed solution. For the reducing power assay, the presence of reductants (i.e. antioxidants) in the extracts causes reduction of the Fe^{3+}/ferricyanide complex to the Fe^{2+} state. Therefore, it can be monitored by measuring the formation of Perl's Prussian blue coloration having a maximum absorbance at 700 nm.

Figure 4 depicts the concentration-dependent response curves of the fractions of *Mallotus philippinensis* bark extracts for the antiradical activity against DPPH$^{\bullet}$. The results are expressed as the decrease in the absorbance of the DPPH$^{\bullet}$ solution at 517 nm. The antiradical activities of fractions III–VI were several times higher than

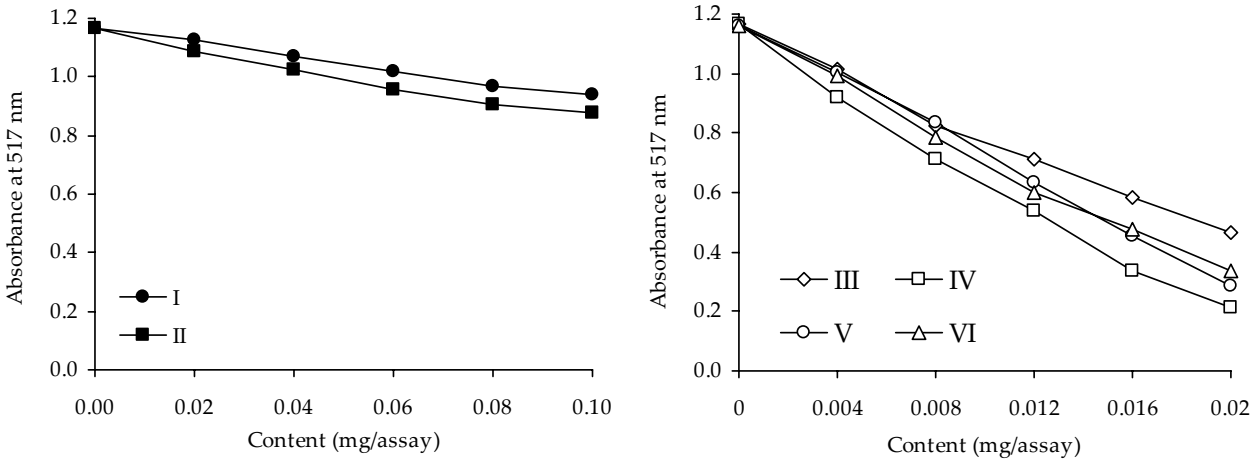

Figure 4. Scavenging effect of phenolic fractions of *Mallotus philippinensis* bark extract on the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•), as measured by changes in absorbance at 517 nm

those of fractions I and II. Like in the case of total antioxidant activity, fraction IV was found as the strongest scavenger of DPPH•. Fraction IV also exhibited the strongest reducing power that was ~ 10 times higher than those observed in fractions I and II (Figure 5).

Antiradical activity and reducing power in fractions III–VI were similar to those of the extract from the leaves of bearberry (*Arctostaphylos uva-ursi*), which is also a rich source of condensed tannins (Amarowicz *et al.* 2004b). A strong antiradical activity against DPPH• is typical for the tannins-rich plant extracts (Muir 1995; Chen & Chang 1995; Amarowicz *et al.* 1999, 2000a, b; Shahidi *et al.* 1997, 2001).

The antioxidant and antiradical properties of plant bark have been reported in several studies. Cui *et al.* (2005) noted that the addition of pine (*Pinus massoniana*) bark extract to a linoleic acid emulsion acted as a strong inhibitor of the peroxidation process. Procyanidins obtained from pine bark showed a strong antiradical activity (Virgili *et al.* 1998; Packer *et al.* 1999). Simić *et al.* (2003) described the inhibition of lipid peroxidation in liposomes, induced by Fe^{2+}/ascorbate system of *Larus nobilis* bark extract. The methanol extract from *Yucca periculosa* demonstrated scavenging properties towards DPPH• in TLC autographic and spectrophotometric assays (Torres *et al.* 2003). The extract of cat's claw (*Uncaria tomentosa*) bark was an effective scavenger of DPPH• (Sandoval *et al.* 2000). Ju *et al.* (2004) reported the extract from the dried bark of *Betula platyphylla* var. *japonica* as a strong DPPH• scavenger and lipid peroxidation inhibitor.

The RP-HPLC chromatograms of the phenolic fractions of *Mallotus philippinensis* bark extract

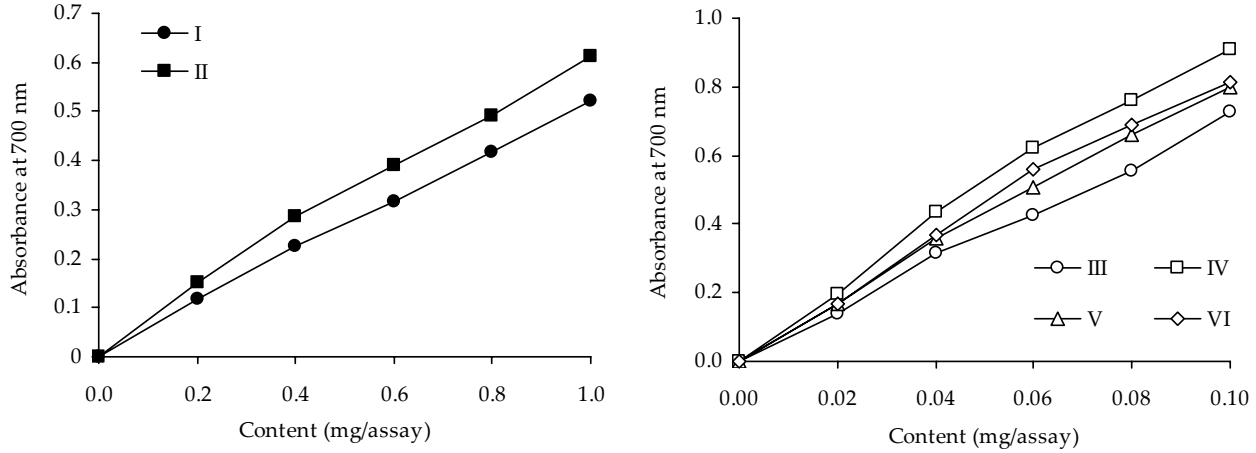

Figure 5. Reducing power of phenolic fraction of *Mallotus philippinensis* bark extracts, as measured by changes in absorbance at 700 nm

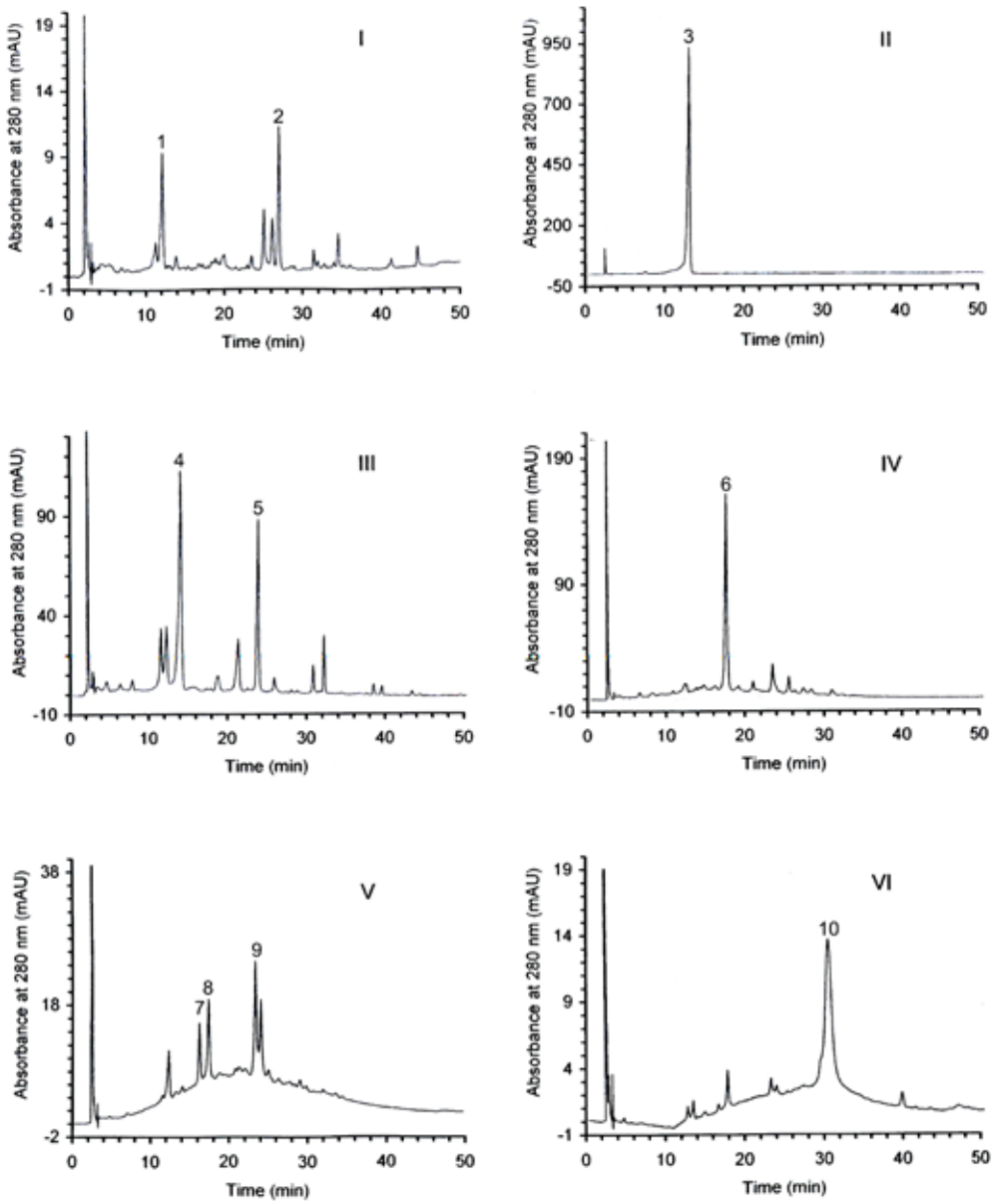

Figure 6. HPLC chromatograms of phenolic fractions of *Mallotus philipinensis* bark extract

were characterised by ten (1–10) dominant peaks (Figure 6). Three main peaks were recorded on the chromatogram as coming from fraction V, two peaks from fraction I, and two from fraction III. On the chromatograms of fractions II, IV, and VI, only one main peak was observed. The lack of a flat base line recorded on the chromatograms of fractions V and VI confirms the presence of condensed tannins in these fractions. The compounds giving peaks 1 and 3 in the chromatograms were characterised by an absorption maximum at 272 nm and a shoulder at 306 nm. At 272 nm, a maximum was recorded with the compound giving peak 8, and at 278 nm with peaks 5 and 7. The compounds attributed to peaks 4, 6, and 9 were characterised by maxima of UV spectra at 274, 278, and 276 nm, respectively. The maxima of UV spectra of compounds 2 and 10 were shifted towards a longer wavelength and recorded at 282 nm (2), 282 and 302 nm (10). The UV spectra show the absence of hydroxycinnaminates, flavonols and flavanols in the fractions. UV spectra of those phenolic compounds are characterised by the maxima at wavelengths of 320–330 nm (phenolic acids) and 340–350 nm (flavonols and flavanols) (Weidner *et al.* 1999; Mabry *et al.* 1970). The strong absorption band at 278 and 282 nm (peak 2 and 5) can be attributed to catechins or procyanidins (Amarowicz & Shahidi 1995).

The ESI-MS spectrum in the positive mode of the compound separated from the *Mallotus philippinensis* bark using a semi-preparative HPLC

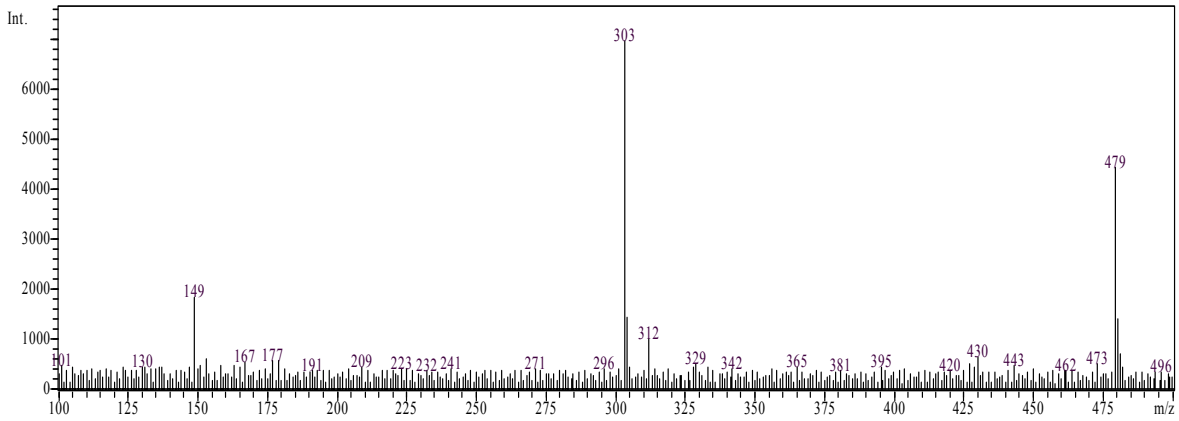

Figure 7. ESI-MS spectrum of the main compound separated from fraction II of the *Mallotus philippinensis* bark ex-

showed a $[M+H]^+$ at an *m/z* of 479 and dominant peaks of 303 and 304 where the glucuronic acid moiety was cleaved from the glycoside (Figure 7). Mass spectra indicated that the compound has a molecular weight of 478. Molecular weight of the aglycon was calculated to be 303. Most probably it was a derivative of bergenin whose MW is 328 (Takahashi *et al*. 2003).

CONCLUSION

From the results achieved it can be concluded that the fractions separated from the *Mallotus philipinensis* possess strong antioxidant and antiradical properties. After toxicological studies on some eventual harmful compounds present in the extract or its fractions, we suggest to use this material as natural antioxidant for food, functional foods, or nutraceuticals. Because of the presence of condensed tannins in *Mallotus philippinensis* bark, sensory studies are needed before practical applications.

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References

Alasalvar C., Karamać M., Amarowicz R., Shahidi F. (2006): Antioxidant and antiradical activities in extracts of hazelnut kernel (*Corylus avellan* L.) and hazelnut leafy green leafy cover. Journal of Agricultural and Food Chemistry, **54**: 4826–4832.

Amakura Y., Yoshida T. (1996): Tannins and related polyphenols of euphorbiaceous plants. 14. Euphorbin I, a new dimeric hydrolyzable tannin from *Euphorbia watanabei*. Chemical and Pharmaceutical Bulletin, **44**: 1293–1297.

Amarowicz R., Shahidi F. (1995). A rapid chromatographic method for separation of individual catechins from green tea. Food Research International, **29**: 71–76.

Amarowicz R., Weidner S. (2001): Content of phenolic acids in rye caryopses determined using DAD-HPLC method. Czech Journal of Food Sciences, **19**: 201–205.

Amarowicz R., Raab B., Karamać M. (1999): Antioxidative activity of an ethanolic extract of evening primrose. Nahrung, **43**: 216–217.

Amarowicz R., Naczk M., Shahidi F. (2000a): Antioxidant activity of crude extracts of canola/rapeseed hulls. Journal American Oil Chemists' Society, **77**: 957–961.

Amarowicz R., Naczk M., Zadernowski R., Shahiid F. (2000b): Antioxidant activity of condensed tannins of beach pea, canola hulls, evening primrose, and faba bean. Journal of Food Lipids, **7**: 195–205.

Amarowicz R., Karamać M., Weidner S., Abe S., Shahidi F. (2002): Antioxidant activity of wheat caryopses and embryos extracts. Journal of Food Lipids, **9**: 201–210.

Amarowicz R., Troszyńska A., Baryłko-Pikielna N., Shahidi F. (2004a): Extracts of polyphenolics from legume seeds – correlation between their total antioxidant activity, total phenolics content, tannins content and astringency. Journal of Food Lipids, **11**: 278–286.

Amarowicz R., Pegg R.B., Rahimi-Moghaddam P., Barl B., Weil J.A. (2004b): Free-radical scavenging

capacity and antioxidant activity of selected plant species from the Canadian prairies. Food Chemistry, **84**: 551–562.

Amarowicz R., Troszyńska A., Shahidi F. (2005): Antioxidant activity of almond extract. Journal of Food Lipids, **11**: 278–286.

An T.Y., Hu L.H., Cheng X.F., Chen Z.L. (2001): Benzopyran derivatives from *Mallotus apelta*. Phytochemistry, **57**: 273–278.

An T.Y., Hu L.H., Cheng X.F., Chen Z.L. (2003): Two new benzopyran derivatives from *Mallotus apelta*. Natural Product Research, **17**: 325–328.

Arfan M., Amin H., Karamać M., Kosińska A., Amarowicz R., Shahidi F. (2007): Antioxidant activity of extracts of *Mallotus philipensis* fruits and bark. Journal of Food Lipids, **14**: 280–297.

Chattopadhyay D., Arunachalam G., Mandal A.B., Sur T.K., Mandal S.C., Bhattacharya S.K. (2002): Antimicrobial and anti-inflammatory activity of folklore: *Mallotus peltatus* leaf extract. Journal of Ethnopharmacplogy, **82**: 229–237.

Cheng X.F., Chen Z.L. (1999): Two new diterpenoids from *Mallotus apelta* Muell. Arg. Journal of Asian Natural Products Research, **1**: 319–325.

Chung Y.-C., Chang C.-T., Chao W.-W., Lin C.-F., Chou S.-T. (2002): Antioxidative activity and safety of the 50% ethanolic extract from red bean fermented by *Bacillus subtilis* IMR-NK1. Journal of Agricultural and Food Chemistry, **50**: 2454–2458.

Cui Y. Xie H., Wang J. (2005): Potential biomedical properties of *Pinus massoniana* bark extract. Phytotherapy Research, **19**: 34–38.

European Food Safety Authority (2004): Discussion Paper on "Botanicals and Biological Preparations widely used as food supplements and related products: Coherent and Comprehensive Risk Assessment and Consumer Information Approaches", Brussels, 25 June 2004. Available at: http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620769656.htm

Huang P.L., Wang L.W., Lin C.N. (1999): New triterpenoids of *Mallotus repandus*. Journal of Natural Products, **62**: 891–892.

Ishii R., Horie M., Saito K., Arisawa M., Kitanaka S. (2001): Inhibitory effects of phloroglucinol derivatives from *Mallotus japonicus* on nitric oxide production by a murine macrophage-like cell line, RAW 264.7, activated by lipopolysaccharide and interferon-gamma. Biochimica Biophysica Acta – General Subjects, **1568**: 74–82.

Ishii R., Horie M., Saito K., Arisawa M., Kitanaka S. (2002): Prostaglandin E-2 production and induction of prostaglandin endoperoxide synthase-2 is inhibited in a murine macrophage-like cell line, RAW 264.7, by *Mallotus japonicus* phloroglucinol derivatives. Biochimica Biophysica Acta – General Subjects, **1571**: 115–123.

Ishii R., Horie M., Saito K., Arisawa M., Kitanaka S. (2003): Inhibition of lipopolysaccharide-induced proinflammatory cytokine expression via suppression of nuclear factor-kappa B activation by *Mallotus japonicus* phloroglucinol derivatives. Biochimica Biophysica Acta – General Subjects, **1620**: 108–118.

Ju E.M., Lee S.E., Hwang H.J., Kim J.H. (2004): Antioxidant and anticancer activity of extracts from *Betula plantyphylla var.* japonica. Life Sciences, **74**: 1013–1026.

Kim H.S., Lim H.K., Chung M.W., Kim Y.C. (2000). Antihepatotoxic activity of bergenin, the major constituent of *Mallotus japonicus*, on carbon tetrachloride-intoxicated hepatocytes. Journal of Ethnopharmacology, **69**: 79–83.

Likhitwitayawuid K., Supudompol B. (2005): A new phloroglucinol dimer from *Mallotus pallidus*. Heterocycles, **65**: 161–164.

Lim H.K., Kim H.S., Choi H.S., Oh S., Jang C.G., Choi J., Kim S.H., Chang M.J. (2000): Effects of acetylbergenin against D-galactosamine-induced hepatotoxicity in rats. Pharmacological Research, **42**: 471–474.

Lim H.K., Kim H.S., Chung M.W., Kim Y.C. (2001a): Protective effects of bergenin, the major constituent of *Mallotus japonicus*, D-galactosamine-intoxicated rat hepatocytes. Journal of Ethnopharmacology, **70**: 69–72.

Lim H.K., Kim H.S., Kim S.H., Chang M.J., Rhee G.S., Choi J. (2001a): Protective effect of acetylbergenin against carbon tetrachloride-induced hepatotoxicity in rats. Archives of Pharmacal Research, **24**: 114–118.

Ma J., Jones S.H., Hecht S.M. (2004): A coumarin from *Mallotus resinosus* mediates DNA cleavage. Journal of Natural Products, **67**: 1614–1616.

Mabry T.J., Markham K.R., Thomas M.B. (1970): The Systematic Identification of Flavonoids. Springer-Verlag, New York-Heidelberg-Berlin: 41–57.

Muir A.D. (1995): Antioxidative activity of condensed tannins. In: Shahidi F. (ed.): Chemistry, Health Effects, and Applications. AOCS Press, Champaign: 204–212.

Oyaizu M. (1986): Studies on products of browning reaction: Antioxidative activities of products of browning reaction prepared from glucosamine. Japanese Journal of Nutrition, **44**: 307–315.

Packer L., Rimbach G., Virgili F. (1999): Antioxidant activity and biological properties of a procyanidin-rich extract from pine (*Pinus maritime*) bark, pycnogenol. Free Radical Biology and Medicine, **27**: 704–724.

Porter L.J., Hirstich L.N., Chan B.G. (1986): The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. Phytochemistry, **25**: 223–230.

Price M.L, Van Scoyoc S., Butler L.G. (1978): A critical evaluation of the vanillic reaction as an asssay for tannin in sorghum grain. Journal of Agricultural and Food Chemistry, **26**: 1214–1218.

Rao V.S., Seshadri T.R. (1947): Kamala dye as an anthelmintic. Proceedings of the Indian Academy of Sciences – Section A, **26**: 178–181.

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

Shahidi F., Amarowicz R., He Y.H., Wettasinghe M. (1997): Antioxidant activity of evening primrose (*Oenothera blennis*): A preliminary study. Journal of Food Lipids, **4**: 75–86.

Shahidi F., Chavan U.D., Naczk M., Amarowicz R. (2001): Nutrient distribution and phenolic antioxidants in air-classified fractions of beach pea (*Lathyrus maritimus* L.). Journal of Agricultural and Food Chemistry, **49**: 926–933.

Sandoval M., Charbonnet R.M., Okuhama N.N., Roberts J., Krenova Z., Trentacosti A.M., Miller M.J.S. (2000): Cat's claw inhibits TNFα production and scavenges free radicals: role in cytoprotection. Free Radical Biology and Medicine, **29**: 71–78.

Simić M., Kundaković T., Kovačević N. (2003): Preliminary assay on the antioxidative activity of *Laurus nobilis* extracts. Fitoterapia, **74**: 613–616.

Strumeyer D.H., Malin M.J. (1975): Condensed tannins in grain sorghum: isolation, fractionation, and characterisation. Journal of Agricultural and Food Chemistry, **23**: 909–914.

Supudompol B., Likhitwitayawuid K., Houghton P.J. (2004): Phloroglucinol derivatives from *Mallotus pallidus*. Phytochemistry, **65**: 2589–2594.

Takahashi H., Kosaka M., Watanabe Y., Nakade K., Fukuyama Y. (2003): Synthesis and neuroprotective activity of bergenin derivatives with antioxidant activity. Bioorganic and Medicinal Chemistry, **11:** 1781–1788.

Tanaka T., Ito T., Iinuma M., Takahashi Y., Naganawa H. (1998): Dimeric chalcone derivatives from *Mallotus philippensis*. Phytochemistry, **48**: 142–1427.

Torres P., Avila J.G., De Vivar A.R., García A.M., Martín J.C., Aranda E., Céspedes C.L. (2003): Antioxidant and insect growth regulatory activities of stilbenes and extracts from *Yucca perculosa.* Phytochemistry, **64**: 463–473.

Van Kiem P., Van Minh C., Huong H.T., Nam N.H., Lee J.J., Kim Y.H. (2004): Pentacyclic triterpenoids from *Mallotus apelta*. Archives of Pharmacal Research, **27**: 1109–1113.

Virgili F., Kobuchi H., Packe L. (1998): Procyanidins extracted from *Pinus maritime* (Pycnogenol®): scavengers of free radical species and modulators of nitrogen monoxide metabolism in activated murine RAW 264.7 macrophages. Free Radical Biology and Medicine, **24**: 1120–1129.

Wei K., Li W., Koike K., Liu L.J., Fu X.W., Lin L.B., Chen Y.J., Nikaido T. (2004): Two new galloylglucosides from the leaves of *Mallotus furetianus*. Chemical and Pharmaceutical Bulletin, **52**: 776–779.

Weidner S., Amarowicz R., Karamać M., Dąbrowski G. (1999): Phenolic acids in caryopses of wheat, rye and triticale that disply different resistance to pre-harvest sprouting. European Food Research and Technology, **210**: 109–113.

Yen G.-C., Chen H.-Y. (1995): Antioxidant activity of various tea extracts in relation to their antimutagenicity. Journal of Agricultural and Food Chemistry, **43**: 27–2.

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