

ANTIOXIDANT ACTIVITY OF THE CRUDE EXTRACTS OF DRUMSTICK TREE (*MORINGA OLEIFERA* LAM.) AND SWEET BROOMWEED (*SCOPARIA DULCIS* L.) LEAVES*Leelavinothan Pari*¹, *Magdalena Karama*², *Agnieszka Kosińska*², *Anna Rybarczyk*², *Ryszard Amarowicz*²¹*Department of Biochemistry, Faculty of Science, Annamalai University, Tamil Nadu, India;*²*Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Division of Food Science, Olsztyn, Poland*

Key words: drumstick tree (*Moringa oleifera* Lam.), sweet broomweed (*Scoparia dulcis* L.), leaves, antioxidant activity, phenolic compounds, phenolic acids, HPLC

The crude extracts of phenolic compounds were obtained from drumstick (*Moringa oleifera* Lam.) and sweet broomweed (*Scoparia dulcis* L.) leaves. The content of total phenolics in the extracts was determined using the Folin-Ciocalteu's phenol reagent. Phenolic compounds present in the crude extracts showed antioxidant properties as revealed by the following determinations: the Total Antioxidant Activity (TAA), DPPH radical scavenging activity, and reducing power. The content of total phenolics in the extract of *Moringa oleifera* leaves was 118 mg/g. The lower content of the total phenolics was noted for the *Scoparia dulcis* leaves extract: 88 mg/g. The extract of *Moringa oleifera* was characterized by the higher value of the TAA (0.636 μ mol Trolox/mg) than *Scoparia dulcis* (0.432 μ mol Trolox/mg). For the extracts of *Moringa oleifera* also the stronger antiradical activity against DPPH radical and reducing power were noted. Phenolic acids (derivatives of caffeic, *p*-coumaric or ferulic acids) were the dominant phenolic constituents of *Moringa oleifera* leaves extract.

INTRODUCTION

Free radicals play an important role in the pathogenesis of several human diseases, such as cancer, rheumatoid arthritis, and cardiovascular diseases [Hertog *et al.*, 1997]. Natural antioxidants present in food of plant origin protect against these radicals and are therefore important tools in obtaining and preserving good health [Dell Agli *et al.*, 2004; Scoorbrate *et al.*, 2005]. Strong epidemiological evidence suggests that regular consumption of fruits and vegetables, which are a rich source of the antioxidants, can reduce cancer and coronary heart diseases [Block *et al.*, 1992; Middleton *et al.*, 2000].

Moringa oleifera Lam. (drumstick tree, horseradish tree) is an indigenous tree from northwestern India and is often cultivated in hedges and home yards. The tree is valued mainly for the tender pods, which are esteemed as a vegetable [Ramachandran *et al.*, 1980]. Flowers and young leaves are also eaten as vegetables. The leaves are a rich source of essential amino acids such as methionine, cysteine, tryptophan, and lysine [Makkar & Becker, 1997]. Decoctions and extracts made from the leaves are also variously employed in native medicine [Morton, 1991]. Ashokumar & Pari [2003] reported that the alcoholic extract of *Moringa oleifera* reduced a toxicity of some drugs in rats. Estrella *et al.* [2000] reported that *Moringa oleifera* leaves increased breast milk production among young mothers. Most of the Philippines women consume

Moringa leaves mixed in chicken or shellfish soups to enhance breast milk production. In southern India, village people use the fresh leaves to prepare cow and buffalo ghee from butter fat. It has been found that there is a significant increase in the shelf life of ghee and that *Moringa* leaves can be a good source of natural antioxidants.

Scoparia dulcis L., commonly known as "sweet broomweed", is widely used in Indian folk medicine for the treatment of diabetes mellitus. The herb *Scoparia dulcis* is also used in Brazilian folk medicine to treat bronchitis, gastric disorders, insect bites and skin wounds, and in oriental medicine to treat hypertension. Freire *et al.* [1991] have found that administration of the crude leaf extract of *Scoparia dulcis* have analgesic and anti-inflammatory properties. Scopadulcic acid B (SA-B), a novel diterpenoid, is the main ingredient of the biologically active compounds of *Scoparia dulcis* and its debenzoyl derivative, diacetyl scopadol (DAS), has been shown to inhibit gastric H⁺, K⁽⁺⁾-ATPase [Asano *et al.*, 1990] and antiviral effect [Hayashi *et al.*, 1988]. Recently, Pari & Latha [2005] and Ratnasooriya *et al.* [2005] have reported that the aqueous extract of *Scoparia dulcis* possesses antidiabetic and antioxidant properties.

The aim of the present study was to evaluate antioxidant activity of the crude extracts of drumstick tree (*Moringa oleifera* Lam.) and sweet broomweed (*Scoparia dulcis* L.) leaves using several assays.

MATERIALS AND METHODS

Chemicals. 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). Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox), caffeic acid, *p*-coumaric acid, ferulic acid, and (+)catechin were obtained from Sigma (Poznań, Poland).

Plant material. Leaves of *Moringa oleifera* and *Scoparia dulcis* were collected from Neyveli, Cuddalore District, Tamil Nadu, India. The plants were identified and authenticated at the Herbarium of Botany Directorate at the Annamalai University.

Preparation of plant extracts. Fresh leaves of *Scoparia dulcis* were chopped into small pieces and soaked overnight in water at solid material to water ratio of 1:3 (w/v) [Pari & Latha, 2004]. This suspension was filtered and the filtrate was lyophilized. For *Moringa oleifera* leaves the methanol extraction was used under similar conditions. The solvent was evaporated using a rotary evaporator. Both extracts were stored at 4°C until needed.

Total phenolics. The content of total phenolic compounds in extracts was estimated using the Folin-Ciocalteu's phenol reagent [Naczk & Shahidi, 1989]. (+)Catechin was used as a standard.

Total Antioxidant Activity (TAA). The Total Antioxidant Activity of the extracts was determined according to the Trolox equivalent antioxidant activity (TEAC) assay described by Re *et al.* [1999]. TAA was expressed as μmol Trolox equivalent/mg of extract.

Reducing power. Reducing power of extracts was determined as described by Oyaizu [1986]. The solution of the extracts (0.2–1.0 mg) in 1 mL of distilled water was mixed with 2.5 mL of 0.2 mol/L phosphate buffer (pH 6.6) and 2.5 mL of 1% (w/v) potassium ferricyanide. The mixture was incubated at 50°C for 20 min. Following this, 2.5 mL of 10% (w/v) trichloroacetic acid was added and the mixture was then centrifuged at $1750 \times g$ for 10 min. A 2.5 mL aliquot of supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% (w/v) FeCl_3 ; the absorbance of the mixture was read at 700 nm.

Scavenging of DPPH radical. Scavenging effect of phenolics from the extracts was monitored as described by Yen & Chen [1995]. A 0.1 mL of methanolic solution containing 0.04 to 0.20 mg of the extracts was mixed with 2 mL of methanol and then 0.25 mL a methanolic solution of DPPH (1 mmol/L) was added. The mixture was vortexed for 1 min, then left standing at room temperature for 20 min and the absorbance of this solution was subsequently read at 517 nm.

HPLC analysis of the crude extracts. For the HPLC

finger print analysis of phenolic compounds present in extracts a Shimadzu system (Shimadzu Corp., Kyoto, Japan) consisting of two LC-10AD pumps, SCL-10A system controller, SPD-M10A photo-diode array detector, and a pre-packed LUNA C18 (4×250 mm, $5 \mu\text{m}$, Phenomenex) were used. A flow rate of 1 mL/min, and gradient elution of acetonitrile-water-acetic acid (5:93:2, v/v/v) [solvent A] and of acetonitrile-water-acetic acid (40:58:2, v/v/v) [solvent B], 0–50 min solvent B from 0 to 100%; and injection volume of 20 μL were applied; whereas the separation of compounds was monitored at 280 and 320 nm.

Separation of phenolic acids from extract. Separation of phenolic acids was carried out according to Amarowicz & Weidner [2001]. The extract (300 mg) was suspended in 10 mL of 2 mol/L NaOH and hydrolysed for 4 h at room temperature under a nitrogen atmosphere. After acidification to pH 2 using 6 mol/L HCl, free phenolic acids and constituents liberated from esters were extracted 5 times into 15 mL of diethyl ether using a separatory funnel. Then, ether was evaporated to dryness. The dry residues of free phenolic acids and phenolic acids liberated from esters were dissolved in 2 mL of methanol and filtered through a $0.45 \mu\text{m}$ filter. The samples obtained in this way were injected onto an HPLC column.

HPLC analysis of phenolic acids. Phenolic acids were analysed using a Shimadzu HPLC system (Shimadzu Corp., Kyoto, Japan) consisting of a LC-10AD pump, SCL-10A system controller and SPD-M10A photo-diode array detector. Phenolic acids separation was done by a prepacked LiChrospher 100 RP-18 column (4×250 mm, $5 \mu\text{m}$; Merck, Darmstadt, Germany). The mobile phase water-acetonitrile-acetic acid (88:10:2; v/v/v) [Amarowicz & Weidner, 2001] was delivered at a rate of 1 mL/min. The detection was monitored at 320 nm.

RESULTS AND DISCUSSION

The content of total phenolics in the the extract of *Moringa oleifera* leaves was 118 mg/g (Table 1). The lower content of the total phenolics was noted for the *Scoparia dulcis* leaves extract: 88 mg/g. The extract of *Moringa oleifera* was characterized by the higher value of the TAA (0.636 μmol Trolox/mg) than the extract of *Scoparia dulcis* (0.432 μmol Trolox/mg). In general, the content of total phenolics in both extracts was higher than in the extracts of plant origin such as legume seeds and cereal grains [Amarowicz *et al.*, 2002; Karamac *et al.*, 2002, 2004; Amarowicz *et al.*, 2004a]. Similar results were determined in the extracts of oil seeds [Amarowicz *et al.*, 1995, 2001]. Higher amounts were found in grapevine leaves extracts [Amarowicz *et al.*, 2007].

TABLE 1. Characteristic of the extracts: content of total phenolics and Total Antioxidant Activity.

Plant	Total phenolics (mg/g)	Total Antioxidant Activity (μmol Trolox/mg)
<i>Moringa oleifera</i>	118 \pm 3	0.636 \pm 0.024
<i>Scoparia dulcis</i>	88 \pm 2	0.432 \pm 0.036

The values of TAA of the crude extracts of *Moringa oleifera* and *Scoparia dulcis* leaves were higher than those of methanolic extracts of cereal [Zieliński & Kozłowska, 2000], plants of *Brassica* family [Ciska *et al.*, 2007], almonds [Amarowicz *et al.*, 2004b], and pea [Amarowicz & Troszyńska, 2003]. Similar value of TAA to this reported in this study were found previously for the extracts of red lentil (0.68 mmol Trolox/g) and broad bean (0.58 mmol Trolox/g) [Amarowicz *et al.*, 2004b]. The extracts of grapevines leaves showed two-three times higher TAA values (0.991-1.225 mmol Trolox/g) [Amarowicz *et al.*, 2007] than the extracts of *Moringa oleifera* and *Scoparia dulcis* leaves.

The strong free radical scavenging effect of both extracts was confirmed in a DPPH assay (Figure 1). The extract of *Moringa oleifera* showed the stronger scavenging effect than the extract of *Scoparia dulcis*. At a concentration of 0.2 mg/assay the extract of *Moringa oleifera* scavenged 65% of DPPH used for the assay whereas the extract of *Scoparia*

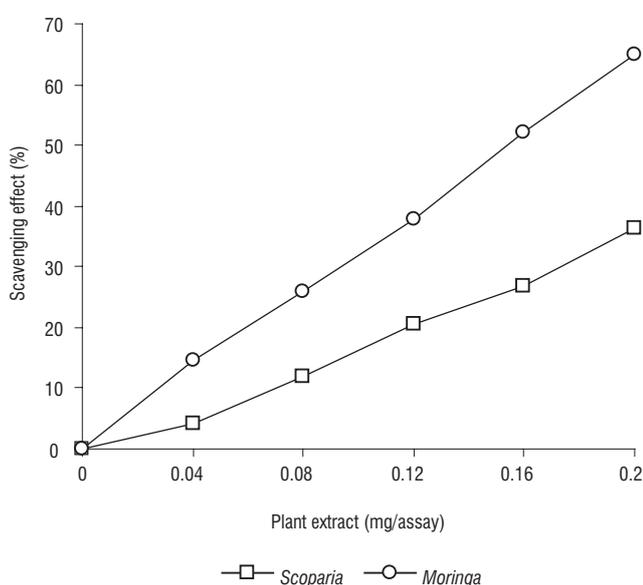


FIGURE 1. Scavenging effect of crude extracts from *Moringa oleifera* and *Scoparia dulcis* leaves on the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), as measured by changes in absorbance at 517 nm.

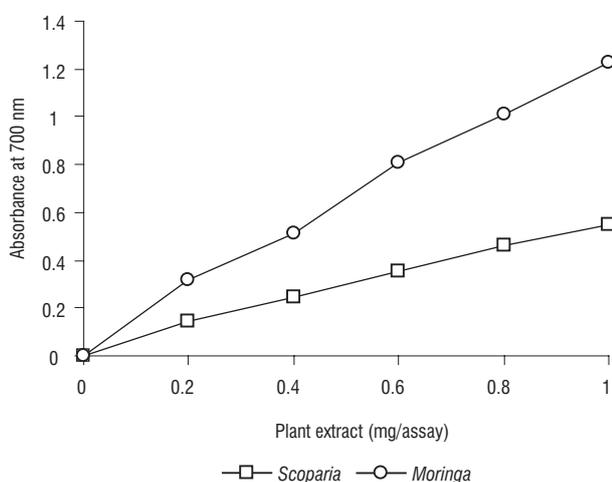


FIGURE 2. Reducing power of crude extracts from *Moringa oleifera* and *Scoparia dulcis*, as measured by changes in absorbance at 700 nm.

dulcis only 36%. The same relationship between two extracts were observed when their reducing power was assayed (Figure 2). The slope of the curve of the *Moringa oleifera* extract was more than two times higher than that of *Scoparia dulcis*. Both extracts offered the stronger antiradical properties and reducing power than the crude extracts of cereal grains [Amarowicz *et al.*, 2002; Karamać *et al.*, 2002, 2004], pea [Amarowicz & Troszyńska, 2003], almonds [Amarowicz *et al.*, 2004b] and red bean [Amarowicz & Troszyńska, 2004]. The stronger results were found for the grapevine leaves extracts [Amarowicz *et al.*, 2007]. Those extracts exhibited the presence of condensed tannins, which are strong antioxidants. The extracts examined in this study were free of tannins. From practical point of view, this is a beneficial property because tannins cause the sensation of astringency [Amarowicz *et al.*, 2004a], which can limit the application of tannin-rich extracts as a component of functional foods.

The HPLC chromatogram of the extract of *Moringa oleifera* leaves was characterized by six peaks recorded at wavelength of 280 nm (Figure 3A) and 320 nm (Figure 3B) with retention times of 13.2; 18.4; 19.2; 23.7; 31.1; 34.4 min. Compounds giving peaks 1-6 on chromatogram from *Moringa oleifera* leaves extracts were characterized by maxima of UV spectra at 324, 325, 326, 312, 354, and 347 nm, respectively (Figure 4). Compounds 1-4 did belong to phenolic acids and were derivatives of caffeic, *p*-coumaric or ferulic acid.

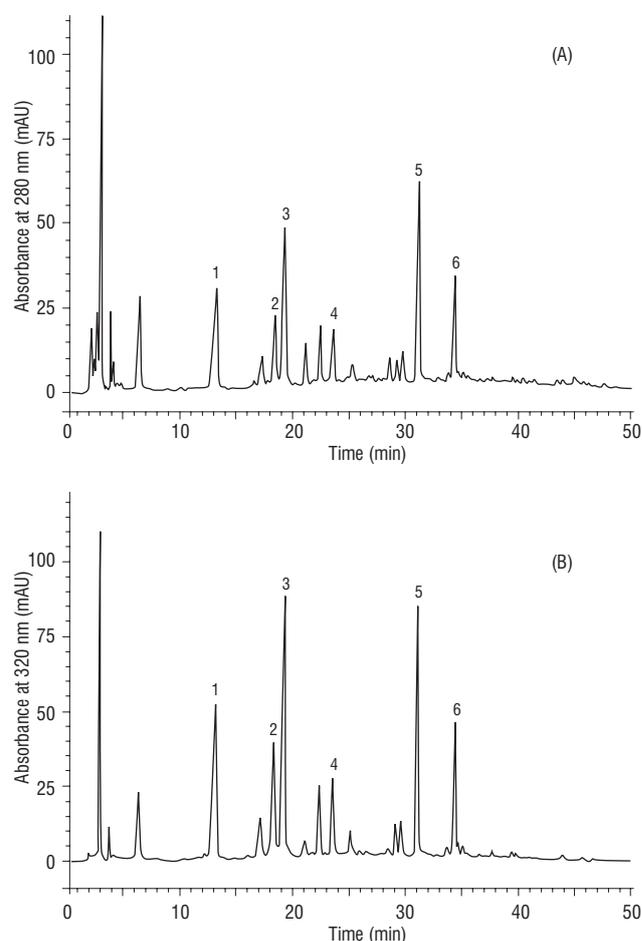


FIGURE 3. HPLC chromatograms of constituents of *Moringa oleifera* leaves extract recorded at wavelength of 280 nm (A) and 320 nm (B).

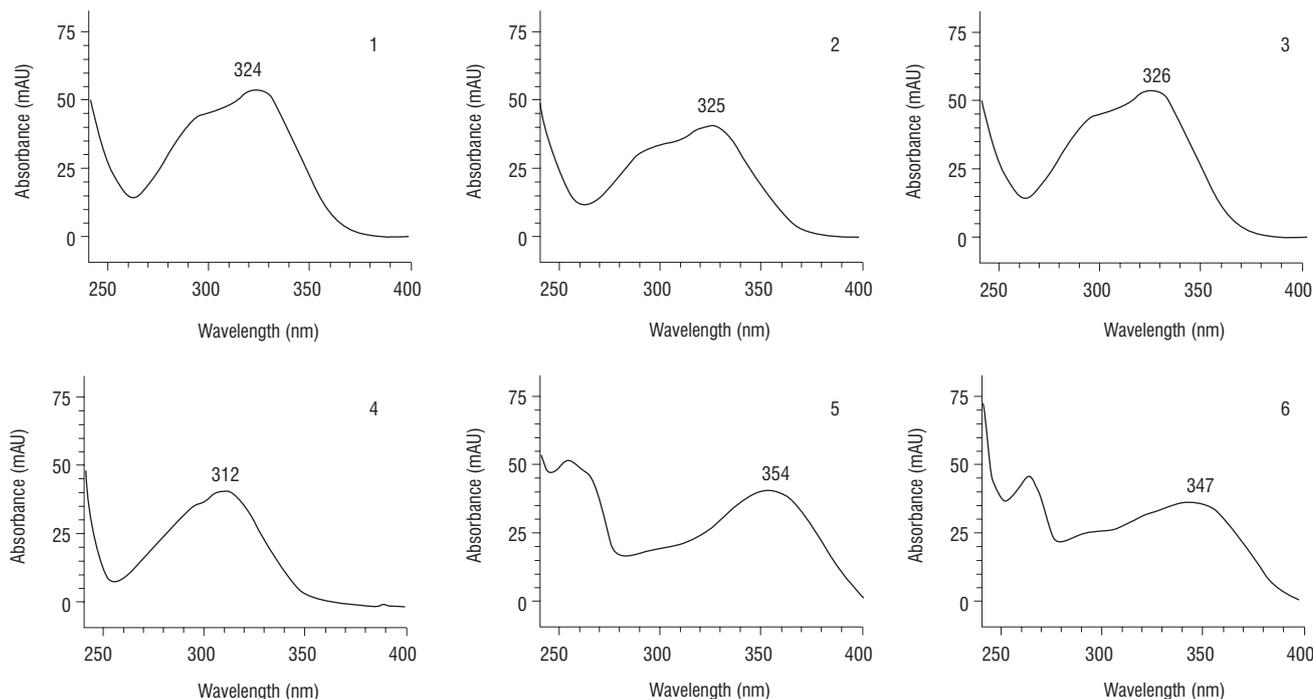


FIGURE 4. UV-DAD spectra of compounds separated from *Moringa oleifera* leaves extract using HPLC method; 1-6 - numbers of peaks corresponding to FIGURE 3.

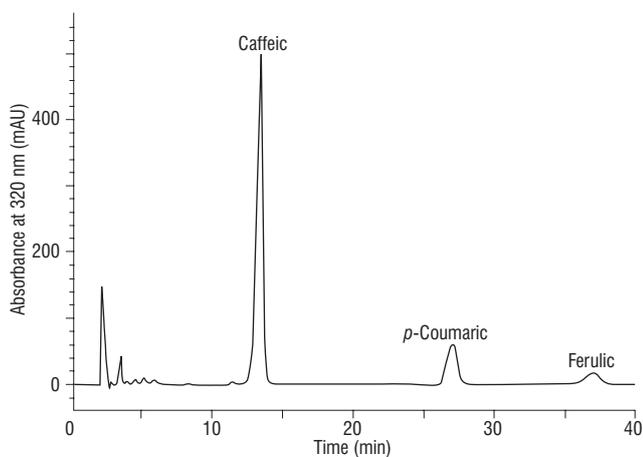


FIGURE 5. HPLC chromatogram of phenolic acids liberated from *Moringa oleifera* leaves extract after basic hydrolysis.

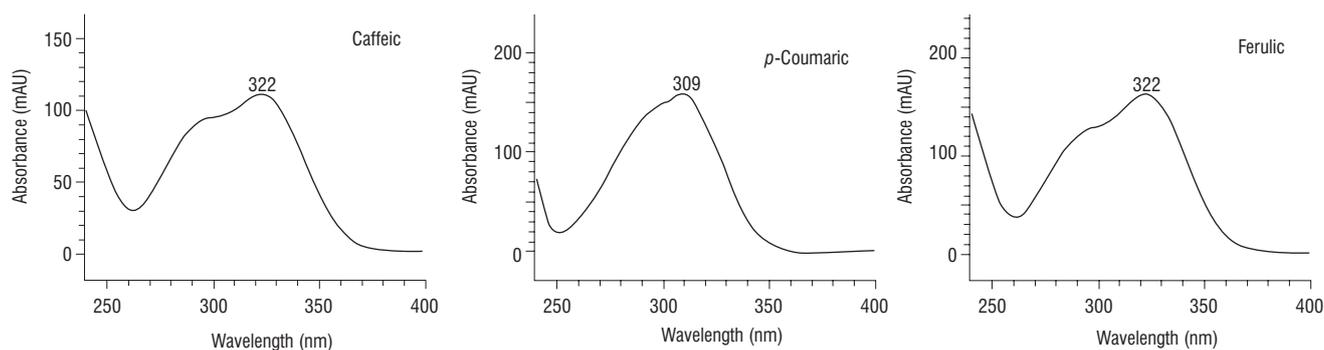


FIGURE 6. UV-DAD spectra of phenolic acids liberated from *Moringa oleifera* leaves extract after basic hydrolysis.

Compounds 5 and 6 were flavanols or flavonols [Mabry *et al.*, 1970].

After the basic hydrolysis of *Moringa oleifera* leaves extracts three phenolic acids were recorded on the chromatogram: caffeic, *p*-coumaric, and ferulic acids. Caffeic acid was the dominant one (Figure 5). Chemical structure of those phenolic acids was confirmed by their UV spectra (Figure 6).

Four main phenolic compounds were detected in *Scoparia dulcis* leaves extract (Figure 7). The peaks recorded on the chromatogram were characterized by retention times of 21.2; 25.1; 32.2; 33.0 min. The spectra of compounds separated from the *Scoparia dulcis* leaves extract (Figure 8) exhibited maxima at UV spectra at 262 nm (1), 270 and 334 nm (2), 280 and 334 nm (3), 287 and 332 nm (4). The interpretation of these spectra is difficult. The strong absorption bands at 334 and 270 nm (compound 2) and 334 and 280 nm (compound 3) point out, that they can be phenolic acids estrified by some other phenolic constituents.

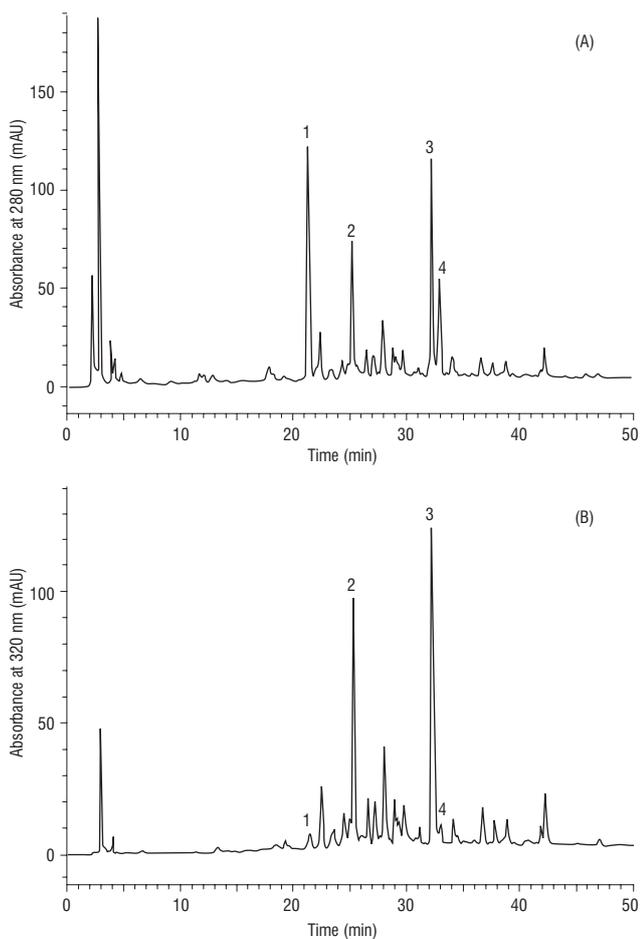


FIGURE 7. HPLC chromatograms of constituents of *Scoparia dulcis* leaves extract recorded at wavelength of 280 nm (A) and 320 nm (B).

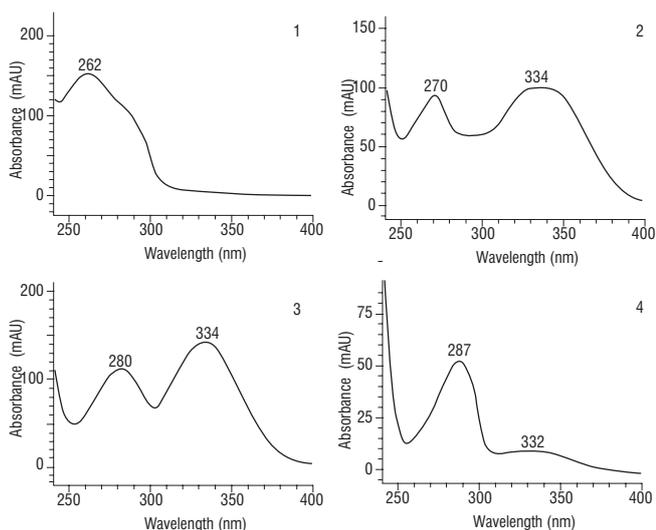


FIGURE 8. UV-DAD spectra of compounds separated from *Scoparia dulcis* leaves extract using HPLC method; 1-4 - numbers of peaks corresponding to FIGURE 7.

CONCLUSIONS

The crude extracts of phenolic compounds obtained from the drumstick (*Moringa oleifera* Lam.) and sweet broom-

weed (*Scoparia dulcis* L.) leaves exhibit the strong antioxidant properties expressed in terms of the free radical scavengers activity and reducing power. The absence of condensed tannins in the extracts allows to apply *Moringa oleifera* and *Scoparia dulcis* leaves as the components of functional foods and use the extract as the nutraceuticals. Phenolic acids are the dominant phenolic constituents of the *Moringa oleifera* leaves extract.

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AKTYWNOŚĆ PRZECIWUTLENIAJĄCA SUROWYCH EKSTRAKTÓW Z LIŚCI *MORINGA OLEIFERA* LAM. I *SCOPARIA DULCIS* L.

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Ekstrakty związków fenolowych uzyskano z liści *Moringa oleifera* Lam i *Scoparia dulcis* L. Zawartość związków fenolowych ogółem oznaczono kolorymetrycznie z odczynnikiem fenolowym Folina i Ciocalteu'a. Do analizy aktywności przeciwutleniającej ekstraktów zastosowano pomiar całkowitej aktywności przeciwutleniającej (TAA), zmierzanie wolnego rodnika DPPH oraz zdolność redukcyjną. Zawartość fenoli ogółem w ekstrakcie z liści *Moringa oleifera* wyniosła 118 mg/g. Niższą wartość zanotowano dla ekstraktu z liści *Scoparia dulcis*: 88 mg/g. Ekstrakt z liści *Moringa oleifera* charakteryzował się wyższą wartością TAA (0.636 μ mol Trolox/mg) niż ekstrakt z liści z *Scoparia dulcis* (0.432 μ mol Trolox/mg). Pierwszy z powyższych ekstraktów wykazywał też silniejszą aktywność przeciwrodnikową wobec DPPH i silniejsze właściwości redukcyjne. W ekstrakcie z liści *Moringa oleifera* dominowały fenolokwasy – pochodne kwasu kawowego, *p*-kumarowego i ferulowego.